doi: 10.13241/j.cnki.pmb.2015.02.042

苦龙胆酯苷的研究进展 *

沈 歆[1] 郭倩倩[2] 曹 蔚[2] 王四旺[2△]

（第四军医大学药学院 1 2010 级药学本科生；2 天然药物学教研室 陕西 西安 710032）

摘要：苦龙胆酯苷是一种裂环烯醚萜苷类化合物，又称为苦龙苷或龙胆苦酯苷。其分子式为：$C_{27}H_{28}O_{14}$，分子量为 576.52，是已知最苦的裂环烯醚萜苷类化合物。目前已证明可从川东獐牙菜、印度獐牙菜、龙胆草以及辐花肋柱花中提取。辐花肋柱花是最新证明的可提取苦龙胆酯苷的植物。苦龙胆酯苷具有助消化，保肝，抗皮肤肿瘤，抗黑热病等药理活性。在古代印度传统医药以及中药藏药中，苦龙胆酯苷的来源植物是治疗消化系统相关疾病的一味常见的草药，如保肝、抗糖尿病等。在体内药代动力学研究中，兔静脉注射苦龙胆酯苷，在血中有较快的清除率（2.62± 0.41 L/h/kg）和广泛的体内组织分布（1.08± 0.44 L/kg）；采用游离、脂质体和囊泡体形式给仓鼠用药具有明显保护肝肾功能且未发现明显不良反应。

关键词：苦龙胆酯苷；提取方法；药理活性

中图分类号：R285 **文献标识码**：A **文章编号**：1673-6273（2015）02-365-03

Research Progress of Amarogentin*

SHEN Xin[1], GUO Qian-qian[2], CAO Wei[2], WANG Si-wang[2△]

(1 The department of undergraduate students; 2 Department of natural medicine, school of pharmacy, the fourth military medical university, Xi'an, Shaanxi, 710032, China)

ABSTRACT: Amarogentin identifies as a secoiridoid glycoside composition. Its formula is $C_{27}H_{28}O_{14}$ and its molecular weight is 576.52. Amarogentin is the most intensely bitter secoiridoid glycoside composition as far as we know. It can be extracted from Swertia davidi Franeh, Swertia chirata,Buch.-Ham, Gentiana lutea L and Lomatogonium rotatum. Among these medical plants Lomatogonium rotatum is the newest one, which is identified containing amarogentin. Amarogentin has been traditionally used for centuries mainly as digestive aid as well as protecting from hepatic injury and also been used for treatment of skin tumor by topical application and for therapy of leishmaniasis. In Ayurveda, Unani, Siddha, Tibetan, and Chinese traditional medicines, the medical plants, which is identified containing amarogentin, are the main ingredient in many herbal preparations to treat the diseases in digestive system, such as protecting from hepatic injury or anti-diabetic. The in vivo pharmacokinetic study shows that amarogentin exhibits a rapid clearance（2.62± 0.41 L/h/kg）and wide distribution（1.08± 0.44 L/kg）after intravenous administration in rabbits; in hamster experimental leishmaniasis model, amarogentin is effective to protect liver and kidney function with no toxicity, in both liposomal and niosomal forms as well as its free form.

Key words: Amarogentin; Extraction; Pharmacological

Chinese Library Classification（CLC）: R285 Document code: A

Article ID: 1673-6273(2015)02-365-03

研究显示，不少植物的有害代谢产物或毒物具有苦味，使得哺乳动物和远古的人类在觅食中通过尝其味来避开这些有毒物质，有人认为这种苦味可以看做是一种对有害或者有毒物的警告；由此可以推论，苦味化合物在人类的进化过程中扮演着不可或缺的作用[1]。近年来，苦味化合物日益受到重视，本文讨论的正是被认为是世界最苦的裂环烯醚萜苷类化合物 " 苦龙胆酯苷 " 的来源及其生物活性。该药多来源于天然产物的有机提取，其中甲醇提取物的提取效率最高，目前还未有文献报道能够完全化学合成苦龙胆酯苷。在古代传统医药中，苦龙胆酯苷的来源植物被广泛用作保肝药，现代医学实验也已证明苦龙胆酯苷的确有预防肝脏癌变的作用。此外通过体内体外实验还证明了苦龙胆酯苷有良好的抗肿瘤作用以及抗黑热病作用。在安全性评价中，苦龙胆酯苷的三种给药形式在有效血药浓度范围内，都证明对实验动物无毒。

1 苦龙胆酯苷的来源

1.1 从植物中提取

川东獐牙菜 Swertia davidi Franeh 又名水灵芝，属龙胆科獐牙菜属植物，为民间传统中草药，主要化学成分有环烯醚萜类、三萜类、黄酮类和呫酮类，临床用于治疗黄疸型肝炎、痢疾等。称取粉碎的川东獐牙菜全草 1.0 g，加 10 mL 甲醇，超声处理 30 min 滤过，收集滤液，滤渣加 8 mL 甲醇，超声处理 30

* 基金项目：陕西省工程技术研究中心重大课题资助项目（2010ZDGC-19）

作者简介：沈歆（1992-），女，本科，电话：18700916601，E-mail：shenxin9204@126.com

△通讯作者：王四旺（1958-），男，教授，博士生导师，中药药理与新药创制，电话：029-84774748，E-mail:wangsiw@fmmu.edu.cn

（收稿日期：2014-05-23 接受日期：2014-06-20）

min,同法提取 2 次,收集、合并滤液,吸取滤液 1 mL,用 0.45 μm 滤膜滤过,即得到粗品苦龙胆酯苷,HPLC 测定其在植物中含量为 0.3%[2,3]。

印度獐牙菜 Swertia chirata,Buch.-Ham 是一种印度传统药用植物,常被用于治疗肝炎,胆囊炎,肺炎,痢疾,疥疮,神经衰弱,苦补剂,退烧药[12]。取印度獐牙菜地上部分用甲醇过夜浸泡,上清液用正丁烷萃取后用硅胶柱提纯,流动相为氯仿:甲醇=19:1,洗脱液中含苦龙胆酯苷 70-75%[4],也有文献中使用丙酮:水 =8:2 提取[5,20]。

龙胆草 Gentiana lutea L 生长于欧洲中南部,山坡的石灰性土壤中,龙胆草根作为一种传统药用植物,其作用有驱虫,消炎,防腐,苦补剂,利胆,通经,退热,制冷和健胃。龙胆草根提取物还作为一种饮料(moxie soda)在美国上市,在欧洲一些国家的开胃酒中也有添加。用甲醇在室温下振摇 48 h 提取,但经 HPLC 检测,苦龙胆酯苷仅占 0.025-0.4%[6,7]。

辐花肋柱花 Lomatogonium rotatum 是一种传统藏医、蒙医用药,在青海分布于海拔 3000-4100 m 的山坡草地、灌丛中。辐花肋柱花常用于治疗肝脏、胆囊及脾脏类疾病。苦龙苷是其主要药用成分,对子宫平滑肌有明显的解痉作用,同时还有镇静作用。称取粉碎至 80 目的样品 0.5 g,加入甲醇 10 mL,超声提取 0.5 h,过滤,滤渣重复提取 1 次,合并滤液,定容至 25 mL 过 0.5 μm 滤膜,得苦龙胆酯苷的含量为 0.044 mg/g[8]。

1.2 通过化学方法合成

Wang Chang Zeng 等人成功通过化学方法,经六步反应成功合成苦龙胆酯苷的部分分子基团 --3- 乙基(3'- 甲氧基苯基)-3- 氧代丙酸叔丁酯,产率为 13.6%。该方法从 3- 甲氧基苯乙酮开始经过成酯,脱甲基,水解,催化氢化等反应得到 3- 乙基(3'- 甲氧基苯基)-3- 氧代丙酸叔丁酯。为了继续合成苦龙胆酯苷,对 3- 乙基(3'- 甲氧基苯基)-3- 氧代丙酸叔丁酯的辅酶 A 和 N- 乙酰半胱氨硫醇酯也进行了合成,最大产率分别为 42%和 17%。但目前还没有人能够完全通过化学方法合成苦龙胆酯苷,仅能合成其的部分分子基团[9]。

2 苦龙胆酯苷的理化性质

2.1 苦龙胆酯苷的分子式

分子式:$C_{27}H_{28}O_{14}$,分子量为 576.52,结构式如图 1[10]。

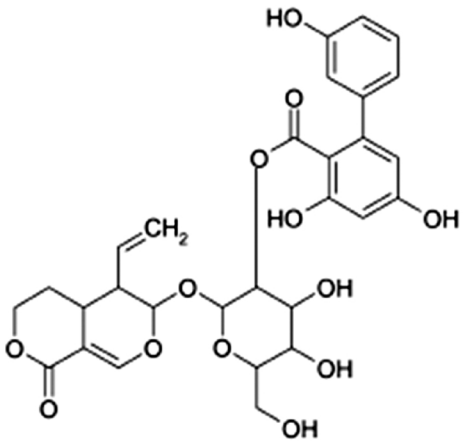

图 1 苦龙胆酯苷化学结构图

Fig. 1 Chemical structure of amarogentin

2.2 苦味

苦龙胆酯苷有极强的苦味,是目前发现最苦的环烯醚萜苷类化合物,要用 200-500 ppm 的圣草素钠盐的苦掩剂来掩盖,其苦度相当于咖啡因的苦度[14]。苦龙胆酯苷对人类苦味受体基因 hTAS2Rs published 家族有激活作用,能激活其中的四个受体:hTAS2R43、hTAS2R46、hTAS2R47 和 hTAS2R50,其中对 hTAS2R47 的阈值浓度最低,与穿心莲内酯对 hTAS2R50 的激动有竞争作用,且是该基因的专属激动剂。

2.3 溶解性

苦龙胆酯苷的极性较大,易溶于水和甲醇,可溶于乙醇、丙酮和正丁醇,难溶于三氯甲烷、乙醚等亲脂性有机溶剂。利用该性质可以有效的从植物中提取、分离苦龙胆酯苷。在已有文献记载中从原植物提取苦龙胆酯苷纯品时,甲醇的提取效率最高,其次是乙醇,水因其沸点较高较少应用于提取,而在动物及细胞实验中多应用水作为溶剂。

3 苦龙胆酯苷的生物活性

3.1 保肝作用

苦龙胆酯苷通过调节 G1/S 细胞周期的控制点和诱导细胞凋亡来预防肝脏癌变。在 CCl_4 诱导发生肝癌的小鼠模型中,一组小鼠在癌变前 15 天给苦龙胆酯苷,另一组在癌变后一周给苦龙胆酯苷,30 周后癌变前给药小鼠无明显肝脏形态和组织结构的改变,也未出现小鼠体重减轻和淋巴细胞渗透等病理变化,而癌变后给药小鼠 30 周后肝脏表面出现小病灶,肝脏结构出现中度发育异常,以及淋巴细胞渗透;癌变前给药小鼠同对照组小鼠相比,增殖细胞显著减少,凋亡数增加,而癌变后给药小鼠同对照组小鼠相比增殖细胞增多,凋亡数减少。由此可以看出,苦龙胆酯苷的预防作用比治疗作用更有效。0.2 mg/kg 是苦龙胆酯苷用药的最有效剂量[15]。獐牙菜提取物能上调解毒酶体系表达和减少肝脏脂质过氧化。Prosenjit Saha 等证明印度獐牙菜正己烷粗提物和乙酸乙酯纯化物(90-95%苦龙胆酯苷)有抑制肝脏脂质过氧化作用[16]。

3.2 抗肿瘤作用

Cox 是细胞内花生四烯酸转变成前列腺素的限速酶,在有丝分裂,致癌基因,肿瘤转移等刺激下诱导 Cox-II 表达可以促进肿瘤细胞增殖,血管生成;细胞凋亡蛋白酶(Procaspase-3)在细胞内作为一个未活化的前体存在,由 p11p17 亚基激活后诱导凋亡。在 DMBA(dimethylbenzanthracene 二甲基苯并蒽)诱导的老鼠皮肤癌模型中,发现苦龙胆酯苷的作用靶点就是 Cox-II 和细胞凋亡蛋白酶(Procaspase-3),通过下调 Cox-II 表达和激活细胞凋亡蛋白酶,来诱导凋亡抑制肿瘤增殖[17]。印度獐牙菜正己烷粗提物和 90-95%的苦龙胆酯苷给予 DMBA 诱导的皮肤癌模型老鼠,发现粗提物和苦龙胆酯苷都表现出了抑制增殖促进凋亡的作用,且粗提物抑制肿瘤发生的效果较好,苦龙胆酯苷抑制肿瘤多样性效果较好[16]。

3.3 抗黑热病作用

杜氏利什曼原虫的无鞭毛体主要寄生在肝、脾、骨髓、淋巴结等器官的巨噬细胞内,常引起全身症状,如发热、肝脾肿大、贫血、鼻衄等。在印度,患者皮肤常有暗色素沉着,并有发热故又称黑热病。因其致病力较强,如不治疗常因并发症而死亡,病

死率可高达 90%以上。已发现苦龙胆酯苷有抑制拓扑异构酶 I 的作用,阻止其与 DNA 结合,抑制寄生虫增殖,表现出抗黑热病功效。Swapna Medda 等给仓鼠注射利什曼原虫后分为四组,分别给游离苦龙胆酯苷,其脂质体,其囊泡体,30 天后发现给药组(游离,脂质体,囊泡体)的寄生虫发生率分别减少了 34%、69%和 90%。给游离苦龙胆酯苷的小鼠在五个剂量组都没有死亡,最佳治疗剂量是 2.5 mg/kg[18]。此外,还发现苦龙胆酯苷的抗黑热病作用有剂量依赖性,同喜树碱有相同的抗病原虫效应[13]。

4 苦龙胆酯苷的药代动力学和安全性评价

4.1 药动学研究

给兔体内静脉注射标准纯度苦龙胆酯苷,血药浓度分析发现其有较快的清除率(2.62± 0.41 L/h/kg)和广泛的体内分布(1.08± 0.44 L/kg)[19]。苦龙胆酯苷体外检测线性良好,冻结、降温、干燥和长时间保存的稳定性良好,回收率>90%,日间和日内准确度及精密度分别为<15%和<10%,LC-MS 最低检测限 0.156 ng/ml[6,7]。

4.2 初步安全性评价

通过测定仓鼠血浆中谷丙转氨酶的浓度,验证苦龙胆酯苷的肝毒性。给予游离苦龙胆酯苷的仓鼠体内谷丙转氨酶稍有上升,而以脂质体和囊泡体形式给药的仓鼠体内该酶浓度较正常仓鼠相比无明显变化,证明苦龙胆酯苷对肝脏无明显毒性反应。通过测定尿液中尿素氮和肌酐浓度,验证苦龙胆酯苷的肾毒性。同样,给予游离苦龙胆酯苷的仓鼠尿液中尿素氮和肌酐浓度,较正常小鼠有所上升,但是以脂质体和囊泡体形式给药的仓鼠体内尿素氮和肌酐浓度恢复到正常水平,证明苦龙胆酯苷无肾毒性。此外,仓鼠解剖病理分析,发现脾脏染色后未见病理变化[18]。印度獐牙菜正己烷粗提物和乙酸乙酯纯化物(90-95%苦龙胆酯苷),用 IC_{50} 浓度 10 mg 和 0.5 mg 给正常小鼠腹腔注射,未造成小鼠死亡[16]。

5 结语

以上阐述了苦龙胆酯苷的来源,初步的理化性质和生物活性以及药代动力学和安全性评价。现已证实苦龙胆酯苷有助于消化,保肝,抗皮肤肿瘤,抗黑热病等疗效和生物安全性,并在体外及动物实验中已引人关注。但苦龙胆酯苷是否具有临床应用前景和开发成新型保肝药或抗肿瘤新药尚有大量的研究工作。

参 考 文 献(References)

[1] Maik Behrens, Anne Brockhoff, Claudia Batram, et al. The Human Bitter Taste Receptor hTAS2R50 is Activated by the Two Natural Bitter Terpenoids Andrographolide and Amarogentin [J]. Agricultural and Food Chemistry, 2009, 57: 9860-9866

[2] 谭桂山, 徐平声, 田华咏, 等. 川东獐牙菜化学成分的研究 [J]. 中国药学杂志, 2000, 35(7): 441-443
Tan Gui-shan, Xu Ping-sheng, Tian Hua-yong, et al. Studies on the chemical constituents of Swertia davidi [J]. Chin Pharm J, 2000, 35 (7): 441-443

[3] 周慧, 杨瑞, 郑一敏, 等. 川东獐牙菜中苦龙胆酯苷含量测定研究 [J]. 中国药业, 2001, 20(3): 5-6
Zhou Hui, Fang Rui, Zheng Yi-min, et al. Determination of Amarogenti in Swertia Davidi Franch. by HPLC [J]. China Pharmaceuticals, 2001, 20(3): 5-6

[4] Susanna Phoboo, Marcia Da Silva Pinto, Ana Cristina Lopes Barbosa, et al. Phenolic-Linked Biochemical Rationale for the Anti-Diabetic Properties of Swertia chirayita (Roxb. ex Flem.) Karst [J]. Phytotherapy Research, 2012, 1002(10): 4714-4722

[5] Pradeep Pratap Singh, Ambika, Shive Murat Singh Chauhan. Activity-guided isolation of antioxidant xanthones from Swertia chirayita (Roxb.) H. Karsten (Gentianaceae) [J]. Natural Product Research, 2012, 26(18): 1682-1686

[6] Branislav Nastasijevic, Tamara Lazarevic ′ -Pas˘ ti, Suzana Dimitrijevic ′ -Brankovic, et al. Inhibition of myeloperoxidase and antioxidative activity of Gentiana lutea extracts [J]. Journal of Pharmaceutical and Biomedical Analysis, 2012, 66: 191-196

[7] Chandrasekhar Akileshwari, Puppala Muthenna, Branislav Nastasijevic, et al. Inhibition of Aldose Reductase by Gentiana lutea Extracts [J]. Experimental Diabetes Research, 2012, 1155 (10): 147965-147972

[8] 俞青芬, 林鹏程. 反相高效液相色谱法测定辐花肋柱花中苦龙苷含量的研究 [J]. 安徽农业科学, 2007, 35(4): 967-968
Yu Qing-fen, Lin Peng-cheng. Determination of Amarogentin in Lomatogonium rotatum by RP-HPLC [J]. Journal of Anhui Agri. Sci., 2007, 35(4): 967-968

[9] Chang-Zeng Wang, Ulrich H. Maier, Meinhart H. Zenk. Synthesis of 3,3′ ,5-Trihydroxybiphenyl-2-carboxylic Acid, a Component of the Bitterest Natural Product Amarogentin and Its Coenzyme A and N-Acetyl Cysteamine Thiol Esters [J]. American Chemical Society and American Society of Pharmacognosy, 2000, 63: 371-374

[10] Ivana Citov, Markus Ganzera, Hermann Stuppner, et al. Determina - tion of gentisin, isogentisin, and amarogentin in Gentiana lutea L. by capillary electrophoresis[J]. J. Sep. Sci., 2008, 31: 195-200

[11] Satyendra Suryawanshi, R.K. Asthana, R.C. Gupta. Simultaneous estimation of mangiferin and four secoiridoid glycosides in rat plasma using liquid chromatography tandem mass spectrometry and its application to pharmacokinetic study of herbal preparation[J]. Journal of Chromatography, 2007, 858: 211-219

[12] Sushma Koul, K. A. Suri, P. Dutt, et al. Protocol for In Vitro Regeneration and Marker Glycoside Assessment in Swertia chirata Buch-Ham [J]. Protocol for In Vitro Regeneration and Marker Glycoside, 2009, 547: 139-153

[13] Sutapa Ray, Hemanta K. Majumder, Ajit K. Chakravarty, et al. Amarogentin,a Naturally Occurring Secoiridoid Glycoside and a Newly Recognized Inhibitor of Topoisomerase I from Leishmania donouani[J]. J . Nut. Prod., 1996, 59: 27-29

[14] Jakob P.Ley, Gerhard Krammer, Gerald Reinders, et al. Evaluation of Bitter Masking Flavanones from Herba Santa (Eriodictyon californicum (H. & A.) Torr., Hydrophyllaceae)[J]. Agricultural and Food Chemistry, 2005, 53: 6061- 6066

[15] Debolina Pal, Subhayan Sur, Suvra Mandal, et al. Prevention of liver carcinogenesis by amarogentin through modulation of G1/S cell cycle check point and induction of apoptosis [J]. Carcinogenesis, 2012, 33: 2424-2431

(下转第 346 页)

controls beta-tubulin III expression and is associated with paclitaxel-based treatment response and progression-free survival in ovarian cancer patients[J]. Endocr Relat Cancer, 2011, 18(1): 85-95

[5] Li S D, Zhang JR, Wang YQ, et al. The role of microRNAs in ovarian cancer initiation and progression [J]. J Cell Mol Med, 2010, 14(9): 2240-2249

[6] Mateescu B, Batista L, Cardon M, et al. miR-141 and miR-200a act on ovarian tumorigenesis by controlling oxidative stress response[J]. Nat Med, 2011, 17(12): 1627-1635

[7] Torres A, Torres K, Maciejewski R, et al. MicroRNAs and their role in gynecological tumors[J]. Med Res Rev, 2011, 31(6): 895-923

[8] Stroup DF, Berlin JA, Morton SC, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group[J]. JAMA, 2000, 283(15): 2008-2012

[9] Knobloch K, Yoon U, Vogt PM. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement and publication bias[J]. J Craniomaxillofac Surg, 2011, 39(2): 91-92

[10] Zhang L, Riethdorf S, Wu G, et al. Meta-analysis of the prognostic value of circulating tumor cells in breast cancer [J]. Clin Cancer Res, 2012, 18(20): 5701-5710

[11] Marchini S, Cavalieri D, Fruscio R, et al. Association between miR-200c and the survival of patients with stage I epithelial ovarian cancer: a retrospective study of two independent tumour tissue collections[J]. Lancet Oncol, 2011, 12(3): 273-285

[12] 李晓翠,严沁,万菁,等. miR-200a 在卵巢癌患者血清中的表达及临床意义[J]. 现代妇产科进展, 2012, 21(12): 953-956
Li Xiao-cui, Yan Qin, Wan Jing, et al. Expression of miR-200a in serum of ovarian epithelial carcinoma and its clinical significance. [J]. Progress in Obstetrics and Gynecology, 2012, 21(12): 953-956

[13] 李林霞,李双弟,杨懿霞,等. miRNA-200c 在上皮性卵巢癌细胞株及肿瘤组织中的表达变化及意义[J]. 第二军医大学学报, 2011, 32 (6): 612-616
Li Lin-xia, Li Shuang-di, Yang Yi-xia, et al. Change of miRNA-200c expression in ovarian cancer and its clinical significance [J]. Academic Journal of Second Military Medical University, 2011, 32 (6): 612-616

[14] Nam EJ, Yoon H, Kim SW, et al. MicroRNA expression profiles in serous ovarian carcinoma[J]. Clin Cancer Res,2008,14(9):2690-2695

[15] Dersimonian R, Laird N. Meta-analysis in clinical trials [J]. Control Clin Trials, 1986, 7(3): 177-188

[16] Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses[J]. BMJ, 2003, 327(7414): 557-560

[17] Gould RB, Bracken MB, Rimm DL. Tissue biomarkers for prognosis in cutaneous melanoma: a systematic review and meta-analysis [J]. J Natl Cancer Inst, 2009, 101(7): 452-474

[18] Egger M, Davey SG, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test[J]. BMJ, 1997, 315(7109):629-634

[19] Lin S, Pan L, Guo S, et al. Prognostic role of microRNA-181a/b in hematological malignancies: a meta-analysis [J]. PLoS One, 2013, 8 (3): e59532

[20] 程妮,朱耀魁,张磊,等. 微 RNA 在卵巢上皮性癌发生发展中作用及其临床意义[J]. 生命科学, 2012, 24(5): 428-432
Cheng Ni, Zhu Yao-kui, Zhang Lei, et al. The roles of miRNAs in the pathogenesis of epithelial ovarian cancer and its clinical significance [J]. Chinese Bulletin of Life Sciences, 2012, 24(5): 428-432

[21] Holschneider CH, Berek JS. Ovarian cancer: epidemiology, biology, and prognostic factors[J]. Semin Surg Oncol, 2000, 19(1): 3-10

[22] van Jaarsveld MT, Helleman J, Berns EM, et al. MicroRNAs in ovarian cancer biology and therapy resistance [J]. Int J Biochem Cell Biol, 2010, 42(8): 1282-1290

[23] Hayes DF, Isaacs C, Stearns V. Prognostic factors in breast cancer: current and new predictors of metastasis [J]. J Mammary Gland Biol Neoplasia, 2001, 6(4): 375-392

[24] Shih KK, Qin LX, Tanner EJ, et al. A microRNA survival signature (MiSS) for advanced ovarian cancer[J]. Gynecol Oncol, 2011, 121(3): 444-450

[25] Li X, Zhang Y, Zhang Y, et al. Survival prediction of gastric cancer by a seven-microRNA signature[J]. Gut, 2010, 59(5): 579-585

(上接第 367 页)

[16] Prosenjit Saha, Suvra Mandal, Ashes Das, et al. Evaluation of the Anticarcinogenic Activity of Swertia chirata Buch.Ham, an Indian Medicinal Plant, on DMBA-induced Mouse Skin Carcinogenesis Model[J]. Phytotherapy Research, 2004, 18: 373-378

[17] Prosenjit Saha, Suvra Mandal, Ashes Das, et al. Amarogentin can reduce hyperproliferation by downregulation of Cox-II and upregulation of apoptosis in mouse skin carcinogenesis model [J]. Cancer Letters, 2006, 244: 252-259

[18] Swapna Medda, Sibabrata Mukhopadhyay, Mukul Kumar Basu. Evaluation of the in-vivo activity and toxicity of amarogentin, an antileishmanial agent, in both liposomal and niosomal forms [J]. Journal of Antimicrobial Chemotherapy, 1999, 44: 791-794

[19] Satyendra Suryawanshi, R. K. Asthana, R. C. Gupta. Assessment of Systemic Interaction between Swertia chirata Extract and its Bioactive Constituents in Rabbits [J]. Phytotherapy Research, 2009, 23: 1036-1038

[20] Satyendra Suryawanshi, Nitin Mehrotra, R. K. Asthana, et al. Liquid chromatography/tandem mass spectrometric study and analysis of xanthone and secoiridoid glycoside composition of Swertia chirata, a potent antidiabetic [J]. Rapid Communications in Mass Spectrometry, 2006, 20: 3761-3768