

# Studies on the Immunomodulatory and Antioxidant Activities of Germinated Brown Rice Extracts

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## ABSTRACT

Germinated brown rice (GBR) contains various nutrients needed by human body, such as  $\gamma$ -aminobutyric acid, vitamin E, food fiber, antioxidants, inositol and long-chain fatty acids. In this study, different solvents (phosphate buffer solution (PBS), aqueous alkali and ethanol) were used for extraction, so as to study the growth inhibition ability on human leukemic U937 cells, immunomodulatory activity, and antioxidative activity of the GBR extracts. As for the antioxidation, the GBR extracts (PGBR) obtained by PBS exhibited higher DPPH free radical scavenging ability, reducing power and superoxide anion scavenging ability than did those obtained by aqueous alkali (AGBR) and ethanol (EGBR). However, EGBR exhibited the highest Trolox equivalent antioxidative capacity and ferrous ion chelating ability. As for the cell growth inhibition ability and immunomodulatory activity, the growth inhibition rates of U937 cells incubated in the conditioned mediums stimulated by PGBR, AGBR and EGBR for 1 day under a concentration of 800  $\mu$ g/mL were 49.83, 57.39 and 51.23 %, respectively. After stimulation for 3 days under the same concentration, the growth inhibition rates were 58.51, 62.23 and 60.77 %, respectively, where the inhibition effect of AGBR was the highest. The cytokine secretion of the conditioned medium of human mononuclear cells (MNCs) stimulated by PGBR, AGBR and EGBR for 1 and 3 days was also determined in this study. The IL-1 secretion of MNC stimulated by PGBR, AGBR and EGBR (at 1000  $\mu$ g/mL) for 1 day was 1293.47, 1572.93 and 2094.71 pg/mL, respectively; and for 3 days was 1797.56, 1861.63 and 2613.95 pg/mL, respectively. According to the results, IL-1 secretion increased with increasing stimulation day. EGBR was the most effective to stimulate the mononuclear cell conditioned medium (MNC-CM) for IL-1 secretion. The TNF- secretion of MNC stimulated by PGBR, AGBR and EGBR (at 1000  $\mu$ g/mL) for 1 day was 1950.38, 546.30 and 1365.02 pg/mL, respectively; and for 3 days was 2346.54, 914.82 and 1881.10 pg/mL, respectively. PGBR was the most effective for the TNF- secretion. The IFN- secretion of MNC stimulated by PGBR, AGBR and EGBR (at 1000  $\mu$ g/mL) for 1 day was 853.30, 911.75 and 893.46 pg/mL, respectively; and for 3 days was 1461.14, 1135.47 and 907.91 pg/mL, respectively. PGBR was also the most effective for the IFN- secretion. The NO contents of MNC-CM stimulated by PGBR, AGBR and EGBR (at 100  $\mu$ g/mL) for 1 day were 0.54, 0.46 and 0.52  $\mu$ M/mL, respectively; and for 3 days were 0.80, 0.68 and 0.66  $\mu$ M/mL, respectively. The greatest growth index of MNC, up to 1.02~1.04, was achieved by treatment of GBR extracts at 100  $\mu$ g/mL for 1 day. After treatment of GBR extracts at 100  $\mu$ g/mL for 3 days, PGBR and AGBR could inhibit the proliferation of the MNC. Under a concentration of 500  $\mu$ g/mL, the extracts could kill MNCs, where PGBR was the most significant.

Keywords : Germinated brown rice、 Immunomodulatory activity、 Antioxidative activity、 Cytokines、 Human leukemic U937 cells、 Human nuclear cells

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## REFERENCES

- 1.中國國家標準。CNS13446。
- 2.丁穎。1949。中國古來粳秈稻種栽培及分佈之探討與栽培稻種分類法預報。中山大學農學院農林研究委員會專刊。農藝專刊。
- 3.江淑華。2005。牛初乳及其酵素水解物之抗氧化性與其蛋白質組成之相關性研究。私立大葉大學生物產業科技學系博士論文，彰化。
- 4.宮華婷。2006。初乳之免疫調節作用及對人類白血病細胞(U937)之抑制效果。國立台灣大學食品科技研究所碩士論文，台北。
- 5.汪呈因。1983。稻作學與米。徐氏基金會出版。台灣，台北。
- 6.朱礮英。2002。富含-胺基丁酸發芽糙米之研製。國立嘉義大學食品科學系碩士論文。
- 7.李平篤、李門輝。1993。水稻種子發芽發育過程中醱代謝相關酵素活性之變化。中國農業化學會誌31: 265-273。
- 8.胡長達。2003。經不同方式發芽之糙米其化學組成成分、理化特性與抗氧化活性之探討。國立中興大學食品科學系碩士論文。
- 9.高景輝、湯文通 1977b。種子的生理。科學農業 25(5/6): 165-169。
- 10.徐麗嵐。2002。以柳松菇與鴻喜菇誘導人類白血病細胞(U937)分化及對Balb/c 鼠皮下移植CT26 腫瘤之抑制效果。國立台灣大學食品科技研究所碩士論文。台北，台灣。
- 11.歐馨婷。2001。多種蔬菜抑制人類白血病細胞(U937)增殖與誘導其分化之探討。國立台灣大學食品科技研究所碩士論文。台北，台灣。
- 12.曾慶瀛、余哲仁、洪云利。1994。烏豆、薏仁和糙米應用於早餐穀粉之製造。中華生質能源學會會誌13: 102-109。
- 13.雷少平。1980。米糠油中米糠醇與對烏腳病之膳食治療之研究。國立台灣大學農業化學研究所碩士論文。
- 14.鄭心嫻、謝明哲、朱哲輝。1991。白米、糙米及添加聚糊精的白米飲食對青年男子代謝的影響。中華民國營養學會雜誌。16: 157-175。
- 15.藍以政。1998。血液疾病簡介。內科新知。
- 16.盧訓。1991。穀類科學與加工，pp.92。國立中興大學。台中，台灣。
- 17.莊榮輝。1985。水稻蔗糖合成之研究。國立台灣大學農業化學研究所博士論文，台北。
- 18.曹巧吟。2003。樟芝中免疫調節蛋白的純化與其生理活性之探討。國立臺灣大學園藝學研究所碩士論文，台北。
- 19.Ahmad, R., Ahmad, S. and Baig, M. A. 1996. Enzymic studies of germinated cereal grains. Pak. J. Sci. Res. 48(3-4): 71-73.
- 20.Arnao B.M., Cano A, Hernandez-Ruiz J, Garcia-Canovas. F. and Acosta, M. 1996: Inhibition by L - ascorbic acid and other antioxidants of the 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) oxidation catalyzed by peroxidase: a new approach for determining total antioxidant status of food. Ana. Biol. Chem. 236: 255-261.
- 21.Aruoma, O. I., Murcia, A., Butler, J. and Halliwell, B. 1993. Evaluation of antioxidant and prooxidant actions of gallic acid and its derivatives. J. Agric. Food Chem. 41: 1880-1885.
- 22.AOAC. (1995). Official method of Analysis, 14th ed. Association of Official American Chemists, Washington, D.C., U.S.A.
- 23.Bogdan, C. 2000. The function of nitric oxide in the immune system. in Handbook of Experimental Pharmacology. volume: Nitric Oxide (ed. Mayer, B.). p. 443-492.
- 24.Bourne, L. and Rice-Evans, C. A. 1997. The effect of the phenolic antioxidant ferulic acid on the oxidation of low density lipoprotein depends on the pro-oxidant used. Free Radic. Res. 27: 337-344.
- 25.Centeno, C., Viveros, A., Brenes, A., Canales, R., Lozano, A. and Cuadra, C. 2001. Effect of several germination conditions on total P, phytate P, phytase, and acid phosphatase activities and inositol phosphate esters in rye and barley. J. Agric. Food Chem. 49: 3208-3215.
- 26.Champagne, E. T., Hron, R. J. and Abraham, G. 1991. Stabilizing brown rice product by aqueous ethanol extraction. Cereal Chem. 68: 267-271.
- 27.Chen, Y. Y. and Chang, H. M. 2004. Antiproliferative and differentiating effects of polysaccharide fraction from fu-ling (Poria cocos) on human leukemic U937 and HL-60 cells. Food Chem. Toxicol. 42: 759-769.
- 28.Cushman, D. W. and Cheung, H. 1971. Spectrophotometric assay and properties of the angiotensin converting enzyme of rabbit lung. Biochem. Pharmacol. 20: 1637-1648.
- 29.Decker, E. A. and Welch, B. 1990. Role of ferritin as a lipid

oxidants. *J. Chem. Soc. Faraday Trans. 94*: 1971-1978. 30. Dexter, T. M. and Heyworth, C. M. 1994. Growth factors and the molecular control of hematopoiesis. *Eur. J. Clin. Microbiol. Infect Dis.* 13: 3-8. 31. Dinarello, C. A. 1991. Interleukin-1 and interleukin-1 antagonism. *Blood* 77: 1627-1752. 32. Empson, K. L., Labuza, T. P. and Graf, E. 1991. Phytic acid as a food antioxidant. *J. Food Sci.* 56: 560-563. 33. Fang, Y. Z., Yang, S. and Wu, G. Y. 2002. Free radicals, antioxidants, and nutrition. *Nutrition* 18: 872-879. 34. Goldsby, R. A., Kindt, T. J., Osborne, B. A. and Kuby, J. 2003. Immunology. 5th ed. W. H. Freeman Co., New York. 35. Jantova, S., Cipak, L. and Letasiova, S. 2007. Berberine induces apoptosis through a mitochondrial/caspase pathway in human promonocytic U937 cells. *Toxicology in Vitro* 21:25 – 31. 36. Gauldie, R. W., West, I. F., Mulligan K. P. and Coote, G. W. 1992. Structure and chemical composition of the scales of the Orange Roughy *Hoplostethus atlanticus* and the Oreodories. *Tissue and Cell* 23: 677-708. 37. Halliwell, B. and Gutteridge, J. 1998. Free radicals in biology and medicine. pp. 316-319. Oxford university press, London, UK. 38. Isaacs, A. and Lindenmann, J. 1957. Virus interference I. The interferon. *Proc R Soc Lond B Biol. Sci.* 147: 258-267. 39. Kahlon, T. S., Chow, F. I., Chiu, M. M., Hudson, C. A. and Sayer, R. N. 1996. Cholesterol-lowering by rice bran and rice bran oil unsaponifiable matter in hamsters. *Cereal Chem.* 73: 69-73. 40. Kaushansky, K., Lin, N. and Adamson, J. W. 1988. Interleukin-1 stimulates fibroblasts to synthesize granulocyte- macrophage and granulocyte colony-stimulating factors. Mechanism for the hematopoietic response to inflammation. *J. Clin Invest.* 81: 92-97. 41. Krishnadasan, B., Naidu, B., Byrne, K., Fraga, C., Verrier, E. and Mulligan, M. 2003. The role of proinflammatory cytokines in lung ischemia – reperfusion injury. *J. Thorac. Cardiovasc Surg.* 125 (2): 261 – 272. 42. Kikunaga, S., Katoh, Y., and Takahashi, M. 1991. Biochemical change in phosphorus compounds and in the activity of phytase and  $\alpha$ -amylase in the rice (*Oryza sativa*) grain during germination. *J. Sci. Food Agric.* 56: 335-343. 43. Kikuchi, A., Kaibuchi, K., Hori, Y., Nonaka, H., Sakoda, T., Kawamura, M., Mizuno, T. and Takai, Y. 1992. Molecular cloning of the human cDNA for a stimulatory GDP/GTP exchange protein for c-Ki-ras p21 and smg p21. *Oncogene*, 7: 289-293. 44. Kiyoshi Yamazaki, M. D., Joseph, A., Murray, M. D. and Hirohito Kita, M. D. 2008. Innate immunomodulatory effects of cereal grains through induction of IL-10. *J. Allergy Clin. Immunol.* 121: 172-178. 45. Kobayashi, S., Ueda, K. and Komano, T. 1990. The effect of metal ions on the DNA damage induced by hydrogen peroxide. *Agric Biol. Chem.* 54: 69-76. 46. Lander, H. M., Sehajpal, P., Levine, D. M. and Novogrodsky, A. 1993. Activation of human peripheral blood mononuclear cells by nitric oxide-generating compound. *J. Immunol.* 150: 1509-1516. 47. Legdeur, M. C., Bontje, P. M., Ossenkoppele, G. J., Beelen, R. H., van de Loosdrecht, A. A., Broekhoven, M. G., Langenhuijsen, M. M., Thijsen, S. F., Hofstee, H. and Schuurhuis, G. J. 1996. The role of BCL-2 and bax protein in monocyte-mediated apoptosis in human leukemic cell lines. *Exp. Hematol.* 24: 1530-1539. 48. Lindmark-Mansson, H. and Akesson, B. 2000. Antioxidative factors in milk. *Br. J. Nutri.* 84: S103-S110. 49. Lieu, C. W., Lee, S. S. and Wang, S. Y. 1992. The effect of *Ganoderma lucidum* on induction of differentiation in leukemic U937 cells. *Anticancer Res.* 12: 1211-1216. 50. Lieu, C.W., Lee, S.S. and Wang, S.Y. 1992. The effect of *Ganoderma lucidum* on induction of differentiation in leukemic U937 cells. *Anticancer Res.* 12(4): 1211-1216. 51. Liang, C. W., Lai, Y. C. and Chu, Y. H. 2004. A study of the effects on nine Chinese herbs on proinflammatory cytokines production in two cell culture models. *J. Chin. Med.* 15(4): 293-304. 52. Lotem, J. and Sachs, L. 1987. Regulation of cell-surface receptors for hematopoietic differentiation-inducing protein MGI-2 on normal and leukemic myeloid cells. *Int. J. Cancer* 40(4): 532-539. 53. Maillard, M. N., Soum, M. H., Boivin, P. and Berset, C. 1996. Antioxidant activity of barley and malt: Relationship with phenolic content. *Lebensmittel-Wissenschaft und-technologie.* 29: 238-244. 54. Maines, M. D., Costa, L. G., Reed, D. J., Sassa, S. and Sipes, I. G. 1999. Current protocols in toxicology. John Wiley and Sons, New York. 55. Marero, L. M., Payumo, E. M., Aguinaldo, A. R., Matsumoto, I., and Homma, S. (1991) Antinutritional factors in weaning foods prepared from germinated cereals and legumes. *Lebensm. Wiss. U. Technol.* 24: 177-181. 56. Maruyama, S. and Suzuki, H. 1982. A peptides inhibitor of angiotensin I-converting enzyme in the tryptic hydrolysate of casein. *Agric. Biol. Chem.* 46: 1393-1394. 57. Maruyama, S., Nakagomi, K. Tomozuka, N. and Suzuki, H. 1985. Isolation and characterization of angiotensin I-converting enzyme inhibitor derived from an enzymatic hydrolysate of casein. II. Isolation and bradykinin-potentiating activity on the uterus and the ileum of rats. *Agric. Biol. Chem.* 49: 1405-1409. 58. Matsudaira, P. 1990. Limited N-terminal sequence analysis. *Methods in enzymology.* 182: 602-613. 59. Miyoshi, S., Ishikawa, H., Kaneko, T., Fukui, F., Tanaka, H. and Maruyama, S. 1991. Structures and activity of Angiotensin I -converting enzyme inhibitors in an  $\alpha$ -Zein hydrolysate. *Agric. Biol. Chem.* 55: 1313-1318. 60. Morgera, S., Haase, M., Rocktaschel, J., Bohler, T., Heymann, C. V., Vargas-Hein, O., Krausch, D., Zuckermann-Becker, H., Muller, J. M., Kox, W. J. and Neumayer, H. H. 2003. High permeability haemofiltration improves peripheral blood mononuclear cell proliferation in septic patients with acute renal failure. *Nephrol Dial Transplant* 18: 2570 – 2576. 61. Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* 65: 55-63. 62. Mullally, M. M., Meisel, H. and Fitzgerald, R. J. 1996. Synthetic peptides corresponding to  $\alpha$ -lactalbumin and  $\alpha$ -lactoglobulin sequences with angiotensin-I-converting enzyme inhibitory activity. *Bio. Chem. Hoppe Seyler.* 377: 259-260. 63. Munker, R. and Koeffler, H.P. 1987. Tumor necrosis factor: recent advances. *Klin Wochenschr* 65(8): 345-52. 64. Nagai, T., & Inoue, R. (2003). Preparation and the functional properties of water extract and alkaline extract of royal jelly. *Food Chem.* 84, 181-186. 65. Naldini, A., Pucci, A. and Carraro, F. 2001. Hypoxia induces the expression and release of interleukin 1 receptor antagonist in mitogen-activated mononuclear cells. *Cytokine* 13: 334 – 341. 66. Nakadai, T., Nansuno, S. and Iguchi. 1972. The action of peptidase from *Aspergillus oryzae* in digestion of soybean protein. *Agric. Biol. Chem.* 36: 261. 67. Olsson, I. L., Breitman, T. R. and Gallo, R. C. 1982. Priming of human myeloid leukemic cell lines HL-60 and U937 with retinoic acid for differentiation effects of cyclic adenosine 3' :5' -monophosphate-inducing agents  $\alpha$ -lymphocyte-derived differentiation factor. *Cancer Res.* 42: 3928-3933. 68. Old, L. J. 1988. Tumor necrosis factor. *Sci. Am.* 258: 59 – 75. 69. Onozaki, K., Urawa, H., Tamatani, T., Iwamura, Y., Hashimoto, T., Baba, T., Suzuki, H., Yamada, M., Yamamoto, S. and Oppenheim, J.J. 1988. Synergistic interactions of interleukin 1, interferon-beta, and tumor necrosis factor in terminally differentiating a mouse myeloid leukemic cell line (M1). Evidence that interferon-beta is

an autocrine differentiating factor. *J. Immunol.* 140(1): 112-119. 70.Olsson, I. L. and Breitman, T. R. 1982. Induction of differentiation of human histiocytic lymphoma cell line U937 by retinoic acid cyclic adenosine 3' : 5' -monophosphate-inducing agents. *Cancer Res.* 42: 3924-3927.

71.Oshima, G., Shimabukuro, H. and Nagasawa, K. 1979. *Biochim. Biophys. Acta.* 566: 128-137. 72.Ozols, J. 1990. Amino acid analysis. *Methods in enzymology.* 182: 587-601. 73.Pan, Y., He, C., Wang, H., Ji, X., Wang, K. and Liu, P. 2010. Antioxidant activity of microwave-assisted extract of *Buddleia officinalis* and its major active component. *Food Chem.* 121: 497-502. 74.Ralph, P., Harris, P.E., Punjabi, C.J., Welte, K., Litcofsky, P.B., Ho, M.K., Rubin, B.Y., Moore, M.A. and Springer, T.A. 1983. Lymphokine inducing " terminal differentiation " of the human monoblast leukemia line U937: a role for interferon- . *Blood* 62(6): 1169-1175. 75.Payne, C. M., Bernstein, C., Bernstein, H., Gerner, E. W. and Garewal, H. 1999. Reactive nitrogen species in colon carcinogenesis. *Antioxid. Redox. Signal* 1: 449 – 67. 76.Perdon, A. A., Del-Rosario, E. J. and Juliano, B. O. 1975. Solubilization of starch synthetase bound to *Oryza sativa* (rice) starch granules. *Phytochem.* 14: 949-951. 77.Pestka, S., Langer, J.A., Zoon, K.C. and Samuel, C.E. 1987. Interferons and their actions. *Annu. Rev. Biochem.* 56: 727-777. 78.Powrie, F. and Coffman, R.L. 1993. Cytokine regulation of T-cell function: potential therapeutic intervention. *Immunology Today* 14(6): 270-274. 79.Ralph, P., Harris, P. E., Punjabi, C. J., Welte, K., Litcofsky, P. B., Ho, M. K., Rubin, B. Y., Moore, M. A. and Springer, T. A. 1983. Lymphokine inducing " terminal differentiation " of the human monoblast leukemia line U937: a role for interferon- . *Blood* 62: 1169-1175. 80.Ramamoorthy, P. K. and Bono, A. 2007. Antioxidant activity, total phenolic and flavonoid content of *Morinda citrifolia* fruit extracts from various extraction processes. *J. Engi. Sci. Tech.* 2: 70-80. 81.Regnier, F. E. and Chicz, R. M. 1990. High-performance liquid chromatography: effective protein purification by various chromatographic modes. *Methods in enzymology* 182:392-421. 82.Robak, J. and Gryglewski, I. R. 1988. Flavonoids are scavengers of superoxide anions. *Biochem. Pharma.* 37: 837-841. 83.Salgame, P., Abrams, J.S., Clayberger, C., Goldstein, H., Convit, J., Modlin, R.L. and Bloom, B.R. 1991. Differing lymphokine profiles of functional subsets of human CD4 and CD8 T cell clones. *Sci.* 254(5029): 279-282. 84.Sharma, R. D. and Rukmini, C. 1986. Rice bran oil and hypocholesterolemia in rats. *Lipid* 21: 715-723. 85.Slupphaug, G., Kavli, B. and Krokkan, H. E. 2003. The interacting pathways for prevention and repair of oxidative DNA damage. *Mutat. Res.* 531: 231-251. 86.Smith, C., Halliwell, B. and Aruoma, O. I. 1992. Protection by albumin against the prooxidant actions of phenolic dietary components. *Food Chem. Toxic.* 30: 483-489. 87.Sriskandan, S., Mckee, A., Hall, L. and Cohen, J. 1996. Comparative effects of clindamycin and ampicillin on superantigenic activity of *Streptococcus pyogenes*. 88.Sugano, M. and Tsuji, E. 1997. Rice bran oil and cholesterol metabolism. *J. Nutr.* 127: 521-524. 89.Sun, T. and Ho, C. T. 2007. Antioxidant activities of buckwheat extracts. *Food Chem.* 90: 743-749. 90.Sundstrom, C. and Nilsson, K. 1976. Establishment characterization of a human histiocytic lymphoma cell line (U-937). *Int. J. Cancer* 17: 565-577. 91.Takeda, K., minowada, J. and Bloch, A. 1993. Differential ability of mitogen-stimulated human leukocyte-conditioned media to induce Fc receptors in human leukemia cells. *Cell Immunol.* 79: 288-297. 92.Tamatani, T., Kimura, S., Hashimoto, T. and Onozaki, K. 1989. Purification of guinea pig tumor necrosis factor (TNF): comparison of its antiproliferative and differentiative activities for myeloid leukemic cell lines with those of recombinant human TNF. *J. Biochem.* 105(1): 55-60. 93.Tarr, M. and Samson, T. 1997. Oxygen free radicals in tissue damage. *React. Kinet. Catal. Lett.* 60: 221-212. 94.Thomas, J., Liu, F. and Link, D. C. 2002. Mechanisms of mobilization of hematopoietic progenitors with granulocyte colony-stimulating factor. *Curr. Opin. Hematol.* 9: 183-189. 95.Vassalli, P. 1992. The pathophysiology of tumor necrosis factors. *Annu. Rev. Immunol.* 10: 411-52. 96.Wang, H. X., Ng, T. B., Ooi, V. E., Liu, W. K. and Chang, S. T. 1996. A polysaccharide-peptide complex from cultured mycelia of the mushroom *Tricholoma mongolicum* with immunoenhancing and antitumor activities. *Biochem. Cell Biol.* 74: 95-100. 97.Wahl, S. M., McCartney-Francis, N., Chan, J., Dionne, R. and Ta, L. 2003. Orenstein JM. Nitric oxide in experimental joint inflammation. Benefit or detriment? *Cells Tissues Organs* 174: 26-33. 98.Wright, S. C., Kumar, P., Tam, A. W., Shen, N., Varma, M. and Larrick, J. W. 1992. Apoptosis and DNA fragmentation precede TNF-induced cytolysis in U937 cells. *J. Cell Biochem.* 48: 344-355. 99.Yagi, K. 1989. A simple fluorometric assay for lipid peroxides in blood serum or plasma, in *CRC hand book of free radicals and antioxidants in biomedicine*, Vol III, p. 215. 100.Yen, G. C., Chen, H. Y. and Peng, H. H. 1997. Antioxidant and prooxidant effect of various tea extracts. *J. Agric. Food Chem.* 45: 30-34. 101.Yildirim, A., Oktay, M. and Bilaloglu, V. 2001. The antioxidant activity of the leaves of *cydonia vulgaris*. *TURK J. Med. Sci.* 31: 23-27.