

飲食成分對乙醯氨酚引致的肝損傷及人類肝腫瘤細胞株影響的研究

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摘要

本研究第一部分探討了組氨酸和肌醇對於乙醯氨酚引致的肝損傷的Balb/cA小鼠的保護作用。組氨酸或肌醇，分別以 0.5、1 或 2 g/L 的濃度被加入飲用水中 4 週。小鼠的急性肝損傷是以腹腔內注射乙醯氨酚來產生。研究結果發現，乙醯氨酚的處理顯著減少肝臟中的穀胱甘醇以及抗壞血酸的水平，顯著增加肝臟中的丙二醛(malonyldialdehyde, MDA) 水平、活性氧物質(ROS)、氧化型穀胱甘醇(GSSG)，並且顯著減少肝臟中的穀胱甘醇過氧化酶(GPX)、過氧化氫(CAT)、以及超氧化物歧化酶(SOD)的活性(P < 0.05)。然而，預先攝食組氨酸或肌醇能顯著減少乙醯氨酚引致的氧化壓力。其機轉是透過增加GSH，減少MDA、ROS和GSSG的形成，以及維持肝臟中GPX、CAT和SOD活性(P < 0.05)。乙醯氨酚的處理能增加肝臟中的IL-6、IL-10、TNF- α ，以及單核細胞趨化蛋白-1 (MCP-1) (P < 0.05)。預先攝食組氨酸或肌醇能顯著降低乙醯氨酚引致的cytokines增加(P < 0.05)。然而，齊墩果酸和熊果酸只有在 4 μ mol/L 和 8 μ mol/L 時能顯著降低Huh7細胞株的細胞存活率，以及增加DNA fragmentation (P < 0.05)。齊墩果酸或熊果酸處理顯著降低HepG2、Hep3B和HA22T細胞株的粒線體膜電位，而且呈現concentration-dependent的趨勢(P < 0.05)。齊墩果酸和熊果酸的處理顯著降低HepG2、Hep3B、HA22T、以及Huh7肝癌細胞株的Na⁺-K⁺-ATPase活性以及VEGF的水平，而且呈現concentration-dependent的趨勢(P < 0.05)。齊墩果酸和熊果酸能顯著增加HepG2、Hep3B和HA22T細胞株的caspase-3和caspase-8的活性，而且呈現concentration-dependent的趨勢(P < 0.05)。另外，齊墩果酸和熊果酸能顯著減少HepG2、Hep3B、以及Huh7細胞株的細胞粘附以及ICAM-1的水平，而且呈現concentration-dependent的趨勢(P < 0.05)。這一部分的研究顯示齊墩果酸和熊果酸有強效的抗癌效果，能夠造成肝癌細胞株的凋亡。

關鍵詞：組氨酸、肌醇、乙醯氨酚、肝損傷、齊墩果酸、熊果酸、凋亡、肝腫瘤

目錄

封面內頁 簽名頁 中文摘要.....	iii	英文摘要.....	iii
.....v 誌謝.....	vii	目錄.....	vii
.....viii 圖目錄.....	xii	表目錄.....	xiv
1. 緒論.....	1	2. 文獻回顧.....	3
2.1 藥物性肝損傷.....	3	2.1.1 藥物性肝損傷流行病學.....	3
2.1.2 藥物性肝損傷機轉.....	6	2.1.3 造成藥物性肝損傷的藥物.....	7
2.1.4 乙醯氨酚引致的肝損傷.....	9	2.1.5 乙醯氨酚代謝機轉.....	10
2.1.6 乙醯氨酚引致的肝損傷的相關研究.....	12	2.1.7 組氨酸和肌醇.....	17
2.2 肝癌.....	20	2.2.1 台灣肝癌現況.....	20
2.2.2 肝癌危險因子.....	21	2.2.3 肝癌的預防.....	28
2.2.4 肝癌治療.....	30	2.2.5 肝癌細胞株的相關研究.....	35
2.2.6 齊墩果酸和熊果酸.....	38	3. 材料與方法.....	41
3.1 組氨酸和肌醇在乙醯氨酚引致的肝損傷的保護作用.....	41	3.1.1 材料.....	41
3.1.2 實驗設計.....	41	3.1.3 丙氨酸轉氨酶，天門冬氨酸轉氨酶和C-反應蛋白的分析.....	42
3.1.4 生育酚及抗壞血酸的測量.....	42	3.1.5 穀胱甘醇和氧化型穀胱甘醇含量，超氧化物歧化酶，過氧化氫和穀胱甘醇過氧化酶活性測定.....	42
3.1.6 脂質氧化和活性氧物質的測定.....	42	3.1.7 Cytokines的測量.....	43
3.1.8 CYP2E1的活性測定.....	43	3.1.9 MCP-1, TNF- α , 和GPX mRNA表現的半定量聚合鏈反應.....	45
3.1.10 統計分析.....	46	3.2 齊墩果酸和熊果酸對人類肝腫瘤細胞株的影響.....	46
3.2.1 材料.....	46	3.2.2 肝癌細胞株培養.....	47
3.2.3 實驗設計.....	47	3.2.4 細胞存活試驗.....	48
3.2.5 測量 DNA fragmentation.....	48	3.2.6 測定粒線體膜電位.....	48
3.2.7 Na ⁺ -K ⁺ -ATPase活性測定.....	50	3.2.8 caspases活性測定.....	50
3.2.9 粘附試驗.....	51	3.2.10 分析血管內皮生長因子.....	51
3.2.11 統計分析.....	51	4. 結果與討論.....	53
4.1 組氨酸和肌醇在乙醯氨酚引致的肝損傷的保護作用.....	53	4.1.1 組氨酸或肌醇處理對ALT和AST的影響.....	53
4.1.2 組氨酸或肌醇處理對CRP的影響.....	56	4.1.3 穀胱甘醇、氧化型穀胱甘醇、超氧化物歧化酶、過氧化氫、和穀	56

胱甘?過氧化?活性測定...56	4.1.4脂質氧化和活性氧物質 (ROS) 的測定.....61	4.1.5 cytokines的測量.....
.....61	4.1.6 CYP2E1的活性測定.....65	4.1.7 MCP-1, TNF- α , 和GPX mRNA的表現.....
... 65	4.2 齊墩果酸和熊果酸對人類肝腫瘤細胞株的影響.....68	4.2.1 細胞存活 (cell viability) 試驗.....68
4.2.2 DNA fragmentation.....70	4.2.3粒線體膜電位.....73	4.2.4 Na ⁺ -K ⁺ -ATPase活性.....
.....75	4.2.5 caspases活性.....75	4.2.6粘附試驗.....77
4.2.7 血管內皮生長因子.....80	5. 結論.....86	5.1 組氨酸和肌?在乙醯氨基酚引致的肝損傷的保護作用.....86
5.2 齊墩果酸和熊果酸對人類肝腫瘤細胞株的影響.....87	參 考 文 獻.....89	論 文 學 術 發 表.....
...113	圖目錄 圖1 乙醯氨基酚的化學結構式.....5	圖2 乙醯氨基酚的代謝路徑.....
...11	圖3 組氨酸的化學結構式.....18	圖4 肌?的化學結構式.....19
圖5 凋亡的訊息傳導路徑.....34	圖6 齊墩果酸的化學結構式.....39	圖7 熊果酸的化學結構式.....40
圖8 CYP2E1催化的p-nitrophenol羥基化反應.....44	圖9 MTT assay.....49	圖10 小鼠血清中ALT和AST的水平與給予組氨酸或肌?處理間的關係.....55
圖11 小鼠血清中CRP的水平與給予組氨酸或肌?處理間的關係.....57	圖12 小鼠肝臟中GPX、CAT 以及SOD的活性與給予組氨酸或肌?處理間的關係.....60	圖13 小鼠肝臟微粒體CYP2E1的活性與給予組氨酸或肌?處理間的關係.....66
圖14 小鼠肝臟中TNF-alpha以及 MCP-1相對mRNA的表現與給予組氨酸或肌?處理間的關係... ..67	圖15 小鼠肝臟中GPX相對mRNA的表現與給予組氨酸或肌?處理間的關係.....69	圖16 齊墩果酸 (OA) 或熊果酸 (UA) 對人類正常肝細胞株及肝癌細胞株的細胞存活的影響.....71
圖17 齊墩果酸 (OA) 或熊果酸 (UA) 對人類正常肝細胞株及肝癌細胞株的DNA fragmentation的影響.....72	圖18 齊墩果酸 (OA) 或熊果酸 (UA) 對人類正常肝細胞株及肝癌細胞株的粒線體膜電位的影響.....74	圖19 齊墩果酸 (OA) 或熊果酸 (UA) 對人類正常肝細胞株及肝癌細胞株的Na ⁺ -K ⁺ -ATPase活性的影響.....76
圖20 齊墩果酸 (OA) 或熊果酸 (UA) 對人類正常肝細胞株及肝癌細胞株的caspase-3的影響.....78	圖21 齊墩果酸 (OA) 或熊果酸 (UA) 對人類正常肝細胞株及肝癌細胞株的caspase-8的影響.....79	圖22 齊墩果酸 (OA) 或熊果酸 (UA) 對人類HepG2、Hep3B、Huh7和HA22T肝癌細胞株的細胞黏附的影響.....81
圖23 齊墩果酸 (OA) 或熊果酸 (UA) 對人類HepG2、Hep3B、Huh7和HA22T肝癌細胞株的ICAM-1水平的影響.....82	圖24 齊墩果酸 (OA) 或熊果酸 (UA) 對人類HepG2、Hep3B、Huh7和HA22T肝癌細胞株的VEGF水平的影響.....83	表目錄 表1 攝食0.5、1 或 2 g/L 組氨酸 (His) 或肌? (Car) 的小鼠, 在第1週及第4週的飲水量(WI, mL/mouse/d) 以及體重(g).....54
表2 以0.5、1 或 2 g/L 組氨酸 (His) 或肌? (Car) 前處理之後再給予或不給予乙醯氨基酚(APAP) 的小鼠, 其肝中GSH (nmol/mg protein), GSSG (nmol/mg protein), α -tocopherol (nmol/g tissue), 以及 ascorbic acid (nmol/mg tissue) 的含量.....58	表3 以0.5、1 或 2 g/L 組氨酸 (His) 或肌? (Car) 前處理之後再給予或不給予乙醯氨基酚(APAP) 的小鼠, 其肝中MDA (μ mol/L) 及ROS(nmol/mg protein) 的含量.....
.....62	表4 以0.5、1 或 2 g/L 組氨酸 (His) 或肌? (Car) 前處理之後再給予乙醯氨基酚(APAP) 的小鼠, 其肝中TNF- α , IL-6, IL-10, 以及MCP-1的含量(pg/mg protein).....63	

參考文獻

- Adami, H.O., Chow, W.H., Nyren, O. et al. 1996. Excess risk of primary liver cancer in patients with diabetes mellitus. *J Natl Cancer Inst.* 88: 1472-1477.
- Aithal, P.G. and Day, C.P. 1999. The natural history of histologically proved drug induced liver disease. *Gut.* 44: 731 – 735.
- Ahmed, M.B., Khater, M.R. 2001. Evaluation of the protective potential of *Ambrosia maritima* extract on acetaminophen-induced liver damage. *J Ethnopharmacol.* 75: 169-174.
- Ahn, J. and Flamm, S.L. 2004. Hepatocellular carcinoma. *Dis Mon.* 50: 556-573.
- Albano, E. 2002. Free radical mechanisms in immune reactions associated with alcoholic liver disease. *Free Radic BiolMed.* 32: 110 – 114.
- Andrade, R.J., Lucena, M.I., Fernandez, M.C. et al. 2005. Drug-induced liver injury: an analysis of 461 incidences submitted to the Spanish registry over a 10-year period. *Gastroenterology.* 129: 512 – 521.
- Anikeeva, N., Somersalo, K., Sims, T.N., Thomas, V.K., Dustin, M.L., Sykulev, Y. 2005. Distinct role of lymphocyte function-associated antigen-1 in mediating effective cytolytic activity by cytotoxic T lymphocytes. *Proceedings of the National Academy of Sciences of the United States of America* 102, 6437 – 6442.
- Beasley, R.P., Hwang, L.Y., Lin, C.C. et al. 1981. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22 707 men in Taiwan. *Lancet.* 2: 1129-1133.
- Berry, J.G., Pidd, K., Roche, A.M. et al. 2007. Prevalence and patterns of alcohol use in the Australian workforce: findings from the 2001 National Drug Strategy Household Survey. *Addiction.* 102: 1399 – 1410.
- Bjornsson, E., Kalaitzakis, E., Av Klinteberg, V. et al. 2007. Long-term follow-up of patients with mild to moderate druginduced liver injury. *Aliment Pharmacol Ther.* 26: 79 – 85.
- Bjornsson, E. and Olsson, R. 2005. Outcome and prognostic markers in severe druginduced liver disease. *Hepatology.* 42: 481 – 489.
- Bjornsson, E. and Olsson, R. 2006. Suspected druginduced liver fatalities reported to the WHO database. *Dig Liver Dis.* 38: 33 – 38.
- Blazka, M.E., Wilmer, J.L., Holladay, S.D. et al. 1995. Role of proinflammatory cytokines in acetaminophen hepatotoxicity. *Toxicol Appl Pharmacol.* 133: 43 – 52.
- Blazka, M.E., Elwell, M.R., Holladay, S.D. et al. 1996. Histopathology of acetaminophen-induced liver changes: role of interleukin 1 alpha and tumor necrosis factor alpha. *Toxicol Pathol.* 24: 181 - 189.
- Boldyrev, A.A.

and Severin, S.E. 1990. The histidine-containing dipeptides, carnosine and anserine: distribution, properties and biological protein kinase C activity significance. *Adv Enzyme Regul.* 30:175 – 194. 16.Bosch, F.X., Ribes, J., Diaz, M., Cleries, R. 2004. Primary liver cancer: worldwide incidence and trends. *Gastroenterology.* 127: S5eS16. 17.Bourdi, M., Masubuchi, Y., Reilly, T.P. et al . 2002. Protection against acetaminophen-induced liver injury and lethality by interleukin 10: role of inducible nitric oxide synthase. *Hepatology.* 35: 289 - 298. 18.Braga. C., La Vecchia, C., Negri, E., Franceschi, S. 1997. Attributable risks for hepatocellular carcinoma in northern Italy. *Eur J Cancer.* 33: 629-634. 19.Bravi, F., Bosetti, C., Tavani, A. et al. 2007. Coffee drinking and hepatocellular carcinoma risk: a meta-analysis. *Hepatology.* 46: 430-435. 20.Bressac, B., Kew, M., Wands, J., Ozturk, M. 1991. Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. *Nature.* 350: 429-431. 21.Bruix, J. and Sherman, M. 2005. Management of hepatocellular carcinoma. *Hepatology.* 42:1208 – 1236. 22.Bruix, J., Barrera, J.M., Calvet, X. et al. 1989. Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. *Lancet.* 2: 1004-1006. 23.Bussieres, J.F. and Habra, M. 1995. Application of International Consensus Meeting Criteria for classifying drug-induced liver disorders. *Ann Pharmacother.* 29: 875 – 878. 24.Cavin, C., Holzhauser, D., Constable, A. et al. 1998. The coffee-specific diterpenes cafestol and kahweol protect against aflatoxin B1-induced genotoxicity through a dual mechanism. *Carcinogenesis.* 19: 1369-1375. 25.Chadalapaka, G., Jutooru, I., McAlees, A. et al. 2008. Structure -dependent inhibition of bladder and pancreatic cancer cell growth by 2- substituted glycyrrheticin and ursolic acid derivatives. *Bioorganic and Medicinal Chemistry Letters.* 18: 2633 – 2639. 26.Chalasan, N., Fontana, R.J., Bonkovsky, H.L. et al. 2008. Causes, clinical features, and outcomes from a prospective study of drug-induced liver injury in the United States. *Gastroenterology.* 135: 1924 – 1934. 27.Chandrasekaran, V.R., Hsu, D.Z., Liu, M.Y. 2009. The protective effect of sesamol against mitochondrial oxidative stress and hepatic injury in acetaminophen-overdosed rats. *Shock.* 32(1):89-93. 28.Chang, M.H., Chen, C.J., Lai, M.S. et al. 1997. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. *N Engl J Med.* 336: 1855-1859. 29.Chang, M.H., Chen, C.J., Lai, M.S. et al. 1997. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. *N Engl J Med.* 336: 1855-1859. 30.Chen, C.H., Su, W.W., Yang, S.S. et al. 2006. Long-term trends and geographic variations in the survival of patients with hepatocellular carcinoma: analysis of 11,312 patients in Taiwan. *J Gastroenterol Hepatol.* 21: 1561-1566. 31.Cheng, A.L., Kang, Y.K., Chen, Z. et al. 2009. Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebocontrolled trial. *Lancet Oncol.* 10: 25 – 34. 32.Chen, J., Song, X., Zhang, H. et al. 2006. Sophorolipid produced from the new yeast strain *Wickerhamiella domercqiae* induces apoptosis in H7402 human liver cancer cells. *Appl Microbiol Biotechnol.* 72(1): 52-59. 33.Colombo, M., Kuo, G., Choo, Q.L. et al. 1989. Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. *Lancet.* 2: 1006-1008. 34.Cook, B.L. and Liesveld, J. 2008. Alcohol-related health problems. In: Wallace RB, Kohatsu N, eds. *Public Health and Preventive Medicine*, p.999 – 1012. McGraw-Hill Companies, Inc, New York , USA. 35.Cucchiari, M., Kammer, A.R., Grabscheid, B. et al. 2000. Vigorous peripheral blood cytotoxic T cell response during the acute phase of hepatitis C virus infection. *Cell Immunol.* 203: 111-123. 36.Cui, T., Li, J.Z., Kayahara, H. et al. 2006. Quantification of the polyphenols and triterpene acids in Chinese hawthorn fruit by highperformance liquid chromatography. *Journal of Agricultural and Food Chemistry.* 54: 4574 – 4581. 37.Dambach, D.M., Durham, S.K., Laskin, J.D., Laskin, D.L. 2006. Distinct roles of NF-kappaB p50 in the regulation of acetaminophen-induced inflammatory mediator production and hepatotoxicity. *Toxicol Appl Pharmacol* 211:157 – 165. 38.Darwish, M.A., Raouf, T.A., Rushdy, P. et al. 1993. Risk factors associated with a high seroprevalence of hepatitis C virus infection in Egyptian blood donors. *Am J Trop Med Hyg.* 49: 440-447. 39.Davila, J.A., Morgan, R.O., Shaib, Y. et al. 2005. Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. *Gut.* 54: 533-539. 40.De Abajo, F.J., Montero, D., Madurga, M. et al. 2004. Acute and clinically relevant druginduced liver injury: a population based case-control study. *Br J Clin Pharmacol.* 58: 71 – 80. 41.Delladetsima, J.K., Rassidakis, G., Tassopoulos, N.C. et al. 1996. Histopathology of chronic hepatitis C in relation to epidemiological factors. *J Hepatol.* 24: 27-32. 42.De Mitri, M.S., Poussin, K., Baccarini, P. et al. 1995. HCV-associated liver cancer without cirrhosis. *Lancet.* 345: 413-415. 43.De Valle, M.B., Av Klinteberg, V., Alem, N. et al. 2006. Drug-induced liver injury in a Swedish University hospital out-patient hepatology clinic. *Aliment Pharmacol Ther.* 24: 1187 – 1195. 44.Donato, F., Gelatti, U., Limina, R.M., Fattovich, G. 2006. Southern Europe as an example of interaction between various environmental factors: a systemic review of the epidemiologic evidence. *Oncogene.* 25: 756 – 770. 45.Duh, M.S., Walker, A.M., Kronlund, K.H. Jr. 1999. Descriptive epidemiology of acute liver enzyme abnormalities in the general population of central Massachusetts. *Pharmacoepidemiol Drug Saf.* 8: 275—283. 46.El-Serag, H.B. 2001. Global epidemiology of hepatocellular carcinoma. *Clin Liver Dis.* 5: 87e107. 47.DI-Refaie, A., Savage, K., Bhattacharya, S. et al. 1996. HCV-associated hepatocellular carcinoma without cirrhosis. *J Hepatol.* 24: 277-285. 48.DI Gohary, A., Hassan, A., Nooman, Z. et al. 1995. High prevalence of hepatitis C virus among urban and rural population groups in Egypt. *Acta Trop.* 59: 155-161. 49.Einbond, L.S., Zhimizu, M., Ma, H., Wu, H.A., Goldsberry, S., Sicular, S., Panjikaran, M., Genovese, G., Cruz, E., 2008. Actein inhibits the Na+ – K+-ATPase and enhances the growth inhibitory effect of digitoxin on human breast cancer cells. *Biochemical and Biophysical Research Communications* 375, 608 – 613. 50.El Far, M.A., Atwa, M.A., Yahya, R.S., El Basuni, M.A. 2006. Evaluation of serum levels of p53 in hepatocellular carcinoma in Egypt. *Clin Chem Lab Med.* 44: 653-656. 51.Ezzat, S., Abdel-Hamid, M., Eissa, S.A. et al. 2005. Associations of pesticides, HCV, HBV, and hepatocellular carcinoma in Egypt. *Int J Hyg Environ Health.* 208: 329-339. 52.Fabregat, I., Roncero, C., Fernandez, M. 2007. Survival and apoptosis: a dysregulated balance in liver cancer. *Liver Int.* 27: 155-162. 53.Farinati, F., Serqio, A., Baldan, A. et al. 2009. Early and very early hepatocellular carcinoma: when and how much do staging and choice of treatment really matter? A multi-center study. *BMC Cancer.* 9: 33-44. 54.Feldstein, A.E., Gores, G.J. 2005. Apoptosis in alcoholic and

nonalcoholic steatohepatitis. *Front Biosci.* 10: 3093-3099. 55. Fontana, R.J., Watkins, P.B., Bonkovsky, H.L. et al. 2009. Drug-Induced Liver Injury Network (DILIN) prospective study: rationale, design and conduct. *Drug Saf.* 32: 55 – 68. 56. Frank, C., Mohamed, M.K., Strickland, G.T. et al. 2000. The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. *Lancet.* 355: 887-891. 57. Furnkranz, A., Schober, A., Bochkov, V.N., Bashtrykov, P., Kronke, G., Kadl, A., Binder, B.R., Weber, C., Leitinger, N. 2005. Oxidized phospholipids trigger atherogenic inflammation in murine arteries. *Arter Thromb Vascular Biol* 25:633 – 638. 58. Fukuda, K., Shibata, A., Hirohata, I. et al. 1993. A hospital-based case-control study on hepatocellular carcinoma in Fukuoka and Saga Prefectures, northern Kyushu, Japan. *Jpn J Cancer Res.* 84: 708-714. 59. Gao, X., Deeb, D., Jiang, H. et al. 2007. Synthetic triterpenoids inhibit growth and induce apoptosis in human glioblastoma and neuroblastoma cells through inhibition of pro-survival Akt, NF- κ B and Notch1 signaling. *Journal of Neuro-oncology.* 84: 147 – 157. 60. Galan, M.V., Potts, J.A., Silverman, A.L., Gordon, S.C. 2005. Hepatitis in a United States tertiary referral center. *J Clin Gastroenterol.* 39:64 – 67. 61. Gho, Y.S., Kim, P.N., Li, H.C., Elkin, M., Kleinman, H.K. 2001. Stimulation of tumor growth by human soluble intercellular adhesion molecule-1. *Cancer Research* 61: 4253 – 4257. 62. Guioetto, A., Calderan, A., Ruzza, P., Borin, G. 2005. Carnosine and carnosine-related antioxidant: a review. *Curr Med Chem.* 12: 2293 – 2315. 63. Gunawan, B. and Kaplowitz, N. 2004. Clinical perspectives on xenobiotic-induced hepatotoxicity. *Drug Metab Rev.* 36: 301 – 312. 64. Groopman, J.D., Scholl, P., Wang, J.S. 1996. Epidemiology of human aflatoxin exposures and their relationship to liver cancer. *Prog Clin Biol Res.* 395: 211-222. 65. Gruner, N.H., Gerlach, T.J., Jung, M.C. et al. 2000. Association of hepatitis C virus-specific CD8+ T cells with viral clearance in acute hepatitis C. *J Infect Dis.* 181: 1528-1536. 66. Hartleb, M., Biernat, L., Kochel, A. 2002. Drug-induced liver damage: a three-year study of patients from one gastroenterological department. *Med Sci Monit* 8: CR292—CR296. 67. Hassan, M.M., Zaghloul, A.S., El-Serag, H.B. et al. 2001. The role of hepatitis C in hepatocellular carcinoma: a case control study among Egyptian patients. *J Clin Gastroenterol.* 33: 123-126. 68. Hayashi, P.H. 2009. Causality assessment in drug-induced liver injury. *Semin Liver Dis.* 29: 348 – 356. 69. Hazai, E., Vereczkey, L., Monostory, K. 2002. Reduction of toxic metabolite formation of acetaminophen. *Biochem Biophys Res Commun* 291:1089 – 1094. 70. Higuchi, S., Matsushita, S., Maesato, H., Osaki, Y. 2007. Japan: alcohol today. *Addiction.* 102: 1849 – 1862. 71. Hsu, C.C., Lin, C.C., Liao, T.S., Yin, M.C. 2006. Protective effect of *s*-allyl cysteine and propyl cysteine on acetaminophen-induced hepatotoxicity in mice. *Food Chem Toxicol.* 44: 393 – 397. 72. Hsu, C.C., Lin, K.Y., Wang, Z.H. et al. 2008. Preventive effect of *Ganoderma amboinense* on acetaminophen-induced acute liver injury. *Phytomedicine.* 15: 946-950. 73. Hsu, Y.L., Kuo, P.L., Tzeng, T.F. et al. 2008. Huang-lian-jie-du-tang, a traditional Chinese medicine prescription, induces cell-cycle arrest and apoptosis in human liver cancer cells in vitro and in vivo. *J Gastroenterol Hepatol.* 23: e290-e299. 74. Huang, J.F., Yu, M.L., Lee, C.M. et al. 2007. Sustained virological response to interferon reduces cirrhosis in chronic hepatitis C: a 1,386-patient study from Taiwan. *Aliment Pharmacol Ther.* 25: 1029-1037. 75. Hubbard, A.K., Rothlein, R. 2000. Intercellular adhesion molecule-1 (ICAM-1) expression and cell signaling cascade. *Free Radical Biology and Medicine* 28: 1379 – 1386. 76. Hwang, H.J., Kwon, M.J., Kim, I.H. et al. 2008. Chemoprotective effects of a protein from the red algae *Porphyra yezoensis* on acetaminophen-induced liver injury in rats. *Phytother Res.* 22: 1149-1153. 77. Imamura, H., Matsuyama, Y., Tanaka, E. et al. 2003. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. *J. Hepatol.* 38: 200 – 207. 78. Ishida, Y., Kondo, T., Ohshima, T. et al. 2002. A pivotal involvement of IFN- γ in the pathogenesis of acetaminophen-induced acute liver injury. *FASEB J.* 16: 1227-1236. 79. Ju, C., Reilly, T.P., Bourdi, M., Radonovich, M.F., Brady, J.N., George, J.W., Pohl, L.R. 2002. Protective role of Kupffer cells in acetaminophen-induced hepatic injury in mice. *Chem Res Toxicol* 15:1504 – 1513. 80. Ives, N. 2004. By reconsidering its message and its mission, the maker of Tylenol practices what it preaches. *New York Times*, March 17: C8. 81. Ju, C. 2005. Immunological mechanisms of drug-induced liver injury. *Curr Opin Drug Discov Devel.* 8: 38 – 43. 82. Kane, M. 1995. Global programme for control of hepatitis B infection. *Vaccine.* 13 Suppl 1: S47-S49. 83. Kang, H.M., Lee, S.K., Shin, D.S., Lee, M.Y., Han, D.C., Baek, N.I., Son, K.H., Kwon, B.M. 2006. Dehydrotrametenolic acid selectively inhibits the growth of H-ras transformed rat2 cells and induces apoptosis through caspase-3 pathway. *Life Science* 78: 607 – 613. 84. Kao, J.H. and Chen, D.S. 2005. Changing disease burden of hepatocellular carcinoma in the Far East and Southeast Asia. *Liver Int.* 25: 696-703. 85. Kassi, E., Papoutsis, Z., Pratsinis, H. et al. 2007. Ursolic acid, a naturally occurring triterpenoid, demonstrates anticancer activity on human prostate cancer cells. *Journal of Cancer Research and Clinical Oncology.* 133: 493 – 500. 86. Khan, M.H., Farrell, G.C., Byth, K. et al. 2000. Which patients with hepatitis C develop liver complications? *Hepatology.* 31: 513-520. 87. Kim, D.K., Baek, J.H., Kang, C.M., Yoo, M.A., Sung, J.W., Chung, H.Y., Kim, N.D., Choi, Y.H., Lee, S.H., Kim, K.W. 2000. Apoptotic activity of ursolic acid may correlate with inhibition of initiation of DNA replication. *International Journal of Cancer* 87: 629 – 636. 88. Kim, N.D., Kwak, M.K., Kim, S.G. 1997. Inhibition of cytochrome P450 2E1 expression by 2-(allylthio)pyrazine, a potential chemoprotective agent: hepatoprotective effects. *Biochem Pharmacol.* 53: 261 – 269. 89. Kim, R., Emi, M., Tanabe, K., Murakami, S., Uchida, Y., Arihiro, K., 2006. Regulation and interplay of apoptotic and non-apoptotic cell death. *Journal of Pathology* 208: 319 – 326. 90. Kiyosawa, K., Sodeyama, T., Tanaka, E. et al. 1990. Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. *Hepatology.* 12: 671-675. 91. Kobayashi, M., Tanaka, E., Sodeyama, T. et al. 1996. The natural course of chronic hepatitis C: a comparison between patients with genotypes 1 and 2 hepatitis C viruses. *Hepatology.* 23: 695-699. 92. Kuper, H., Tzonou, A., Kaklamani, E. et al. 2000. Tobacco smoking, alcohol consumption and their interaction in the causation of hepatocellular carcinoma. *Int J Cancer.* 85: 498-502. 93. Lai, J.T., Fang, H.L., Hsieh, W.T., Lin, W.C. 2008. Protective effect of a fermented substance from *Saccharomyces cerevisiae* on liver injury in mice caused by acetaminophen. *Biosci Biotechnol Biochem.* 72: 2514-2520. 94. Lai, M.Y., Leung, H.W., Yang, W.H. et al. 2007. Up-regulation of matrix metalloproteinase family gene involvement in ursolic acid-induced human non-small carcinoma cell apoptosis. *Anticancer Research.* 27: 145 – 153. 95. Lammert, C., Einarsson, S., Saha, C. et al. 2008. Relationship

between daily dose of oral medications and idiosyncratic drug-induced liver injury: search for signals. *Hepatology*. 47: 2003 – 2009. 96. Langston, W., Chidlow Jr., J.H., Booth, B.A., Barlow, S.C., Lefer, D.J., Patel, R.P., Keivil, C.G. 2007. Regulation of endothelial glutathione by ICAM-1 governs VEGF-mediated eNOS activity and angiogenesis. *Free Radical Biology and Medicine* 42: 720 – 729. 97. Larrey, D. 2000. Drug-induced liver diseases. *J Hepatol*. 32: 77 - 88. 98. Larrey, D. 2002. Epidemiology and individual susceptibility to adverse drug reactions affecting the liver. *Semin Liver Dis*. 22: 145 – 155. 99. La Vecchia, C., Negri, E., Decarli, A. et al. 1988. Risk factors for hepatocellular carcinoma in northern Italy. *Int J Cancer*. 42: 872-876. 100. La Vecchia, C., Ferraroni, M., Negri, E. et al. 1989. Coffee consumption and digestive tract cancers. *Cancer Res*. 49: 1049-1051. 101. Lazerow, S.K., Abdi, M.S., Lewis, J.H. 2005. Drug-induced liver disease 2004. *Curr Opin Gastroenterol*. 21: 283 – 292. 102. Lazarou, J., Pomeranz, B.H., Corey, P.N. 1998. Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. *JAMA*. 279: 1200 – 1205. 103. Lee, S.S., Buters, J.T., Pineau, T. et al. 1996. Role of CYP2E1 in the hepatotoxicity of acetaminophen. *J Biol Chem*. 271: 12063 – 12067. 104. Lee, W.M. 2003. Drug-induced hepatotoxicity (review). *N Engl J Med*. 349: 474 – 485. 105. Lee, W.M. and Senior, J.R. 2005. Recognizing drug-induced liver injury: current problems, possible solutions. *Toxicol Pathol*. 33: 155 – 164. 106. Lewis, J.H. 2002. The rational use of potentially hepatotoxic medications in patients with underlying liver disease. *Expert Opin Drug Saf*. 1: 159 – 172. 107. Lee, Y.T., Hsu, C.C., Lin, M.H. et al. 2005. Histidine and carnosine delay diabetic deterioration in mice and protect human low density lipoprotein against oxidation and glycation. *Euro J Pharm*. 513: 145 – 150. 108. Li, A.P. 2002. A review of the common properties of drugs with idiosyncratic hepatotoxicity and the “ multiple determinant hypothesis ” for the manifestation of idiosyncratic drug toxicity. *Chem Biol Interact*. 142: 7 – 23. 109. Liaw, Y.F., Tai, D.I., Chu, C.M. et al. 1986. Early detection of hepatocellular carcinoma in patients with chronic type B hepatitis. A prospective study. *Gastroenterology*. 90: 263-267. 110. Lin, S.Y., Liu, J.D., Chang, H.C. et al. 2002. Magnolol suppresses proliferation of cultured human colon and liver cancer cells by inhibiting DNA synthesis and activating apoptosis. *J Cell Biochem*. 84: 532-544. 111. Liu, C.J. and Kao, J.H. 2007. Hepatitis B virus-related hepatocellular carcinoma: epidemiology and pathogenic role of viral factors. *J Chin Med Assoc*. 70: 141-145. 112. Liu, C.J., Kao, J.H., Chen, D.S. 2005. Therapeutic implications of hepatitis B virus genotypes. *Liver Int*. 25: 1097-1107. 113. Liu, W.H., Liu, T.C., Yin, M.C. 2008. Beneficial effects of histidine and carnosine on ethanol-induced chronic liver injury. *Food Chem Toxicol*. 46: 1503 – 1509. 114. Liu, Z.X., Govindarajan, S., Kaplowitz, N. 2004. Innate immune system plays a critical role in determining the progression and severity of acetaminophen hepatotoxicity. *Gastroenterology*. 127: 1760 – 1774. 115. Llovet, J.M., Fuster, J., Bruix, J. 1999. Intention-to-treat analysis of surgical treatment for early hepatocellular carcinoma: resection versus transplantation. *Hepatology*. 30: 1434 – 1440. 116. Llovet, J. M., Real, M.I., Montana, X. et al. 2002. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomized controlled trial. *Lancet*. 359: 1734 – 1739. 117. Lo, C.M., Nqan, H., Tso, W.K. et al. 2002. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. *Hepatology*. 35: 1164 – 1171. 118. Lu, B., Rutledge, B.J., Gu, L. et al. 1998. Abnormalities in monocyte recruitment and cytokine expression in monocyte chemoattractant protein 1 – deficient mice. *J Exp Med*. 187: 601 – 608. 119. Lu, S.N., Su, W.W., Yang, S.S. et al. 2006. Secular trends and geographic variations of hepatitis B virus and hepatitis C virus-associated hepatocellular carcinoma in Taiwan. *Int J Cancer*. 119: 1946-1952. 120. Majer, B.J., Hofer, E., Cavin, C. et al. 2005. Coffee diterpenes prevent the genotoxic effects of 2-amino-1-methyl-6-phenylimidazo[4,5- b]pyridine (PhIP) and N-nitrosodimethylamine in a human derived liver cell line (HepG2). *Food Chem Toxicol*. 43: 433-441. 121. Makin, A.J. and Williams, R. 1997. Acetaminophen-induced hepatotoxicity: predisposing factors and treatments. *Adv Intern Med*. 42: 453 – 483. 122. Manyike, P.T., Kharasch, E.D., Kalthorn, T.F., Slattery, J.T. 2000. Contribution of CYP2E1 and CYP3A to acetaminophen reactive metabolite formation. *Clin Pharmacol Ther*. 67: 275 – 282. 123. Margolis, H.S. 1998. Hepatitis B virus infection. *Bull World Health Organ*. 76 Suppl 2: 152-153. 124. Masubuchi, Y., Bourdi, M., Reilly, T.P. et al. 2003. Role of interleukin-6 in hepatic heat shock protein expression and protection against acetaminophen-induced liver disease. *Biochem Biophys Res Commun*. 304: 207 - 212. 125. Masubuchi, Y., Suda, C., Horie, T. 2005. Involvement of mitochondrial permeability transition in acetaminophen-induced liver injury in mice. *J Hepatol*. 42: 110 – 116. 126. Mazzaferro, V., Reqalia, E., Doci, R. et al. 1996. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N. Engl. J. Med*. 334: 693 – 699. 127. McKillop, I.H. and Schrum, L.W. 2005. Alcohol and liver cancer. *Alcohol* 5: 195 – 203. 128. Michielsen, P.P., Francque, S.M., van Dongen, J.L. 2005. Viral hepatitis and hepatocellular carcinoma. *World J Surg Oncol*. 3: 27-44. 129. Mitra, A., Kulkarni, A.P., Ravikumar, V.C., Bourcier, D.R. 1991. Effect of ascorbic acid esters on hepatic glutathione levels in mice treated with a hepatotoxic dose of acetaminophen. *J Biochem Toxicol* 6: 93 – 100. 130. Meier, Y., Cavallaro, M., Roos, M. et al. 2005. Incidence of drug-induced liver injury in medical inpatients. *Eur J Clin Pharmacol*. 61: 135 – 143. 131. Mohamed-Ali, V., Armstrong, L., Vlark, D., Bolton, C.H., Pinkney, J.H. 2001. Evidence for the regulation of levels of plasma adhesion molecules by inflammatory cytokines and their soluble receptors in type 1 diabetes. *J Int Med* 250: 415 – 421. 132. Montalto, G., Cervello, M., Giannitrapani, L. et al. 2002. Epidemiology, risk factors, and natural history of hepatocellular carcinoma. *Ann N Y Acad Sci*. 963: 13-20. 133. Montella, M., Polesel, J., La Vecchia, C. et al. 2007. Coffee and tea consumption and risk of hepatocellular carcinoma in Italy. *Int J Cancer*. 120: 1555-1559. 134. Morgan, T.R., Mandayam, S., Jamal, M. 2004. Alcohol and hepatocellular carcinoma. *Gastroenterology*. 127: S87 – S96. 135. Nelson, S.D. 1990. Molecular mechanisms of hepatotoxicity caused by acetaminophen. *Semin Liver Dis*. 10: 267 – 278. 136. Nishioka, K., Watanabe, J., Furuta, S. et al. 1991. A high prevalence of antibody to the hepatitis C virus in patients with hepatocellular carcinoma in Japan. *Cancer*. 67: 429-433. 137. Ni, Y.H., Chang, M.H., Huang, L.M. et al. 2001. Hepatitis B virus infection in children and adolescents in a hyperendemic area: 15 years after mass hepatitis B vaccination. *Ann Intern Med*. 135: 796-800. 138. Nuñez, M. 2006. Hepatotoxicity of antiretrovirals: incidence, mechanisms and management. *J Hepatol*. 44: S132 – S139. 139. Ohfuji, S., Fukushima, W., Tanaka, T. et al. 2006.

Coffee consumption and reduced risk of hepatocellular carcinoma among patients with chronic type C liver disease: A case-control study. *Hepatol Res.* 36: 201-208.

140. Okada, K., Kamiyama, I., Inomata, M. et al. 1976. e antigen and anti-e in the serum of asymptomatic carrier mothers as indicators of positive and negative transmission of hepatitis B virus to their infants. *N Engl J Med.* 294: 746-749.

141. Oliveira, F.A., Chaves, M.H., Almeida, F.R., Lima, R.C. Jr., Silva, R.M., Maia, J.L., Brito, G.A., Santos, F.A., Rao, V.S. 2005. Protective effect of alpha- and beta-amyrin, a triterpene mixture from *Protium heptaphyllum* (Aubl.) March. trunk wood resin, against acetaminophen-induced liver injury in mice. *J Ethnopharmacol.* 98: 103-108.

142. Ostapowicz, G., Fontana, R.J., Schiodt, F.V. et al. 2002. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. *Ann Intern Med.* 137: 947 - 954.

143. Ostapowicz, G.A., Fontana, R.J., Schiodt, F.V. et al., and the Acute Liver Failure Study Group. 2002. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. *Ann Intern Med.* 137: 945 – 954.

144. Oz, H.S., McClain, C.J., Nagasawa, H.T. et al. 2004. Diverse antioxidants protect against acetaminophen hepatotoxicity. *J Biochem Molecular Toxicol.* 18: 361 – 368.

145. Parikh, S. and Hyman, D. 2007. Hepatocellular cancer: a guide for the internist. *Am J Med.* 120: 194-202.

146. Park, B.K., Kitteringham, N.R., Maggs, J.L. et al. 2005. The role of metabolic activation in drug-induced hepatotoxicity. *Annu Rev Pharmacol Toxicol.* 45: 177 – 202.

147. Parkin, D.M. 2001. Global cancer statistics in the year 2000. *Lancet Oncol.* 2: 533-543.

148. Poon, R.T.P., Ng, I.O., Lau, C., Zhu, L.X., Yu, W.C., Lo, C.M., Fan, S.T., Wong, J., 2001. Serum vascular endothelial growth factor predicts venous invasion in hepatocellular carcinoma: a prospective study. *Annals of Surgery* 233: 227 – 235.

149. Poynard, T., Bedossa, P., Opolon, P. 1997. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet.* 349: 825-832.

150. Rajasekaran, S.A., Barwe, S.P., Rajasekaran, A.K. 2005. Multiple functions of Na, KATPase in epithelial cells. *Seminars in Nephrology* 25: 328 – 334.

151. Rakitsky, V.N., Koblyakov, V.A., Turusov, V.S. 2000. Nongenotoxic (epigenetic) carcinogens: pesticides as an example. A critical review. *Teratog Carcinog Mutagen.* 20: 229-240.

152. Rashid, M., Goldin, R., Wright, M. 2004. Drugs and the liver. *Hosp Med.* 65: 456 – 461.

153. Reilly, T.P., Brady, J.N., Marchick, M.R. et al. 2001. A protective role for cyclooxygenase-2 in drug-induced liver injury in mice. *Chem Res Toxicol.* 14: 1620 - 1628.

154. Ruhl, C.E. and Everhart, J.E. 2005. Coffee and caffeine consumption reduce the risk of elevated serum alanine aminotransferase activity in the United States. *Gastroenterology.* 128: 24-32.

155. Russo, M.W., Galanko, J.A., Shrestha, R. et al. 2004. Liver transplantation for acute liver failure from drug induced liver injury in the United States. *Liver Transpl.* 10: 1018 – 1023.

156. Sakinah, S.A., Handayani, S.T., Hawariah, L.P. 2007. Zerumbone induced apoptosis in liver cancer cells via modulation of Bax/Bcl-2 ratio. *Cancer Cell Int.* 7: 4-14.

157. Sarih, M., Bouchrit, N., Benslimane, A. 2000. Different cytokine profiles of peripheral blood mononuclear cells from patients with persistent and self-limited hepatitis C virus infection. *Immunol Lett.* 74: 117-120.

158. Seeff, L.B., Buskell-Bales, Z., Wright, E.C. et al. 1992. Long-term mortality after transfusion-associated non-A, non-B hepatitis. The National Heart, Lung, and Blood Institute Study Group. *N Engl J Med.* 327: 1906-1911.

159. Sgro, C., Clinard, F., Ouazir, K. et al. 2002. Incidence of drug-induced hepatic injuries: a French population-based study. *Hepatology.* 36: 451 – 455.

160. Sharp, G.B., Lagarde, F., Mizuno, T. et al. 2005. Relationship of hepatocellular carcinoma to soya food consumption: a cohort-based, case-control study in Japan. *Int J Cancer.* 115: 290-295.

161. Shayiq, R.M., Roberts, D.W., Rothstein, K. et al. 1999. Repeat exposure to incremental doses of acetaminophen provides protection against acetaminophen-induced lethality in mice: an explanation for high acetaminophen dosage in humans without hepatic injury. *Hepatology.* 29: 451 – 463.

162. Shepard, C.W., Finelli, L., Alter, M.J. 2005. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis.* 5: 558-567.

163. Stetler-Stevenson, W.G., Aznavoorian, S., Liotta, L.A. 1993. Tumor cell interaction with the extracellular matrix during invasion and metastasis. *Annual Review of Cell Biology* 54: 541 – 573.

164. Sun, J.J., Zhou, X.D., Liu, Y.K., Tang, Z.Y., Feng, J.X., Zhou, G., Xue, Q., Chen, J. 1999. Invasion and metastasis of liver cancer: expression of intercellular adhesion molecule-1. *Journal of Cancer Research and Clinical Oncology* 125: 28 – 34.

165. Suruki, R.Y., Mueller, N., Hayashi, K. et al. 2006. Host immune status and incidence of hepatocellular carcinoma among subjects infected with hepatitis C virus: a nested case-control study in Japan. *Cancer Epidemiol Biomarkers Prev.* 15: 2521-2525.

166. Takahashi, K., Mizuarai, S., Araki, H., Mashiko, S., Ishihara, A., Kanatani, A., Itadani, H., Kotani, H. 2003. Adiposity elevates plasma MCP-1 levels leading to the increased CD11b-positive monocytes in mice. *J Biol Chem* 278: 46654 – 46660.

167. Takikawa, H., Murata, Y., Horiike, N. et al. 2009. Drug-induced liver injury in Japan: an analysis of 1676 cases between 1997 and 2006. *Hepatol Res.* 39: 427 – 431.

168. Tanaka, K., Hara, M., Sakamoto, T. et al. 2007. Inverse association between coffee drinking and the risk of hepatocellular carcinoma: a case-control study in Japan. *Cancer Sci.* 98: 214-218.

169. Tang, C., Lu, Y.H., Xie, J.H., Wang, F., Zou, J.N., Yang, J.S., Xing, Y.Y., Xi, T. 2009. Downregulation of surviving and activation of caspase-3 through the PI3K/Akt pathway in ursolic acid-induced HpeG2 cell apoptosis. *Anticancer Drugs* 20: 249 – 258.

170. The, S.H., Christein, J., Donohue, J. et al. 2005. Hepatic resection of hepatocellular carcinoma in patients with cirrhosis: Model of End-Stage Liver Disease (MELD) score predicts perioperative mortality. *J. Gastrointest. Surg.* 9: 1207 – 1215.

171. Tomita, M., Dragoman, M., Worcester, H., Conran, P., Santoro, T.J. 2004. Proinflammatory cytokine genes are constitutively overexpressed in the heart in experimental systemic lupus erythematosus: a brief communication. *Exp Biol Med* 229: 971 – 976.

172. Tong, M.J., el-Farra, N.S., Reikes, A.R., Co, R.L. 1995. Clinical outcomes after transfusion-associated hepatitis C. *N Engl J Med.* 332: 1463-1466.

173. Trotter, J.F., Wachs, M., Everson, G.T. and Kam, I. 2002. Adult-to-adult transplantation of the right hepatic lobe from a living donor. *N. Engl. J. Med.* 346: 1074 – 1082.

174. Tsai, S.L., Liaw, Y.F., Chen, M.H. et al. 19