北蟲草子實體微波萃取物基因毒性之安全性評估 = Genotoxicity analysis of Microwave-Extract of Cordyceps militaris by Ames..

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摘 要

北蟲草（Cordyceps militaris）原名北冬蟲夏草，又名蛹蟲草，與冬蟲夏草（Cordyceps sinensis）同為蟲草屬，經各方研究發現北蟲草與冬蟲夏草其醫用價值類似，可抗腫瘤、真菌、消炎及增加免疫力。因北蟲草價格便宜，所以市場佔有率已漸漸取代傳統冬蟲夏草。目前市面上有關探求北蟲草基因毒性資料甚少，且往往僅著重於北蟲草與冬蟲夏草活性比較。本研究目的為檢測北蟲草微波萃取物細胞毒性及基因毒性。樣品為子實體水萃取物及基座乙醇萃取物。本研究選用安姆測試 (Ames Test) 及鼷鼠淋巴瘤 tk+/- 分析法作為安全性評估平台。本研究 Ames Test 為平板混合試驗，並選用5株 Samonella typhimurium (TA97a、TA98、TA100、TA102及TA1535) 做為測試菌株。鼷鼠淋巴瘤細胞突變試驗則以 L5178Y tk+/- 細胞接種於96-well 作為測試方法。於 Ames Test 之毒性測試發現，北蟲草萃取物具有明顯抑制細菌生長。當北蟲草子實體水萃取物濃度為5 mg/plate 時，TA100 及 TA98 存活率百分分別為41.1% 和52.7%。而北蟲草基座乙醇萃取物濃度為0.63 mg/plate時，TA100及TA98存活率百分率分別為40.8%及49.1%。基因毒性試驗結果顯示，北蟲草經微波萃取處理萃取物，無論是否經 S9 mix 誘導，均不具有微生物及體外哺乳類細胞基因突變毒性。本試驗亦針對北蟲草萃取物進行抑癌測試，以 XTT 作為細胞存活指示劑，萃取液分別處理CHO-K1 （中國倉鼠細胞） 及 MB-MDA231 （乳癌細胞），結果發現北蟲草萃取物均有明顯毒殺 MB-MDA231 的能力，子實體水萃取物及基座乙醇萃取物IC50 分別為29 μg/ml及27 μg/ml。但對正常細胞不具有毒殺力。

關鍵詞 : 北蟲草、微波萃取、安姆測試、鼷鼠淋巴瘤 tk+/- 分析

目錄

圖目錄

表目錄

參考文獻

1. 呂青 。2000。北蟲草 (Cordyceps militaris) 產多糖條件的篩選及多糖的生物活性研究。中國海洋大學。2. 汪太初、李瑞雪、賈鴻英及胡飛。2009。蟲草素的研究應用進展。現代農業科技。332-333。3. 胡昆。2006。硒對蛹蟲草[Cordyceps militaris（Fr.）Link.]的生物學功效及降脂抗氧化功能的影響。沈陽農業大學。4. 夏敏及溫魯。2006。微波法提取蟲草素研究。食品科學。27:248-251。5. 曹慶穗、嚴俊文、褚芳及李超。2008。均勻設計優選蟲草素微波輔助提取工藝研究。江蘇農業科學。6。6. 陳登科。2004。超臨界二氧化碳萃取蛹蟲草蟲草素之探討。朝陽科技大學應用化學系。7. 楊杰及陳順志。2008。蟲草素研究進展。中國生化藥物雜誌。 414-417。8. 陳美璇。2007。台灣產之不同筋骨草屬蛻皮甾酮，總黃酮及抗氧化能力的分析比較。大葉大學生物產業科技學系。9. 溫魯、翁梁、朱明偉及劉森琴。2008。不同林區蛹蟲草活性成分含量的比較。林農科學。10. 劉波及鄧中平。2005。tk 基因突?的分子機制研究。癌變畸變突變。17:190-192。11. 劉彥威、劉娜及劉利強。2004。冬蟲夏草有效成分的研究進展。動物醫學進展。51-53。12. 盧翠文。2007。北冬蟲夏草液體培養條件的優化與誘變育種。廣西師範大學。13. Amacher, D. E., Paillet, S. C., Turner, G. N., Ray, V. A., and Salsburg, D. S. (1980). Point mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells. II. Test validation and interpretation. Mutat Res 72, 447-474.14. Ames, B. N., McCann, J., and Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutat Res 31, 347-364.15. Caldwell, J. (1993). Perspective on the usefulness of the mouse lymphoma assay as an indicator of a genotoxic carcinogen: ten compounds which are positive in the mouse lymphoma assay but are not genotoxic carcinogens. Teratog Carcinog Mutagen 13, 185-190.16. Chen, Y. J., Shiao, M. S., Lee, S. S., and Wang, S. Y. (1997). Effect of Cordyceps sinensis on the proliferation and differentiation of human leukemic U937 cells. Life Sci 60, 2349-2359.17. Cheung, J. K., Li, J., Cheung, A. W., Zhu, Y., Zheng, K. Y., Bi, C. W., Duan, R., Choi, R. C., Lau, D. T., Dong, T. T., et al. (2009). Cordysinocan, a polysaccharide isolated from cultured Cordyceps, activates immune responses in cultured T-lymphocytes and macrophages: signaling cascade and induction of cytokines. J Ethnopharmacol 124, 61-68.18. Clements,

J. (2000). The mouse lymphoma assay. Mutat Res 455, 97-110.19. Clive, D., Johnson, K. O., Spector, J. F., Batson, A. G., and Brown, M. M. (1979). Validation and characterization of the L5178Y/TK+/- mouse lymphoma mutagen assay system. Mutat Res 59, 61-108.20. Cunningham, K. G., Manson, W., Spring, F. S., and Hutchinson, S. A. (1950). Cordycepin, a metabolic product isolated from cultures of Cordyceps militaris (Linn.) Link. Nature 166, 949.21. Fani, R., Brilli, M., Fondi, M., and Lio, P. (2007). The role of gene fusions in the evolution of metabolic pathways: the histidine biosynthesis case. BMC Evol Biol 7 Suppl 2, S4.22. Forster, M., Laabs, V., Lamshoft, M., Putz, T., and Amelung, W. (2008). Analysis of aged sulfadiazine residues in soils using microwave extraction and liquid chromatography tandem mass spectrometry. Anal Bioanal Chem 391, 1029-1038.23. Hartman, P. E., Ames, B. N., Roth, J. R., Barnes, W. M., and Levin, D. E. (1986). Target sequences for mutagenesis in Salmonella histidine-requiring mutants. Environ Mutagen 8, 631-641.24. Hozier, J., Scalzi, J., Sawyer, J., Carley, N., Applegate, M., Clive, D., and Moore, M. M. (1991). Localization of the mouse thymidine kinase gene to the distal portion of chromosome 11. Genomics 10, 827-830.25. Huang, B. M., Chuang, Y. M., Chen, C. F., and Leu, S. F. (2000). Effects of extracted Cordyceps sinensis on steroidogenesis in MA-10 mouse Leydig tumor cells. Biol Pharm Bull 23, 1532-1535.26. Isono, K., and Yourno, J. (1974). Chemical carcinogens as frameshift mutagens: Salmonella DNA sequence sensitive to mutagenesis by polycyclic carcinogens. Proc Natl Acad Sci U S A 71, 1612-1617.27. Jagerstad, M., Olsson, K., Grivas, S., Negishi, C., Wakabayashi, K., Tsuda, M., Sato, S., and Sugimura, T. (1984). Formation of 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline in a model system by heating creatinine, glycine and glucose. Mutat Res 126, 239-244.28. Jin, C. Y., Kim, G. Y., and Choi, Y. H. (2008). Induction of apoptosis by aqueous extract of Cordyceps militaris through activation of caspases and inactivation of Akt in human breast cancer MDA-MB-231 Cells. J Microbiol Biotechnol 18, 1997-2003.29. Johnston, H. M., and Roth, J. R. (1979). Histidine mutants requiring adenine: selection of mutants with reduced hisG expression in Salmonella typhimurium. Genetics 92, 1-15.30. Jost, L. M., Kirkwood, J. M., and Whiteside, T. L. (1992). Improved short- and long-term XTT-based colorimetric cellular cytotoxicity assay for melanoma and other tumor cells. J Immunol Methods 147, 153-165.31. Kjellstrom, A., Brantlind, M., and Eldsater, C. (2008). Optimized microwave extraction for trace detection of 2,4,6-trinitrotoluene in soil samples. Chemosphere 71, 1701-1708.32. Koh, J. H., Kim, J. M., Chang, U. J., and Suh, H. J. (2003). Hypocholesterolemic effect of hot-water extract from mycelia of Cordyceps sinensis. Biol Pharm Bull 26, 84-87.33. Kuo, Y. C., Lin, C. Y., Tsai, W. J., Wu, C. L., Chen, C. F., and Shiao, M. S. (1994). Growth inhibitors against tumor cells in Cordyceps sinensis other than cordycepin and polysaccharides. Cancer Invest 12, 611-615.34. Kylin, H., H, H. J. B., and Wittig, R. (1994). Environmental Monitoring of Polychlorinated Biphenyls Using Pine Needles as Passive Samplers. Environ Sci, 1320-1324.35. Lee, S. J., Kim, S. K., Choi, W. S., Kim, W. J., and Moon, S. K. (2009). Cordycepin causes p21WAF1-mediated G2/M cell-cycle arrest by regulating c-Jun N-terminal kinase activation in human bladder cancer cells. Arch Biochem Biophys 490, 103-109.36. Levin, D. E., Marnett, L. J., and Ames, B. N. (1984). Spontaneous and mutagen-induced deletions: mechanistic studies in Salmonella tester strain TA102. Proc Natl Acad Sci U S A 81, 4457-4461.37. Levin, D. E., Marnett, L. J., and Ames, B. N. (1984). Spontaneous and mutagen-induced deletions: mechanistic studies in Salmonella tester strain TA102. Proc Natl Acad Sci U S A 81, 4457-4461.38. Li, S. P., Li, P., Dong, T. T., and Tsim, K. W. (2001). Anti-oxidation activity of different types of natural Cordyceps sinensis and cultured Cordyceps mycelia. Phytomedicine 8, 207-212.39. Li, S. P., Yang, F. Q., and Tsim, K. W. (2006). Quality control of Cordyceps sinensis, a valued traditional Chinese medicine. J Pharm Biomed Anal 41, 1571-1584.40. Li, S. P., Zhao, K. J., Ji, Z. N., Song, Z. H., Dong, T. T., Lo, C. K., Cheung, J. K., Zhu, S. Q., and Tsim, K. W. (2003). A polysaccharide isolated from Cordyceps sinensis, a traditional Chinese medicine, protects PC12 cells against hydrogen peroxide-induced injury. Life Sci 73, 2503-2513.41. Lo, H. C., Tu, S. T., Lin, K. C., and Lin, S. C. (2004). The anti-hyperglycemic activity of the fruiting body of Cordyceps in diabetic rats induced by nicotinamide and streptozotocin. Life Sci 74, 2897-2908.42. Lin, Y.-W., and Chiang, B.-H. (2007). Anti-tumor activity of the fermentation broth of Cordyceps militaris cultured in the medium of Radix astragali. Food Science and Technology, 244-250.43. Lv, Z. M., Jiang, Y. T., Wu, L. J., and Liu, K. (2008). [Chemical constituents from dried sorophore of cultured Cordyceps militaris]. Zhongguo Zhong Yao Za Zhi 33, 2914-2917.44. MacPhee, D. G. (1973). Salmonella typhimurium hisG46 (R-Utrecht): possible use in screening mutagens and carcinogens. Appl Microbiol 26, 1004-1005.45. Maron, D. M., and Ames, B. N. (1983). Revised methods for the Salmonella mutagenicity test. Mutat Res 113, 173-215.46. MARTIN., R. G. (1962). The First Enzyme in Histidine Biosynthesis: The Nature of Feedback Inhibition by Histidine. Received for publication 238, 257-268.47. Ohmori, T., Tamura, K., Ohgane, N., Nakamura, T., Kawanishi, G., Yamada, H., and Nomoto, K. (1989). The correlation between molecular weight and antitumor activity of galactosaminoglycan (CO-N) from Cordyceps ophioglossoides. Chem Pharm Bull (Tokyo) 37, 1337-1340.48. Pare, J. (1991). Microwave-assisted natural products extraction. united states patent.49. Park, B. T., Na, K. H., Jung, E. C., Park, J. W., and Kim, H. H. (2009). Antifungal and Anticancer Activities of a Protein from the Mushroom Cordyceps militaris. Korean J Physiol Pharmacol 13, 49-54.50. Puck, T. T., Cieciura, S. J., and Robinson, A. (1958). Genetics of somatic mammalian cells. III. Long-term cultivation of euploid cells from human and animal subjects. J Exp Med 108, 945-956.51. Qing-jun, J., Sheng-xue, L., and Jin-yi, L. (2004). studies of tk gene mutation by hydroxylammonium nitrate. third military medical university,chingqing, 365-369.52. Riela, S., Bruno, M., Formisano, C., Rigano, D., Rosselli, S., Saladino, M. L., and Senatore, F. (2008). Effects of solvent-free microwave extraction on the chemical composition of essential oil of Calamintha nepeta (L.) Savi compared with the conventional production method. J Sep Sci 31, 1110-1117.53. Shen, Y. D., Shao, X. T., Ni, Y. D., Xu, H., and Tong, X. M. (2009). [Cordyceps sinensis polysaccharide enhances apoptosis of HL-60 cells induced by triptolide]. Zhejiang Da Xue Xue Bao Yi Xue Ban 38, 158-162.54. Sugar, A. M., and McCaffrey, R. P. (1998). Antifungal activity of 3'-deoxyadenosine (cordycepin). Antimicrob Agents Chemother 42, 1424-1427.55. Sugimura, T., Nagao, M., and Wakabayashi, K. (1996). Carcinogenicity of food mutagens. Environ Health Perspect 104 Suppl 3,

429-433.56. Yoshikawa, N., Kunitomo, M., Kagota, S., Shinozuka, K., and Nakamura, K. (2009). Inhibitory effect of cordycepin on hematogenic metastasis of B16-F1 mouse melanoma cells accelerated by adenosine-5'-diphosphate. Anticancer Res 29, 3857-3860.57. Yu, R., Yang, W., and Song, L. (2007). Structural characterization and antioxidant activity of a polysaccharide from the fruiting bodies of cultured Cordyceps militaris. Carbohydrate Polymers, 430-436.58. Zill, E. H., Abert Vian, M., Maingonnat, J. F., and Chemat, F. (2009). Clean recovery of antioxidant flavonoids from onions: optimising solvent free microwave extraction method. J Chromatogr A 1216, 7700-7707.