

Studies of Soybean Sprout Extract to Antioxidant

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ABSTRACT

Soybean sprout extract contain a lot of isoflavone, which are strong antioxidants. Isoflavone can scavenge reactive oxygen species to prevent cancer and cardiovascular diseases of human. In addition, isoflavone, just like estrogen, can help women to decrease the symptoms of menopause. The antioxidant ability and protective potential of lipid peroxidation (LPO) of soybean sprout extract were firstly determined in vitro. In the effect of scavenging β,β -diphenyl- β -picrylhydrazyl (DPPH) free radical, experimental results showed that soybean sprout extract are as good as Vit E and Vit C. In the test of reducing ability of redox, results showed that soybean sprout extract have a strong ability to reduce Fe³⁺ of potassium ferricyanide. By using the thiobarbituric acid reactive substances (TBARs) test, soybean sprout extract have been shown to possess an ability to prevent low-density lipoprotein from being oxidized. After a series of in vitro tests, soybean sprout extract were evaluated to possess antioxidant ability. Therefore, soybean sprout extract were used for further studies on animals. There was no evidence to show that the addition of SBE could rescue the effect of decreasing activity of swine sperms due to the existence of dithyl-dithiocarbamic acid (DDC), which is a superoxide dismutase (SOD) inhibitor. DDC was also added into the feed of mice to evaluate their effects in biofunctions. The glutathione peroxidase (GSH-Px) and catalase (CAT) contents in the livers of mice supplemented with DDC were significantly less than those in the livers of mice without supplemented with DDC. Furthermore, the intake of DDC also cause mice to have less embryos and pup number per farrow ($P < 0.05$). If both SBE and DDC coexist in the feed, the above situations could be significantly improved. For instance, a feed with 2% of SBE can reduce the concentration of blood lipid in rat. The concentrations of plasma total cholesterol and triglyceride of the rats taking a feed with 2% of SBE were less than those in the control group by 22% ($P < 0.05$) and 42% ($P < 0.01$), respectively. Based on the experimental results performed above, we concluded that SBE is an efficient antioxidant and can elevate antioxidant status and increase reproduction performance of mouse. SBE supplement can reduce the concentration of blood lipid of rat. Further studies are needed to prove that SBE may have some other biofunctions.

Keywords : antioxidant ; free radicals ; soybean sprout extract ; superoxide dismutase ; isoflavone ; reactive oxygen species ; lipid peroxidation

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REFERENCES

參考文獻 1.丁克祥、陳華東、韓明向、姚鳳祥、江輝、遲洛英、張愛玲、屠璋武(1994) SOD與衰老及抗衰老中藥對SOD作用的研究。自由基生物學與醫學。2: 42-58。2.陳惠英、顏國欽 (1998) 自由基、抗氧化防禦與人體健康。中華民國營養學會雜誌。23: 105-121。3.陳肇文 (2000) 氧化壓力和動脈硬化之發生 - 氧化壓力在高膽固醇症之角色。臨床醫學。45: 58-61。4.廖哲逸 (1999) 大豆異黃酮之機能與在食品之應用。食品資訊。12:46-51。5.張克堅、秦嶺、林童俊、劉耕陶 (1994) 人參葉總皂甙對老齡大鼠白細胞介導的防禦功能的影響。自由基生物學與醫學。2: 4-8。6.Aitken, R.J., D. Buckingham and D. Harkiss (1993a) Use of a xanthine oxidase free radical generating system to investigate the cytotoxic effects of reactive oxygen species on human spermatozoa, *J. Reprod. Fertil.*, 97:441-450. 7.Aitken, R.J., D. Harkiss and D. Buckingham (1993b) Relationship between iron-catalysed lipid peroxidation potential and human sperm function, *J. Reprod. Fertil.*, 98: 257-265. 8.Aitken, R.J., D. Harkiss and D. Buckingham (1993c) Analysis of lipid peroxidation mechanisms in human spermatozoa, *Mol. Reprod. Dev.*, 35:302-315. 9.Aitken, R.J., D.W. Buckingham, A. Carreras and D.S. Irvine (1996) Superoxide dismutase in human sperm suspensions: relationship with cellular composition, oxidative stress, and sperm function, *Free Radic. Biol. Med.*, 21:495-504. 10.Aitken, R.J. (1999) The Amoroso Lecture. The human spermatozoon-a cell in crisis? *J. Reprod. Fertil.*, 115:1-7. 11.Alkan, I., F. Simsek, G. Haklar, E. Kervancioglu, H. Ozveri, S. Yalcin and A. Akdas (1997) Reactive oxygen species production by the spermatozoa of patients with idiopathic infertility: relationship to seminal plasma antioxidants, *J. Urol.*, 157:140-143. 12.Alvarez, J.G. and B.T. Storey (1984) Lipid peroxidation and the reactions of superoxide and hydrogen peroxide in mouse spermatozoa, *Biol. Reprod.*, 30:833-841. 13.Baker, H.W.G., J. Brindle, D.S. Irvine and R.J. Aitken (1996) Protective effect of antioxidants on the impairment of sperm motility by activated polymorphonuclear leukocytes, *Fertil. Steril.*, 65:411-419. 14.Barnes, S., L. Coward, M. Kirk and J. Sfakianos (1998) HPLC-mass spectrometry analysis of isoflavones, *Exp. Bio. Med.*, 217:254-262. 15.Beconi, M.T., M.A. Affranchino, L.M. Schang and N.B. Beorlegui. (1991) Influence of antioxidants on SOD activity in bovine sperm, *Biochem. Int.*, 23:545-53. 16.Bize, I., G. Santander, P. Cabello, D. Driscoll and C. Sharpe (1991) Hydrogen peroxide is involved in hamster sperm capacitation in vitro, *Biol. Reprod.*, 44:398-403. 17.Delamirande, E. and C. Gagnon (1993) A positive role for the superoxide anion in triggering hyperactivation and capacitation of human spermatozoa, *Int. J. Androl.*, 16:21-25. 18.Dzlezak, J.D. (1986) Antioxidants: the ultimate answer to oxidation, *Food Technol.*, 40:94-102. 19.Fukutake, M., M. Takashashi, K. Ishida, H. Kawamura, T. Sugimura and K. Wakabayashi (1996) Quantification of genistein and genistin in soybeans and soybean products, *Food Chem. Toxi.*, 34:457-461. 20.Gavella, M., V. Lipovac, M. Vu?i? and B. Ro?i? (1996) Superoxide anion scavenging capacity of human seminal plasma, *Int. J. Androl.*, 19: 82-90. 21.Gavella, M., V. Lipovac, M. Vucic and V. Sverko (1999) In vitro inhibition of superoxide anion production and superoxide dismutase activity by zinc in human spermatozoa, *Int. J. Androl.*, 22: 266-274. 22.Garcia, M.C., M.L. Marina and M. Torre (2000) Determination by perfusion reversed-phase high-performance liquid chromatography of the soybean protein content of commercial soybean products prepared directly from whole soybeans, *J. Chromatogr. A.*, 881:37-46. 23.Geva, E., J.B. Lessing, B. Bartoov, L. Lerner-Geva, N. Zabludovsky and A. Amit (1996) The effect of antioxidant treatment on human spermatozoa and fertilization rate in an in vitro fertilization program, *Fertil. Steril.*, 66:430-434. 24.Giese, B. (1996) Antioxidant: tools for preventing lipid oxidation, *Food Technol.*, 50:73-81. 25.Griveau, J.F. and D.L. Lannou (1994) Effects of antioxidants on human sperm preparation techniques, *Int. J. Androl.*, 17:225-231. 26.Griveau, J.F., P. Renard and D.L. Lannou (1994) An in vitro promoting role for hydrogen peroxide in human sperm capacitation, *Int. J. Androl.*, 17:300-307. 27.Griveau, J.F., E. Dumont, P. Renard, J.P. Callegari and D.L. Lannou (1995a) Reactive oxygen species, lipid peroxidation and enzymatic defence systems in human spermatozoa, *J. Reprod. Fertil.*, 103:17-26. 28.Griveau, J.F., P. Renard and D.L. Lannou (1995b) Superoxide anion production by human spermatozoa as a part of the ionophore-induced acrosome reaction process, *Int. J. Androl.*, 18: 67-74. 29.Griveau, J.F. and D.L. Lannou (1997a) Reactive oxygen species and human spermatozoa: physiology and pathology, *Int. J. Androl.*, 20:61-69. 30.Griveau, J.F. and D.L. Lannou (1997b) Influence of oxygen tension on reactive oxygen species production and human sperm function, *Int. J. Androl.*, 20:195-200. 31.Hutabarat, L.S., H. Greenfield and M. Mulholland (2000) Quantitative determination of isoflavones and coumestrol in soybean by column liquid chromatography, *J. Chromatogr.*, 886:55-63. 32.Hwang, J., A. Sevanian, H.N. Hodis and F. Ursini (2000) Synergistic inhibition of LDL oxidation by phytoestrogens and ascorbic acid, *Free Radic. Biol. Med.*, 29:79-89. 33.Jo?wik, M., M. Jo?wik, W. Kuczy?ski and M. Szamatowicz (1997) Nonenzymatic antioxidant activity of human seminal plasma, *Fertil. Steril.*, 68:154-158. 34.Kantola, M., M. Saaranen and T. Vanha-Perttula (1988) Selenium and glutathione peroxidase in seminal plasma of men and bulls, *J. Reprod. Fertil.*, 83:785-794. 35.Kovalski, N.N., E.D. Lamirande and C. Gagnon (1992) Rezactive oxygen species generated by human neutrophils inhibit sperm motility:protective effect of seminal plasma and acavengers, *Fertil. Steril.*, 58:809-816. 36.Kurabayashi, Y. and C. Gagnon (1996) Effect of catalase and thioredoxin addition to sperm incubation medium before in vitro fertilization on sperm capacity to support embryo development, *Fertil. Steril.*, 66:1012-1027. 37.Kurpisz, M., R. Miesel, D. Sanocka and P. Jedrzejczak (1996) Seminal plasma can be a predictive factor for male infertility, *Hum. Reprod.*, 11:1223-1226. 38.Lamirande, E.D. and C. Gagnon (1993) Human sperm hyperactivation and capacitation as parts of an oxidative process, *Free Radic. Biol. Med.*, 14:157-166. 39.Mao, S.J.T., M.T. Yates, A.E. Rechtin, R.L. Jackson and W.A. Van Sickle (1990) Antioxidant

activity of probucol and its analogues in hypercholesterolemic watanabe rabbits, Am. Chem. Sci., 34: 298-302. 40. Marklund, S. and G. Markluand (1974) Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase, Eur. J. Biochem., 47:469-74. 41. Massaeli, H., S. Sobrattee and G.N. Pierce (1999) The importance of lipid solubility in antioxidant and free radical generating systems for determining lipoprotein peroxidation, Free Radic. Biol. Med., 26: 1524-1530. 42. Meng, Q.H., P. Lewis, K. Wahala, H. Adlercreutz and M.J. Tikkannen (1999) Incorporation of esterified soybean isoflavones with antioxidant activity into low-density lipoprotein, Biochim. Biophys. Acta., 1438:369-376. 43. Mennella, M.R.F. and R. Jones (1980) Properties of spermatozoal superoxide dismutase and lack of involvement of superoxides in metal-ion-catalysed lipid-peroxidation and reactions in semen, Biochem. J., 191:289-97. 44. Messina, M. (2000) Soyfoods and soybean phyto-oestrogens (isoflavones) as possible alternatives to hormone replacement therapy (HRT), Eur. J. Canc., 36:s71-s77. 45. Michiels, C. and J. Remacle (1988) Use of the inhibition of enzymatic antioxidant systems in order to evaluate their physiological importance, Eur. J. Biochem., 177:435-441. 46. Namiki M. (1990) Antioxidants/antimutagens in food, Crit. Rev. Food Sci. Nutr., 29:281-300. 47. O ' Flaherty, C.M., N.B. Beorlegui and M.T. Beconi (1999) Reactive oxygen species requirements for bovine sperm capacitation and acrosome reaction, Theriogenology, 52:289-301. 48. Okamoto, G., F. Hayase, and H. Kato (1992) Scavenging of active oxygen speices by glycated proteins, Biosc. Biotechnol. Biochem., 56:928-931. 49. Oyaizu, M. (1986) Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine, Jpn. J. Nutri., 44:307. 50. Palan, P. and R. Naz (1996) Changes in various antioxidant levels in human seminal plasma related to immunoinfertility, Arch. Androl., 36:139-43 51. Robak, J. and R.J. Gryglewski (1988) Flavonoids are scavengers of superoxide anions, Biol. Pharm., 37:837-841. 52. Saija, A., M. Scalese, M. Lanza, D. Marzullo, F. Bonina and F. Castelli (1995) Flavonoids as antioxidant agents: importance of their interaction with biomembranes, Free Radic. Biol. Med., 19:481-486. 53. Shimada, K., K. Fujikawa, K. Yahara, and T. Nakamura (1992) Antioxidative properties of xanthane on the autoxidation of soybean oil in cyclodextrin emulsion, J. Agric. Food Chem., 40:495. 54. Singh, A., P. Kumar G, M. Laloraya, S. Verma and M. Nivsarkar (1991) Superoxide dismutase activity regulation by sperm: a new dimension spermine biochemistry and sperm development, Bio. Bioph. Res. Com., 177:420-432. 55. Storey, B.T., J.G. Alvarez and K.A. Thompson (1998) Human sperm glutathione reductase activity in situ reveals limitation in the glutathione antioxidant defense system due to supply of NADPH, Mol. Reprod. Dev., 49:400-407. 56. Suleiman, S.A., M.E. Ali, Z.M.S. Zaki, E.M.A. El-Malik and M.A. Nasr. (1996) Lipid peroxidation and human sperm motility: protective role of vitamin E, J. Androl., 17:530-537. 57. Thomas, S.R. and R. Stocker (2000) Molecular action of vitamin E in lipoprotein oxidation: implications for atherosclerosis, Free Radic. Biol. Med., 28:1795-805. Review. 58. Verma, A. and K.C. Kanwar (1998) Human sperm motility and lipid peroxidation in different ascorbic acid concentrations: an in vitro analysis, Andrologia, 30: 325-329. 59. Videla, L.A., A. Valenzuela, V. Fernandez and A. Kriz (1985) Differential lipid peroxidative response of rat liver and lung tissues to glutathione deflection induced in vivo by diethyl maleate:effect of the antioxidant flavonoid (+)-cyanidanol-3, Biochem. Int., 10:425-433. 60. Williams, W.B., M.E. Cuvelier and C. Berset (1995) Use of a free radical method to evaluate antioxidant activity, Lebensm-Wiss Technol., 28:25-30. 61. Yamaguchi, T., H. Takamura, T. Matoba and J. Terao. (1998) HPLC method of evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl, Biosci. Biotechnol. Biochem., 62:1201-1204. 62. Yeum, K.J., A.L.D. Anjos Ferreira, D. Smith, N.I. Krinsky and R.M. Russell (2000) The effect of -tocopherol on the oxidative cleavage of -carotene, Free Radic. Biol. Med., 29:105-114.