

Effects of Lippia citriodora Aqueous Extract in Caco-2 Cell Line

林雁中、吳芳禎

E-mail: 9707202@mail.dyu.edu.tw

ABSTRACT

Recently, researches on the induction of apoptosis in cancer cells were considered as a new method. In this study, we investigated the induction of apoptosis in colorectal cancer cell line (Caco-2) and liver cancer cell line (HepG2) of human by the aqueous extract of Lippia citriodora. First, we used XTT (sodium 3' [1-(phenylaminocarbonyl)-3,4-tetrazolium]- bis(4-methoxy-6-nitro) benzene sulfonic acid hydrate) method to observe the viability of Caco-2 and HepG2 cancer cell line which treated by different concentration of Lippia citriodora (10~100 mg/mL). The result showed a range of HepG2 viability from 81.81% to 90.14%, they showed a range of 46.95% to 59.71% on Caco-2 viability. In the morphological assay, the Caco-2 cancer cells exhibited conspicuously changed as compare with HepG2 cancer cell line. In the DNA integrity assay, Caco-2 cancer cells showed extensive DNA fragmentation under treatment. Flow cytometric assay revealed that the cell cycle of Caco-2 cancer cells was arrested at sub-G1 phase and showed a peak in sub-G1 phase (55.01%). The percentage of G1, S and G2/M were decreased according to the increase of Lippia citriodora concentration. Effects of the anti-proliferation and induction of apoptosis in Caco-2 cell line with low toxicity by treatment of aqueous extract of Lippia citriodora were considered as a new strategy of cancer therapy. Lippia citriodora may be a potential drug for treatment of human cancers.

Keywords : Lippia citriodora, Apoptosis, Cell cycle, Caco-2, HepG2

Table of Contents

封面內頁 簽名頁 授權書iii 中文摘要iv 英文摘要vi 誌謝vii 目錄viii 圖目錄xi 表目錄xii 第一章 緒論1 第二章 文獻回顧3 第一節 馬鞭草科(Verbenaceae)3 一、馬鞭草科介紹3 二、檸檬馬鞭草(Lippia citriodora)5 三、馬鞭草科之植物化學成分7 四、馬鞭草科藥理作用研究之文獻考察7 第二節 癌症10 一、癌症簡介10 二、大腸直腸癌(colorectal cancer)之病因11 三、大腸直腸癌好發位置12 四、大腸直腸癌之臨床症狀12 五、大腸直腸癌的治療12 第三節 細胞週期(cell cycle)14 一、細胞週期及其調控14 第四節 細胞凋亡17 一、細胞凋亡背景17 二、細胞凋亡與細胞壞死之生化型態特徵17 三、細胞凋亡的研究方法18 四、細胞凋亡的調控機制及相關因子21 第三章 材料與方法24 第一節 材料與儀器24 一、材料24 二、儀器25 第二節 方法25 一、萃取液製備25 二、細胞培養(cell culture)26 三、細胞生長抑制試驗26 四、細胞型態分析試驗27 五、DNA裂解與電泳28 六、細胞週期分析28 七、統計分析29 第四章 結果與討論30 第一節 細胞生長抑制試驗30 一、人類肝癌細胞株(HepG2)的生長抑制實驗30 二、人類大腸直腸癌細胞株(Caco-2)的生長抑制實驗30 第二節 細胞的型態分析試驗35 第三節 DNA裂解與電泳37 第四節 細胞週期分析40 第五章 結論47 參考文獻49 圖目錄 圖一、檸檬馬鞭草6 圖二、細胞週期15 圖三、細胞凋亡與細胞壞死之生化型態特徵19 圖四、肝癌細胞株(HepG2)存活率32 圖五、大腸直腸癌細胞株(Caco-2)存活率34 圖六、檸檬馬鞭草水萃取液對大腸直腸癌細胞株(Caco-2)型態之影響36 圖七、檸檬馬鞭草水萃取液對肝癌細胞株(HepG2)型態之影響38 圖八、檸檬馬鞭草水萃取液對大腸直腸癌細胞株(Caco-2)的DNA裂解分析39 圖九、檸檬馬鞭草水萃取液對大腸直腸癌細胞株(Caco-2)生長週期的影響41 圖十、檸檬馬鞭草水萃取液對大腸直腸癌細胞株(Caco-2)生長週期subG1 population比例數據化圖42 圖十一、檸檬馬鞭草水萃取液對大腸直腸癌細胞株(Caco-2)生長週期G1 population比例數據化圖44 圖十二、檸檬馬鞭草水萃取液對大腸直腸癌細胞株(Caco-2)生長週期S population比例數據化圖45 圖十三、檸檬馬鞭草水萃取液對大腸直腸癌細胞株(Caco-2)生長週期G2/M population比例數據化圖46 表目錄 表一、肝癌細胞株(HepG2)存活率31 表二、大腸直腸癌細胞株(Caco-2)存活率33

REFERENCES

- 王家俊、張立平、徐昌芬和羅莉。2004。馬鞭草C部位使人絨癌JAR細胞阻滯於G2/M期並誘導細胞凋亡。南京醫科大學學報自然科學版24(6):598-601。
- 甘偉松。1975。藥用植物學。第469-475頁。國立中國醫藥研究所。台灣，台中。
- 何康潔。1996。有計畫的細胞凋亡(apoptosis)其存在，機轉，及與疾病的關係。當代醫學 23(12):785-789。
- 李茂佑。2005。蛋白質體學之大腸直腸癌差異性蛋白質表現分析:5-16。國立中山大學生物醫學科學研究所碩士論文。台灣，高雄。
- 林宗瑤。2002。內毒素與薑黃素對大腸癌細胞的影響:61-66。中山醫學大學生物化學研究所碩士論文。
- 林俊彥。1998。台灣香草植物品種。台南區農業改良場技術專刊124:92-94。
- 張立平、夏邦亮、徐昌芬和羅莉。2005。馬鞭草C部位誘導人絨毛癌JAR細胞凋亡分子機制研究。中國腫瘤臨床 32:1089-1091。
- 梁金銅和張金堅。1996。細胞凋亡的特徵、致癌角色、及研究方法。當代醫學 23(11):885-891。
- 黃千芳、賴士偉和劉秋松。2006。大腸癌的篩檢。基

層醫學 21(7):193-196。 10.黃增泉。1993。植物分類學:台灣維管束植物科誌。第356-359頁。南天書局有限公司。台灣，台北。 11.楊文乾。2001。神奇草藥大圖鑑。第460-473頁。林鬱文化事業有限公司。台灣，台北。 12.楊玲玲、張嘉蓁和王靜瓊。2002。銳葉楊梅萃取物誘導子宮頸癌細胞凋亡。Nutrition Science of Journal 27 (2):109-117。 13.楊淑媚、陳延年和蔡昆道。2004。細胞膜上的死亡受體TRAIL-R1(DR4)在人參皂?Rh2誘導肺腺癌細胞凋亡的過程扮演重要角色。中華醫學雜誌15(4):273-291。 14.謝宜英和張瑩瑩。1999。藥用植物完全指南。第18-99頁。城邦文化事業股份有限公司。台灣，台北。 15.謝佩穎。2006。Actinodaphnine 誘導細胞內nitric oxide 、reactive oxygen species 及降低NF- B 活性導致人類肝癌Mahlavu 細胞株計畫性死亡的研究:38-45。輔英科技大學醫事技術系碩士論文。高雄，台灣。 16.劉家剛和周小雷。2005。馬鞭草化學成分和藥理作用研究發展。廣西中醫藥28(2):1-3。 17.蔡美珍。1995。馬鞭草之本草考察及其鎮痛，抗血小板凝集之藥理研究:32-48。中國醫藥學院中國藥學研究所碩士論文。台中，台灣。 18.Aburano, T., Shuke, N., and Okizaki, A. 2002. Tc-99m mixed leukocytes imaging in inflammatory bowel disease of ulcerative colitis and Crohn's disease. International Congress Series 1228: 189-195. 19.Aufmkolk, M., Ingbar, J. C., Amir, S. M., Winterhoff, H., Sougens, H., Hesch, R.D., and Ingbar, S. H. 1984. Inhibition by certain plant extracts of the binding and adenylate cyclase stimulatory effect bovine thyrotropin in human thyroid membranes. Endocrinology 115(2): 527-534. 20.Attardi, L. D. 2005. The role of p53-mediated apoptosis as a crucial anti-tumor response to genomic instability: lessons from mouse model. Mutation Research 569:145-157. 21.Bedi, A., Barber, J. P., Bedi, G. C., el-Deirv, W. S., Sidransky, D., Vala, M. S., Akhtar, A. J., Hilton, J., and Jones, R. J. 1995. BCR-ABL-mediated inhibition of apoptosis with delay of G2/M transition after DNA damage: a mechanism of resistance to multiple anticancer agents. Blood 86(3): 1148-1158. 22.Chen, S. H., and Wu, M. J. 2003. Remarks on the species of Stachytrarphaeta (Verbenaceae) of Taiwan. Botanical Bulletin of Academia Sinica 44: 167-174. 23.Devi, K. P., Sreepriya, M., Decaki, T., and Balakrshna, K. 2003. Antinociceptive and hypnotic effects of *Premna tomentosa* L. (Verbenaceae) in experimental animals. Pharmacology biochemistry and behavior 75: 261-264. 24.Donovan, M., and Cotter, T. G. 2004. Control of mitochondrial integrity by Bcl-2 family members and caspase-independent cell death. Biochimica et Biophysica Acta 1644:133-147. 25.Doxsey, S., Zimmerman, W., and Mikule, K. 2005. Centrosome control of the cell cycle. Trends in Cell Biology 15:303-310. 26.Duesberg, P., and Rasnick, D. 2000. Aneuploidy, the somatic mutation that makes cancer a species of its own. Cell Motility and the Cytoskeleton 47:81-107. 27.Ghisalberti, E. L. 2000. *Lantana camara* L. (Verbenaceae). Fitoterapia 71:467-486. 28.Goodlett, C. R., and Horn, K. H. 2001. Mechanisms of alcohol-induced damage to the developing nervous system. Alcohol Research and Health 25(3): 175 – 184. 29.Graseo, F., Melero, G., Costa, B. A., Prieto, R., and March, J. G. 1994. Urolithiasis and phytotherapy. International urology and nephrology 26(5):507-511. 30.Hastak, K., Agarwal, M. K., Mukhtar, H., and Agarwal, M. 2005. Ablation of either p21 or Bax prevents p53-dependent apoptosis induced by green tea polyphenol epigallocatechin -3-gallate. Journal of the Federation of American Societies 19:789-791. 31.Kerr, J. F. R., Winterford, C. M., and Harmon, B. V. 1994. Apoptosis. Its significance in cancer and cancer therapy. Cancer 73: 2013- 2016. 32.Kuo, P. L., Hsu, Y. L., Chang, C. H., and Lin, C. C. 2005. The mechanism of ellipticine-induced apoptosis and cell cycle arrest in human breast MCF-7 cancer cell. Cancer Letters 223:293 -301. 33.Lin, H. I., Lee, Y. J., Chen, B. F., Tsai, M. C., Lu, J. L., Chou, C. J., and Jow, G. M. 2005. Involvement of Bcl-2 family, cytochrome c and caspase 3 in induction of apoptosis by beauvericin in human non-small cell lung cancer cells. Cancer Letters 230:248-259. 34.Liu, S., Bishop, W. R., and Liu, M. 2003. Differential effects of cell cycle regulatory protein p21WAF1/Cip1 on apoptosis and sensitivity to cnacer chemotherapy. Drug Resistance Updates 6:183-195 35.Los, M., Mozoluk, M., Ferrari, D., Stepczynska, A., Stroh, C., Renz, A., Herceg, Z., Wang, Z. Q., and Schulze-Osthoff, K. 2002. Activation and caspase-mediated inhibition of PARP: A molecular switch between fibroblast necrosis and apoptosis in death receptor signaling. Molecular Biology of the Cell 13:978-988. 36.McConkey, D. J. 1998. Biochemical determinants of apoptosis and necrosis. Toxicology Letters 99:157-168. 37.Michor, F., Iwasa, Y., Lengauer, C., and Nowak, M. A. 2005. Dynamics of colorectal cancer. Seminars in Cancer Biology 15: 484-493. 38.Nagata, S., Nagase, H., Kawane, K., Mukae, N., and Fukuyama, H. 2003. Degradation of chromosomal DNA during apoptosis. Cell Death and Differentiation 10:108-116. 39.Oommen, S., Anto, R. J., Srinivas, G., and Karunagaran, D. 2004. Allicin (from garlic) induces caspase-mediated apoptosis in cancer cells. European Journal of Pharmacology 485:97-103. 40.Pascual, M. E., Slowing, K., Carretero, E., Sa'nchez Mata, D., and Villar, A. 2001. Lippia: traditional uses, chemistry and pharmacology: a review. Journal of Ethnopharmacology 76: 201-214. 41.Pillai, G. R., Srivastava, A. S., and Hassanein, T. I. 2004. Induction of apoptosis in human lung cancer cell by curcumin. Cancer Letters 208:163-170. 42.Smith, M. J., and Fornace, A. J. 1996. Mammalian DNA damage- inducible genes associated with growth arrest and apoptosis. Mutation Research 340:109-124. 43.Vermes, I., Hannen, C., Steffens-Nakken, H., and Reutelingsperger, C. 1995. A novel assay for apoptosis Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labeled annexin V. Journal of Immunological Methods 184: 39-51. 44.Wang, T. H., and Wang, S. H. 1999. Apoptosis: (2) characteristics of apoptosis. Journal of the Formosan Medical Association 98:531-542. 45.Zamorano-Ponce, E., Morales, C., Ramos, D., Sepulveda, C., Cares, S., Rivera, P., Fernandez, J., and Carballo, MA. 2006. Anti-genotoxic effect *Aloysia triphylla* infusion against acrylamide-induce DNA damage as shown by the comet assay techinque. Mutation Research 603(2):145-150.