

枳 子提取物对 D-半乳糖致亚急性衰老小鼠氧化损伤的保护作用

汪海涛¹, 嵇扬^{2*}, 徐永祥¹, 徐江平¹ (1. 南方医科大学药学院药理学系, 广东 广州 510515; 2. 中国人民解放军总后勤部卫生部药品仪器检验所, 北京 100071)

摘要:目的 观察枳 子提取物 (*Semen hoveniae extract*, SHE)对 D-半乳糖诱导的亚急性衰老小鼠氧化损伤的保护作用,并探讨其保护作用的可能机制。方法 NH小鼠随机分为6组:空白对照组,衰老模型组,维生素 E组(200 mg·kg⁻¹·d⁻¹),SHE低、中、高剂量组(50, 100, 200 mg·kg⁻¹·d⁻¹),小鼠颈背部皮下注射 D-半乳糖(100 mg·kg⁻¹·d⁻¹)造成小鼠亚急性衰老模型,同时按分组灌胃给予相应药物,每天一次,连续给药 45 d后进行实验,以小鼠水迷宫测定衰老小鼠的学习记忆能力;比色法测定小鼠肝脏及脑组织中超氧化物歧化酶(SOD)、谷胱甘肽过氧化物酶(GSH-Px)活力及丙二醛(MDA)含量,蛋白含量测定用考马斯亮蓝法。结果 水迷宫实验中,与模型组小鼠相比,各剂量给药组小鼠到达平台的潜伏期明显缩短,进入盲端的错误次数显著减少;肝、脑组织匀浆测定结果显示:各给药组 SOD、GSH-Px活力显著增加,MDA含量明显减少。结论 不同剂量的 SHE均能改善 D-半乳糖致亚急性衰老小鼠的学习记忆能力,具有延缓衰老的作用,此作用可能部分是通过增加体内抗氧化酶的活性,减少过氧化脂质的生成,提高机体抗氧化能力而导致的。

关键词:枳 子;D-半乳糖;氧化损伤;抗氧化

中图分类号:R965 文献标识码:A 文章编号:1001-2494(2008)08-0591-04

Protective Effect of Semen hoveniae Extract on Oxidative Injury in Subacute Senile Mice Induced by D-galactose

WANG Hai-tao¹, JI Yang^{2*}, XU Yong-xiang¹, XU Jiang-ping¹ (1. Department of Pharmacology, Southern Medical University, Guangzhou 510515, China; 2. Institute for Drug and Instrument Control of Health Department GLD of PLA, Beijing 100071, China)

ABSTRACT: OBJECTIVE To study the protective effect and investigate the underlying mechanisms of *Semen hoveniae* extract (SHE) on oxidative injury in subacute senile mice induced by D-galactose. **METHODS** NH mice were randomly divided into 6 groups: control group, aging model group, vitamin E (positive control) group (200 mg·kg⁻¹·d⁻¹), and SHE groups (50, 100, 200 mg·kg⁻¹·d⁻¹). The subacute aging model of mice were induced by D-galactose (100 mg·kg⁻¹·d⁻¹) following s.c. administration, at the same time, SHE or vitamin E were given ig for 45 d. The learning and memory ability of mice were tested by water maze, the activities of superoxide dismutase (SOD), glutathione (GSH-Px) and the contents of malondialdehyde (MDA) in the brain and liver were measured by colorimetric method. **RESULTS** Compared with the model group, the escape latency was shortened and the times of entry errors were decreased significantly in the mice of SHE treatment groups and vitamin E group. The extract obviously strengthened the activities of SOD and GSH-Px in liver and brain tissue, reduced the content of MDA. **CONCLUSION** SHE could improve the learning and memory ability of senile mice, the extract has significant protective effect on oxidative injury and the

- [15] LI X, LI Y, XU W F. Design, synthesis, and evaluation of novel galblyl pyrrolidine derivatives as potential anti-tumor agents[J]. *Bioorg Med Chem*, 2006, 14: 1287-1293.
- [16] FENG X, DANQ NG S, YONGSU Z. Discussion of the Inhibition of Angiogenesis and its Mechanism. In: Progress of Anti-neoplasm-drugs and Chemotherapy[M]. Beijing Science Publ Corp (抗肿瘤和化疗进展), 2001: pp 243.
- [17] LEROY-DUDAL J, DEMELL IERS C, GALLET O, et al. Transmigration of human ovarian adenocarcinoma cells through endothelial extracellular matrix involves alphav integrins and the participation of MMP2[J]. *Int J Cancer*, 2005, 114: 531-543.
- [18] BOOTH C, HARNDEN P, SELBY P J, et al. Towards defining

roles and relationships for tenascin-C and TGF beta-1 in the normal and neoplastic urinary bladder[J]. *J Pathol*, 2002, 198: 359-368.

- [19] TAN MURA S, ASATO K, FUJISHIRO S H, et al. Specific blockade of the ERK pathway inhibits the invasiveness of tumor cells: down-regulation of matrix metalloproteinase-3/-9/-14 and CD44[J]. *Biochem Biophys Res Commun*, 2003, 304: 801-806.
- [20] TANG Y, NAKADA M T, KESAVAN P, et al. Extracellular matrix metalloproteinase inducer stimulates tumor angiogenesis by elevating vascular endothelial cell growth factor and matrix metalloproteinases[J]. *Cancer Res*, 2005, 65: 3193-3199.

(收稿日期: 2007-04-03)

基金项目: 全军医学科研“十一五”计划重大专项(06MA375)

作者简介: 汪海涛, 男, 硕士研究生 *通讯作者: 嵇扬, 女, 主任药师 Tel: (010) 66949079 E-mail: jiyang0809@yahoo.com.cn

mechanisms probably related to its improvement of antioxidases and reduction of lipid peroxidation.

KEY WORDS: Semen hoveniae; D-galactose; oxidative injury; anti-oxidation

自由基参与多种疾病和损伤的发病机制,自1956年提出衰老的自由基理论以来,关于自由基与衰老关系的研究受到了重视,大量实验证实自由基能与细胞组成物质脂类、蛋白质、核酸等发生反应,产生脂质过氧化物,对细胞产生损害,引发衰老^[1],衰老的自由基机制提示,提高体内抗氧化酶活性或减少体内自由基将有助于防治衰老性疾病。枳子(*Semen hoveniae*)为鼠李科(Rhamnaceae)拐枣属(*Hovenia Thunb*)植物枳(*Hovenia dulcis* Thunb)的干燥成熟种子,有研究表明,枳子有显著的解酒、抗肝纤维化作用^[2-3],有关其延缓衰老方面的研究迄今未见报道,本实验采用皮下注射D半乳糖的方法通过氧化损伤建立亚急性衰老小鼠模型,并观察枳子醇提物(SHE)对衰老小鼠的作用,并对其作用机制进行探讨,以期延缓衰老寻找新的药物,并为枳子的应用和推广提供理论依据。

1 材料与方 法

1.1 药物与试剂

枳子[北京同仁堂(亳州)饮片有限公司,批号:601000024,经第二军医大学陈万生教授鉴定为鼠李科拐枣属枳成熟果实];D半乳糖(Amresco公司);维生素E(Merck公司);超氧化物歧化酶(SOD)、丙二醛(MDA)、谷胱甘肽过氧化物酶(GSH-Px)试剂盒(南京建成生物工程研究所)。羧甲基纤维素(CMC, Sigma公司)。

1.2 仪器

SF-300型高速粉碎机(上海中联制药装备有限公司);100型多功能中药提取罐(武汉制药机械厂);SMG-2型程控水迷宫(中国医学科学院药物研究所);DY89-型电动玻璃匀浆机(宁波新芝生物科技股份有限公司);752紫外光栅分光光度计(上海第三分析仪器厂);低温离心机(上海安亭科学仪器厂)。

1.3 枳子提取物制备

取枳子一次性过粉碎机,破碎成粗粉,粗粉至提取罐内加4倍量体积分数为75%乙醇回流煮提3次,提取液合并后过400目筛并减压浓缩至膏状,真空干燥。以芦丁为对照品,测得枳子中总黄酮质量分数为(2.57±0.03)%。

1.4 实验动物

NIH小鼠,27~30g,雌性(南方医科大学实验

动物中心,合格证号:2006A045)。

1.5 实验方法

1.5.1 亚急性衰老小鼠模型的制备及给药 60只小鼠按体重随机分组^[4],分为空白对照组、模型组、给药组(50,100,200 mg·kg⁻¹·d⁻¹)、阳性对照组,空白组注射无菌生理盐水,其他各组每天颈背部皮下注射D半乳糖100 mg·kg⁻¹·d⁻¹,同时,给药组分别灌服SHE 50,100,200 mg·kg⁻¹·d⁻¹(分别相当于生药0.35,0.70,1.40 g·kg⁻¹·d⁻¹),阳性对照组灌以维生素E 200 mg·kg⁻¹·d⁻¹,SHE及维生素E均用1%CMC配制,空白组和模型组灌以等体积1%CMC。皮下注射D半乳糖及给药均是每天1次,连续45d。

1.5.2 小鼠水迷宫实验^[5] 水迷宫由黑色有机玻璃板组成,共有4个盲端及A、B、C3个入水点,终点区有一高出水面的安全平台,ABC入水点与平台的距离依次增大,即C点距平台最远,水深13cm,水温(23±1)℃,第46d进行小鼠水迷宫训练,训练进行3d,平台周围设置标志物,训练前先将小鼠置平台10s,使其熟悉周围环境,第1天将小鼠放入A入水点,让其寻找平台,第2天放入B入水点,第3天放入C入水点,如240s内未找到平台,则将其引导上平台,第4天进行实验,将小鼠置C入水点,记录其游至平台的时间为潜伏期,超过240s则以240s计。其进入盲端的次数为错误次数,以潜伏期和错误次数作为小鼠学习记忆的成绩。

1.5.3 各组织SOD、MDA、GSH-Px含量测定^[6-7]

水迷宫实验结束后,脱臼处死各组小鼠,迅速取其大脑及肝脏,冰水浴中制成10%匀浆,4℃离心取上清,备用。SOD、MDA、GSH-Px含量测定按试剂盒说明书进行。蛋白定量采用考马斯亮蓝法。

1.5.4 统计学方法 结果用 $\bar{x} \pm s$ 表示,采用SPSS13.0软件进行单因素方差分析。

2 实验结果及分析

2.1 小鼠水迷宫实验

与正常对照比较,小鼠连续给予D半乳糖45d后,模型组小鼠到达平台的时间显著延长,进入盲端的次数显著增多,说明造模成功;与模型组比较,各给药组能显著缩短小鼠到达平台的时间,减少进入盲端的次数,低剂量组略好于高、中剂量。阳性对照组也有同样的效应(表1)。实验结果提示,枳子

能明显改善 D 半乳糖致衰老小鼠的学习记忆能力。

表 1 各组小鼠行为学实验结果, $n = 10$, 聊 ±s

Tab. 1 Comparison of behavioral changes among groups, $n = 10$, 聊 ±s

| Group | Dose/mg · kg ⁻¹ · d ⁻¹ | Latency/s | Error time |
|----------------|--|----------------------|------------------------|
| Normal control | | 30 ±21 ¹⁾ | 3.2 ±2.4 ¹⁾ |
| Model control | 100 | 73 ±43 ³⁾ | 7.6 ±4.0 ³⁾ |
| Vitamin E | 200 | 37 ±21 ¹⁾ | 4.2 ±4.2 ²⁾ |
| SHE | 50 | 38 ±29 ¹⁾ | 3.4 ±1.8 ¹⁾ |
| | 100 | 45 ±26 ²⁾ | 4.6 ±2.4 ²⁾ |
| | 200 | 42 ±23 ²⁾ | 4.3 ±2.4 ²⁾ |

注:与模型组比较, ¹⁾ $P < 0.01$, ²⁾ $P < 0.05$;与对照组比较, ³⁾ $P < 0.01$

Note: compared with model control group, ¹⁾ $P < 0.01$, ²⁾ $P < 0.05$; compared with normal control group, ³⁾ $P < 0.01$

2.2 SHE对衰老小鼠肝、脑中MDA含量, SOD及GSH-Px活性的影响

与正常对照组小鼠相比, D 半乳糖致衰老模型小鼠肝、脑组织中 MDA 含量明显升高, SOD 及 GSH-Px 活性均显著下降, 枳 子各给药组能剂量依赖性降低肝、脑组织中 MDA 的含量, 提高 SOD 及 GSH-Px 的活性 (表 2, 表 3)。

表 2 SHE对衰老小鼠肝脏中MDA含量, SOD及GSH-Px活性的影响, $n = 10$, 聊 ±s

Tab. 2 Effect of SHE on SOD, MDA and GSH-Px levels in liver tissues of aging mice, $n = 10$, 聊 ±s

| Group | Dose/mg · kg ⁻¹ · d ⁻¹ | SOD /U · mg (pμt) ⁻¹ | MDA /nmol · mg ⁻¹ | GSH-Px /U · mg (pμt) ⁻¹ |
|----------------|--|---------------------------------|------------------------------|------------------------------------|
| Normal control | | 181 ±56 ¹⁾ | 1.06 ±0.25 ¹⁾ | 148 ±24 ¹⁾ |
| Model control | 100 | 56 ±22 ³⁾ | 1.58 ±0.42 ³⁾ | 64 ±26 ³⁾ |
| Vitamin E | 200 | 141 ±39 ¹⁾ | 1.01 ±0.48 ¹⁾ | 118 ±30 ¹⁾ |
| SHE | 50 | 106 ±47 ²⁾ | 1.32 ±0.56 | 95 ±34 ²⁾ |
| | 100 | 128 ±36 ²⁾ | 1.16 ±0.35 ²⁾ | 124 ±32 ¹⁾ |
| | 200 | 144 ±63 ¹⁾ | 1.09 ±0.37 ²⁾ | 135 ±40 ¹⁾ |

注:与模型组比较, ¹⁾ $P < 0.01$, ²⁾ $P < 0.05$;与对照组比较, ³⁾ $P < 0.01$

Note: compared with model control group, ¹⁾ $P < 0.01$, ²⁾ $P < 0.05$; compared with normal control group, ³⁾ $P < 0.01$

3 讨论

随着年龄的增加, 由于抗氧化酶活力的不断下降, 体内产生的过量自由基无法得到及时清除, 过剩的自由基可诱发动脉粥样硬化、糖尿病、肿瘤等多种疾病, 也是加速机体衰老的重要因素之一^[8]。同时, 机体的免疫系统功能也逐渐衰退, 从而加速衰老过程^[9], 由于氧自由基与脂质过氧化及衰老的发生密切相关, 因此, 寻求改善自由基、抗氧化平衡的药物成为延缓衰老和防治慢性疾病的重要方向。

在一定时间内连续给小鼠注射 D 半乳糖是人造小鼠衰老模型的经典方法。机体内半乳糖浓度升高, 在醛糖还原酶作用下, 还原成半乳糖醇, 破坏机体抗氧化防御系统, 同时半乳糖被还原成半乳

表 3 SHE对衰老小鼠脑组织中MDA含量, SOD及GSH-Px活性的影响, $n = 10$, 聊 ±s

Tab. 3 Effect of SHE on SOD, MDA and GSH-Px levels in brain tissues of aging mice, $n = 10$, 聊 ±s

| Group | Dose/mg · kg ⁻¹ · d ⁻¹ | SOD /U · mg (pμt) ⁻¹ | MDA /nmol · mg ⁻¹ | GSH-Px /U · mg (pμt) ⁻¹ |
|----------------|--|---------------------------------|------------------------------|------------------------------------|
| Normal control | | 210 ±42 ¹⁾ | 1.8 ±0.3 ¹⁾ | 95 ±49 ¹⁾ |
| Model control | 100 | 106 ±26 ³⁾ | 4.8 ±0.9 ³⁾ | 51 ±12 ³⁾ |
| Vitamin E | 200 | 185 ±44 ¹⁾ | 2.9 ±0.7 ¹⁾ | 86 ±29 ²⁾ |
| SHE | 50 | 172 ±54 ¹⁾ | 3.3 ±0.7 ²⁾ | 83 ±22 ²⁾ |
| | 100 | 176 ±70 ¹⁾ | 2.8 ±0.9 ¹⁾ | 91 ±40 ¹⁾ |
| | 200 | 198 ±69 ¹⁾ | 2.1 ±0.6 ¹⁾ | 92 ±32 ¹⁾ |

注:与模型组比较, ¹⁾ $P < 0.01$, ²⁾ $P < 0.05$;与对照组比较, ³⁾ $P < 0.01$

Note: compared with model control group, ¹⁾ $P < 0.01$, ²⁾ $P < 0.05$; compared with normal control group, ³⁾ $P < 0.01$

糖醇的过程中也产生超氧阴离子, 过氧化物脂质增加, 出现衰老^[10]。有研究显示造模时给予半乳糖的量超过 100 mg · kg⁻¹ · d⁻¹ 时, 其学习记忆的能力不再随剂量的增加而减退^[11], 故本实验中将半乳糖的量定为 100 mg · kg⁻¹ · d⁻¹。

MDA 是脂质过氧化的产物, 其高低间接反应了机体细胞受自由基攻击的严重程度; SOD 对机体的氧化与抗氧化平衡起着至关重要的作用, 此酶能清除超氧阴离子自由基, 保护细胞免收损伤; GSH-Px 是机体内一种重要的催化过氧化氢分解的酶, 其特异的催化还原型谷胱甘肽 (GSH) 对过氧化氢的还原反应, 可以保护细胞膜结构和功能完整的作用^[12], 故本实验选测这几项指标。

因雄性小鼠长期群居, 好咬斗易造成受伤而影响实验结果, 故选用雌性小鼠。枳 子含有山奈酚、斛皮素、双氢杨梅黄素等黄酮类活性成分^[13], 这些成分具有较强的抗氧化及自由基清除活性^[14], 本实验结果显示, SHE 组均能显著改善 D 半乳糖诱导的衰老小鼠的学习记忆能力。低剂量小鼠学习记忆成绩略优于高、中剂量, 但 SOD、GSH-Px、MDA 测定结果均有一定的剂量关系, 这可能与行为学实验的个体差异性有关; 同时, 结果显示, SHE 能减少肝脏及脑组织内 MDA 的生成, 提高 GSH-Px 和 SOD 活性, 并且呈现剂量依赖性, 说明枳 子既能减轻组织或细胞的过氧化损伤, 减少脂质过氧化物的形成, 又能增加体内抗氧化酶的活性以增加对自由基损伤的防御功能, 提示枳 子可通过自由基代谢发挥其保护衰老小鼠氧化损伤的作用。

REFERENCES

- [1] BAROUKIR. Ageing free radicals and cellular stress[J]. *Med Sci (Paris)*, 2006, 22(3): 266-272.

(下转第 605 页)

流失,提高角质层水合作用而促进其经皮渗透。SLN对皮肤组织还具一定亲和性和靶向性,可增加药物在皮肤中的贮留量和时间,在皮肤用药部位形成含药量较高的药物储库,有助于延长释药时间和提高药物经皮渗透量。SLN中的磷脂可与角质层脂质融合,使其结构改变,细胞间隙扩大,经皮渗透性提高;此外,磷脂有助于提高SLN安全性并降低可能因药物引起的过敏反应。

TDDS发展迅速,已成为目前最成功的非口服控释给药系统之一。据预测,未来10年内将有近1/3的现用药开发成TDDS,其关键技术在于有效促进药物经皮渗透。本实验结果表明,NM-SLN具有较好的经皮渗透特性,促透效果优于常用的化学促透剂,作为药物递送微载体可用于乳剂、霜剂、擦剂及膜控储库型贴剂等多种TDDS的设计;与化学促透法联合使用,有助于缩短NM-SLN透皮扩散的 t_{lag} ,进一步提高其经皮渗透性能,从而获得更佳的治疗效果。

REFERENCES

[1] MULLER R H, RADTKE M, WISSNG S A. Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations [J]. *Adv Drug Deliv Rev*, 2002, 54 (Suppl 1): 131-155.

[2] HU F Q, HONG Y, YUAN H. Preparation and characterization of solid lipid nanoparticles containing peptide [J]. *Int J Pharm*, 2004, 273 (1-2): 29-35.

[3] LI J, DONG A J. Function of nanoparticles in transdermal and transmucosal drug delivery systems [J]. *Chin J Chin Mater Med*

(中国中药杂志), 2004, 29 (3): 193-196.

[4] SOUTO E B, WISSNG S A, BARBOSA C M, *et al.* Evaluation of the physical stability of SLN and NLC before and after incorporation into hydrogel formulations [J]. *Eur J Pharm Biopharm*, 2004, 58 (1): 83-90.

[5] WISSNG S A, LIPPACHER A, MULLER R H. Investigations on the occlusive properties of solid lipid nanoparticles (SLN) [J]. *J Cosmet Sci*, 2001, 52 (5): 313-323.

[6] CHEN S, ZHENG J M. The observation on *in vitro* permeation and irritation of nimodipine patches [J]. *J Shenyang Pharm Univ (沈阳药科大学学报)*, 2000, 17 (2): 84-86.

[7] KRISHNA AH Y S, BHASKAR P. Studies on the transdermal delivery of nimodipine from a menthol-based TTS in human volunteers [J]. *Curr Drug Deliv*, 2004, 1 (2): 93-102.

[8] CHEN G L, LI W Z, ZHOU Y, *et al.* Effect of compound penetration enhancers on permeability of nimodipine through rat skin [J]. *Chin J Pharm (中国医药工业杂志)*, 1998, 29 (7): 308-310.

[9] ZHOU J P, ZHANG J S, QI P. Study on penetration enhancers for nimodipine [J]. *J Chin Pharm Univ (中国药科大学学报)*, 1994, 25 (3): 149-152.

[10] ZHOU J P, ZHONG M, HUANG J H. Study on transdermal therapeutic system for nimodipine [J]. *J Chin Pharm Univ (中国药科大学学报)*, 2000, 31 (5): 348-351.

[11] MULLER R H, MADER K, GOHLA S. Solid lipid nanoparticles (SLN) for controlled drug delivery—a review of the state of the art [J]. *Eur J Pharm Biopharm*, 2000, 50 (1): 161-177.

[12] SCHWARZ C, MEHNERT W. Solid lipid nanoparticles (SLN) for controlled drug delivery. Drug incorporation and physicochemical characterization [J]. *J Microencapsulation*, 1999, 16 (2): 205-213.

[13] MEI Z, CHEN H, WENG T, *et al.* Solid lipid nanoparticle and microemulsion for topical delivery of triptolide [J]. *Eur J Pharm Biopharm*, 2003, 56 (2): 189-196.

(收稿日期: 2007-12-15)

(上接第 593 页)

[2] JI Y, LI J, YANG P. Effects of fruits of *Hovenia dulcis* Thunb on acute alcohol toxicity in mice [J]. *J Chin Med Mater (中药材)*, 2001, 24 (2): 126-128.

[3] YE L P, ZHANG H, LIU X L. A morphological study of protective effect of *Hovenia dulcis* Thunb extract on hepatic fibrosis [J]. *J Wuhan Univ (Med Ed)* (武汉大学学报: 医学版), 2005, 26 (3): 293-296.

[4] CHEN Q. *Methodology on Pharmacological Research of Traditional Chinese Medicine (中药药理研究方法学)* [M]. Beijing: People's Medical Publishing House, 1993: 31.

[5] LI N G M, ZHAO H Y, FAN W J, *et al.* Effects of accumulation of lactic acid in atlanto-axial joint on abilities of learning and memory in senile mice [J]. *J Jilin Univ (Med Ed)* (吉林大学学报: 医学版), 2006, 32 (6): 968-970.

[6] ZHANG Z H, CHEN X H, ZHANG L P, *et al.* Antiaging effects of beta-carotene from *dunaliella* saline on fruit flies and rats [J]. *Chin Pharmacol Bull (中国药理学通报)*, 2006, 22 (11): 1324-1328.

[7] ZHANG Z M, GE B, XU A X, *et al.* Antisenile effect of polysaccharides from *Fructus ligustri lucidi* [J]. *Chin J Pharmacol Toxicol (中国药理学与毒理学杂志)*, 2006, 20 (2): 108-111.

[8] FLORA S J. Role of free radical and antioxidants in the health

and disease [J]. *Cell Mol Bio (Noisy-le-grand)*, 2007, 15: 53 (1): 1-2.

[9] DAS R, PONNAPPAN S, PONNUAPPAN U. Redox regulation of the proteasome in T lymphocytes during aging [J]. *Free Radic Biol Med*, 2007, 42 (4): 541-551.

[10] MEHMET G, BAH A O, HILMID. Protective effects of vitamins C and E against endometrial damage and oxidative stress in fluoride intoxication [J]. *Clin Exp Pharmacol Physiol*, 2007, 34: 467-474.

[11] WEI H, LI L, SONG Q, *et al.* Behavioural study of the D-galactose induced aging model in C57BL/6J mice [J]. *Behav Brain Res*, 2005, 157 (2): 245-251.

[12] XU S Y, BAN R L, CHEN X. *Pharmacology Experiment Methodology (药理实验方法学)* [M] // 3rd ed. Beijing: People's Medical Publishing House, 2002: 1465-1466.

[13] SHA M, DING L S. Chemical study on the seeds of *Hovenia acerba* Lindl [J]. *J Chin Pharm Univ (中国药科大学学报)*, 2001, 32 (6): 418-420.

[14] WANG K J, CHEN L Z, LIN *et al.* Antioxidant and radical-scavenging activity of flavonoids from *Solidago Canadensis* [J]. *Chin Pharm J (中国药理学杂志)*, 2006, 41 (7): 493-497.

(收稿日期: 2007-08-01)