RESEARCH ON *CISTANCHE DESERTICOLA* AMELIORATES COLITIS AND COLONRECTAL CANCER IN MICE MODEL

肉苁蓉对肠炎及肠癌小鼠模型的治疗作用研究

贾亚敏 著

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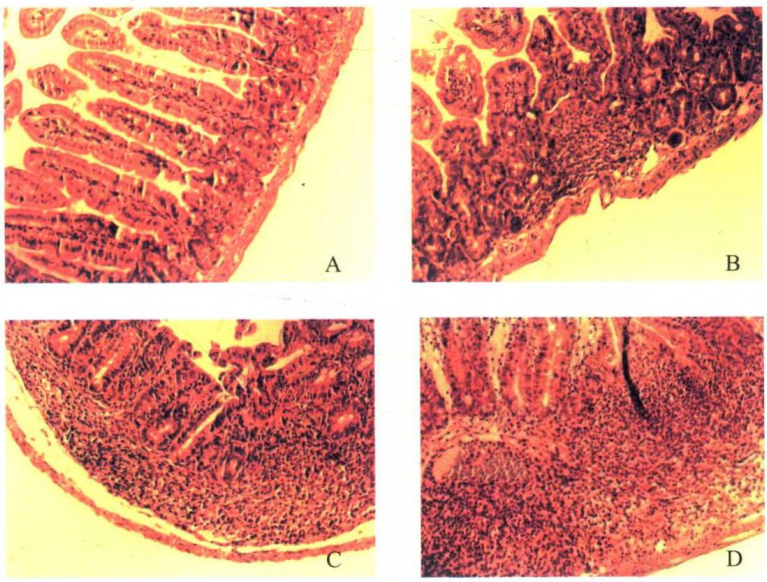

图 3-1　实验小鼠的肠道组织切片

注：A 为正常小鼠的肠道组织：腺窝，杯状细胞存在；B 为异常增生图示：仍有腺窝等组织结构，其显著特征为基层加厚；C 为腺瘤图示：肌层增厚，显著特征是杯状细胞大量消失；D 为肿瘤图示：腺窝和杯状细胞大面积缺失，肌层显著增厚，出现大量炎性细胞浸润。

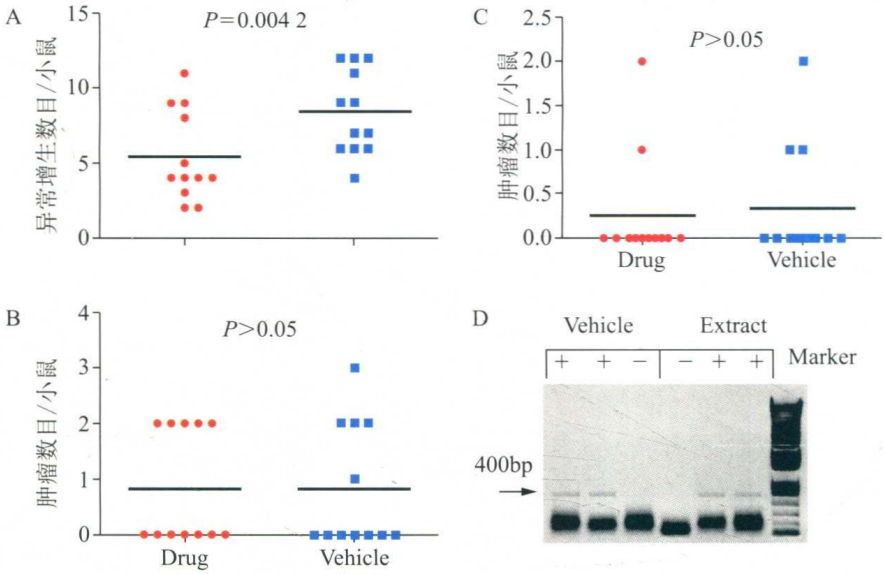

图 3-2　饲喂肉苁蓉水提取物对模型小鼠肠道组织结构及幽门螺杆菌感染的影响

注：A 为饲喂肉苁蓉水提取物对肠道异型增生的影响，每个点代表每只小鼠携带的异常增生的数目，横线是平均值，表示各组中异常增生数目的平均值；B 为饲喂肉苁蓉水提取物对肠道腺瘤的影响；C 为饲喂肉苁蓉水提取物对肠道肿瘤的影响；D 为 16Sr DNA PCR 检测幽门螺杆菌感染结果的示意图，在 400 bp 处有条带，表明有幽门螺杆菌的感染，没有检测到条带，表示没有幽门螺杆菌的感染。

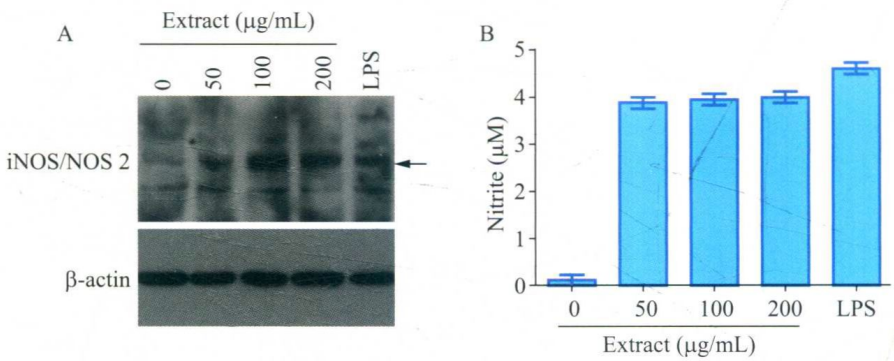

图 3-6　不同浓度肉苁蓉水提取物对 RAW264. 7 细胞 iNOS 蛋白表达及 NO 生成的影响

注：A 为不同浓度的肉苁蓉水提取物增强 RAW264. 7 细胞 iNOS 的表达；B 为不同浓度的肉苁蓉水提取物促进 RAW264. 7 细胞 NO 的生成

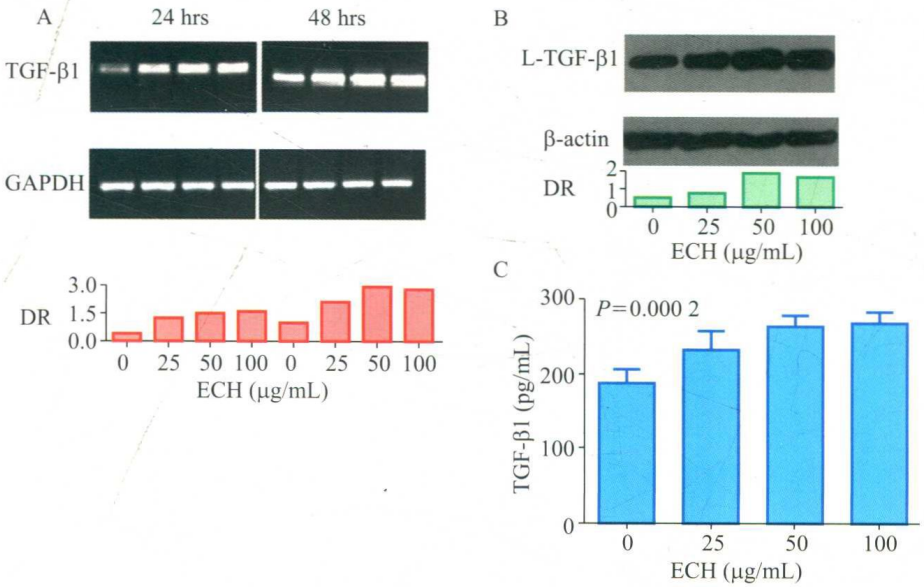

图 4-6　ECH 上调 MODE-K 细胞中 TGF-β 的表达

注：A 为 PCR 检测不同浓度 ECH 处理 MODE-K 细胞 24 h、48 h TGF-β-mRNA 的表达。B 为 Western Blot 检测 TGF-β 蛋白表达水平。C 为 ELISA 测定不同浓度的松果菊苷处理 MODE-K 细胞后细胞液中 TGF-β 分泌水平。

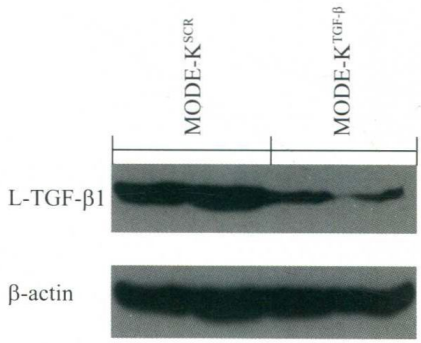

图 4-8　Western Blot 检测 TGF-β1 在 MODE-K^{SCR} 和 MODE-$K^{TGF-\beta}$ 细胞的表达差异

注：其中 MODE-K^{SCR} 细胞的 TGF-β 表达正常，而 MODE-$K^{TGF-\beta}$ 的 TGF-β 表达受阻。

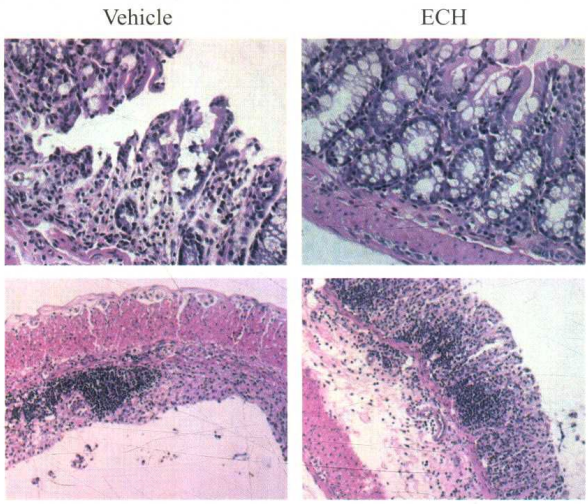

图 5-2 松果菊苷饲喂对肠道上皮组织损伤和浸润组织的影响

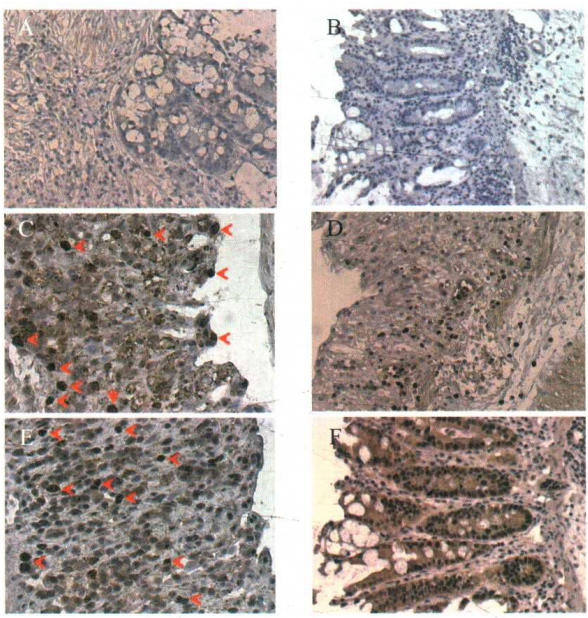

图 5-5 松果菊苷饲喂组小鼠和对照组小鼠的肠道组织切片中 MPO（A-C）和 Ki67（D-F）表达的差异

注：图为 400 倍显微镜下典型肠道组织切片，其中 A 为正常饮食小鼠的肠道组织切片；B 为 3% DSS 处理并饲喂正常饮水的小鼠肠道组织切片，经 MPO 抗体染色标记；C 为 3% DSS 处理并饲喂 ECH 的小鼠肠道组织切片，并经 MPO 抗体染色标记；D 为正常饮食小鼠的肠道组织切片；E 为 3% DSS 处理并饲喂正常饮水的小鼠肠道组织切片，经 Ki67 抗体染色标记；F 为 3% DSS 处理并饲喂 ECH 的小鼠肠道组织切片，并经 Ki67 抗体染色标记。

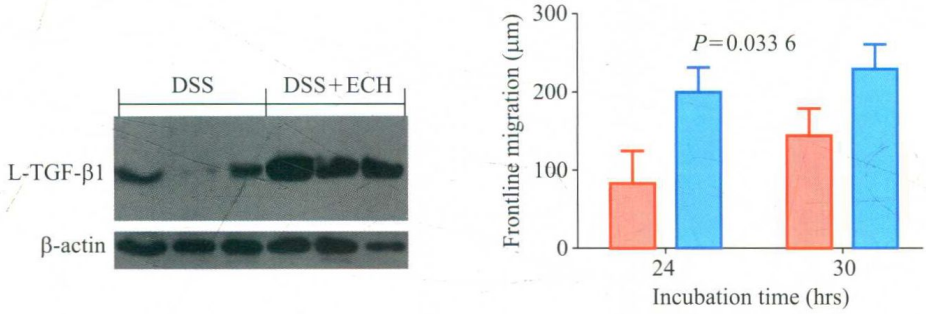

图 5-6　DSS 和松果菊苷处理对肠炎小鼠肠道组织中 TGF-β1 的表达的影响及松果菊苷处理对 MODE-K 细胞培养中刮痕修复能力的影响

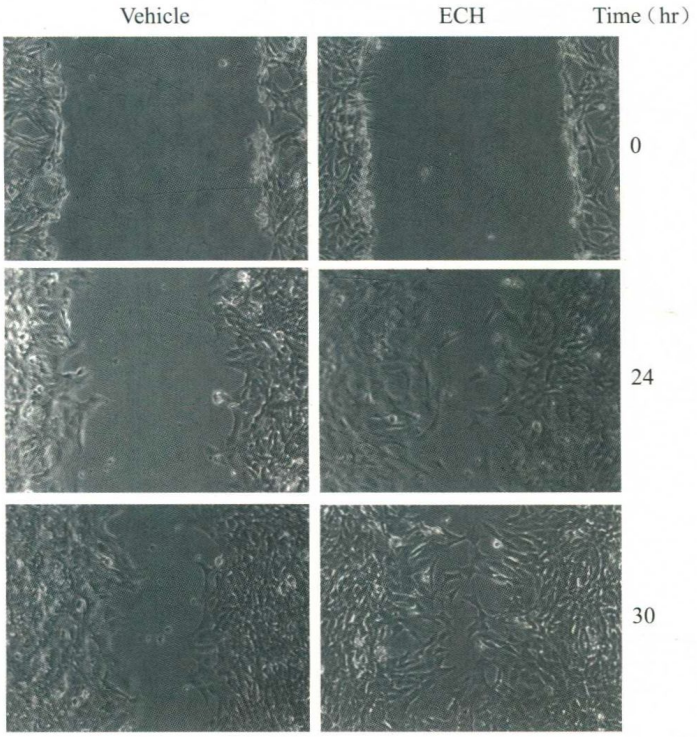

图 5-7　不同培养时间下松果菊苷对 MODE-K 细胞刮痕修复的变化

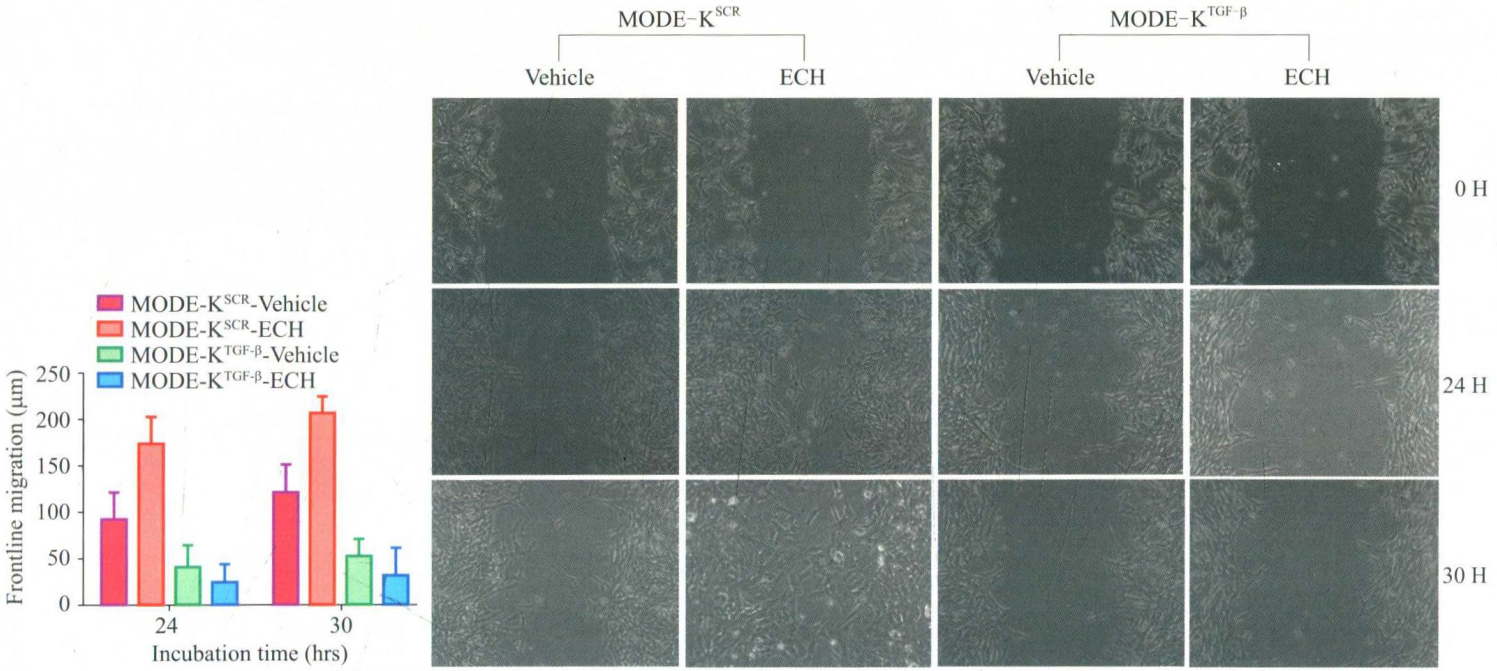

图 5-8　松果菊苷对肠道组织细胞培养过程中阻断 TGF-β1 表达细胞的刮痕修复

内容简介

大肠癌(Colorectal Cancer,简称 CRC)是结肠癌和直肠癌的总称。世界范围内每年有 100 万新增病例,是第三大类型恶性肿瘤,被列为继肺癌和支气管癌、男性前列腺癌、女性乳腺癌之后第三大常见致死肿瘤类型(American Cancer Society,2011)。其发生过程为大肠黏膜上皮在环境或遗传等多种致癌因素作用下发生恶性病变,常伴有扩散和转移。随着更多的研究揭示炎症与癌症之间的联系,结肠炎相关性大肠癌的研究将为揭示大肠癌的发生机制和药物开发治疗评价提供更多的信息途径。而以保护肠道黏膜结构的完整和确保肠道吸收消化功能,提高肠道黏膜自身的修复损伤功能以维持肠道黏膜的屏障功能的思路,也将更多的防病治病的中医理念和智慧融汇其中,将为大肠癌的预防和炎症性肠病(Inflammatory Bowel Disease,简称 IBD)的治疗提供新的依据。

中药肉苁蓉的化学成分研究成果显示,肉苁蓉含有挥发性物质、苯乙醇苷类、多糖类、木质素类、糖苷类等 120 多种化合物。药理学研究揭示,肉苁蓉能提高学习和记忆能力,可用于治疗阿尔茨海默等老年痴呆性疾病;可增强人体免疫力,有抗老化和抗衰老的功效;通过促进大肠蠕动,减少大肠对水分的吸收而改善胃肠道环境,有润肠通便的作用。自肉苁蓉中提取分离的松果菊苷(Echinacoside,缩写为 ECH)对于 H_2O_2 以及肿瘤坏死

因子-α(Tumor Necrosis Factor-α,简称 TNF-α)等细胞因子诱导的神经细胞凋亡有显著的抑制缓解作用。有关肉苁蓉中活性物质的化学结构及其生物活性的研究表明,肉苁蓉具有显著的抗氧化活性,这源自其丰富的酚羟基结构。而炎症性肠炎的发生伴随肠道表皮细胞的损伤及病原微生物的入侵,引起一系列异常免疫反应和氧化应激等反应过程。充分利用我国特色中药材,利用其中特有化学成分防治疾病,是开发炎症性肠病和大肠癌治疗药物的重要途径。

本书应用敲除转化生长因子-β1(TGF-β1)的易患肠癌模型小鼠,研究肉苁蓉水提取物对该模型的治疗作用;用松果菊苷处理 H_2O_2 以及 TNF-α 诱导的 MODE-K 细胞凋亡模型,检测该化合物对肠道表皮细胞的保护作用,进一步揭示保护机制;通过3%右旋葡聚糖硫酸钠(Dextran Sulphate Sodium,简称 DSS)诱导小鼠急性肠炎模型,评价松果菊苷对肠炎的治疗和缓解作用,并探讨松果菊苷的作用机理。具体研究内容和结果如下:

1. 应用 TGF-$\beta1^{+/-}$ $Rag2^{-/-}$ 易患肠癌小鼠模型,按照0.4 g/kg/d 肉苁蓉水提取物干燥粉末的用量饲喂小鼠3个月,发现肉苁蓉水提取物可以显著减少该小鼠肠道发生的与炎症相关的异型增生,减少肠道中幽门螺杆菌的感染;促进脾细胞重量的增加,但不改变脾细胞中 NK 细胞和巨噬细胞的比例;在体内和体外均能增强脾细胞的细胞毒性。

2. 应用培养小鼠巨噬细胞系 RAW264.7 细胞,添加 100 μg/mL 的肉苁蓉水提取物,结果发现肉苁蓉水提取物可以促进该细胞系 NO 的生成,且上调 iNOS 酶的表达;表明肉苁蓉水提取物具有增强巨噬细胞吞噬的能力。

3. 应用培养小鼠肠表皮细胞系MODE-K细胞，添加50 μg/mL的松果菊苷，结果发现添加松果菊苷可以促进细胞增殖并减少凋亡细胞比例；促进细胞TGF-β1蛋白的分泌，上调细胞水平TGF-β1的表达，并增加信使RNA水平的表达；可以减少H_2O_2以及TNF-α所诱导的细胞凋亡；通过上调TGF-β1表达的途径对H_2O_2以及TNF-α诱导的凋亡细胞产生保护作用。

4. 应用3% DSS诱导小鼠急性溃疡性肠炎，按照600 μg/kg松果菊苷/小鼠/天的标准饲喂小鼠，发现松果菊苷可以有效缓解DSS导致的急性溃疡性肠炎症状：体重降低幅度减小，不成形血便减少，小鼠的疾病活动指数降低；减少DSS对肠道表皮结构的损伤，可抑制肠炎发生时对结肠长度的缩短作用；减少结肠肠段中NO的生成；可以维护肠道黏膜结构的完整性，有助于表皮细胞的修复，减少炎症对腺窝和杯状细胞的损伤；增加肠道组织中Ki67阳性细胞的比例，表明可以通过增加表皮细胞的增殖能力缓解DSS对肠道的损伤，减少病原微生物的入侵；促进肠道中TGF-β1的表达。

5. 松果菊苷可以促进原代表皮细胞的增殖。通过细胞刮痕实验，验证添加松果菊苷后对MODE-K细胞刮痕的修复，结果表明松果菊苷对DSS诱导凋亡的MODE-K细胞有保护作用，在刮痕24 h后发挥作用。

本研究中首次发现肉苁蓉对炎症相关肠癌的治疗作用，松果菊苷是其中主要的活性物质；发现松果菊苷可以通过上调TGF-β1表达的方式促进表皮细胞增殖，缓解各种引起凋亡的因素对细胞的损伤；可以有效缓解3% DSS诱导的急性溃疡性肠炎小鼠的症状，可以通过促进肠道组织表皮细胞TGF-β1的

表达，维护肠道黏膜屏障的结构和功能。因此，可继续研究探讨松果菊苷对炎症性肠病和与炎症性肠病相关大肠癌的应用，揭示松果菊苷对细胞因子、趋化因子以及NK-KB等与炎症有关因素的研究，为进一步开发松果菊苷对炎症性肠病以及与炎症性肠病相关的大肠癌的预防和治疗提供更多的背景和依据。

INTRODUCTION

Colorectal cancer included the tumor generated in the colon (intestine) and rectal, ranked behind the lung cancer and bronchial cancer as the third most common cancer in men (the second is prostate cancer) and the third (the second is breast cancer) in women worldwide. There is about 1 million new cases every year (American Cancer Society, 2011). The epidemiological studies suggest that many risk factors associate with this disease, including all kinds of malignant lesions under the environmental and genetic factors, and accompany with diffusion and metastasis. As more and more research explored the connection between the inflammation and cancer, the studies on the colorectal associated cancer will provide more valuable information on the generation mechanism and drug development. Moreover, we aimed to protect the integrity of the colon mucous structure, further assure the function of the absorb ability and digestion, improve the intestine mucous self-restoration and maintain the barrier function of the colon mucous, which will combine the preventive and curative disease essence of traditional medicinal idea and wisdom, and provide new approaches to the preventation and treatment of the colorectal cancer and inflammatory bowel disease.

The research about the *Cistanche destericola*, which is known as

a famous Chinese herb medicine, suggested that there are some volatile component, phenylethanoid glycosides, polysaccharides, lignin and glycosides, with more than 120 kinds of compounds in it. The pharmacological studies showed that the *C. destericola* can improve the study and memory capacity, and treat the Alzheimer's disease, and other agedness disease; can reinforce the immunity and anti aging; can promote the bowel movement and reduce the moisture absorbing ability of the bowel and improve the environment of gastrointestinal tract. Echinacoside, which is a major component of the *C. deserticola*, can significantly protect the neurocyte cells from the H_2O_2 and TNF-α induced apoptosis. The correlative report on the chemistry structure and the bioactivity indicated that the specific anti-oxidation of the Cistanche based on the abundant phenolic hydroxyl structure. Because of the damage of intestine epithelial layer, the invation of pathogenic microorganisms generally induced a series of abnormal immunity reaction and oxidative stress as well as other processes during the inflammatory bowel disease.

This study applied the TGF-$\beta1^{+/-}$ $Rag2^{-/-}$ mice model which is prone to colon cancer, and evaluate the effect of Cistanche extract after the treatment of these mice; treated the apoptosis cell model induced by H_2O_2 and TNF-α on MODE-K cells with Echinacoside, and detected the protective effect to the colon epithelial cell line and studied more about the protection mechanism; induced mice acute colitis model by 3% DSS, treated these mice with ECH and tested the efficacy of ECH to ameliorate the colitis and explore the pathway of ECH. Here are the contents and results as follows:

1. By applying to the TGF-$\beta 1^{+/-}$ $Rag2^{-/-}$ mice model, and treating the mice with Cistanche extract on the concentration of 0.4 g/kg/day for 3 months, we found the Cistanche extract treatment can significantly reduce the hyperplasia associated with the inflammation, and decrease the infection of Helicobactor in the intestine; that the Cistanche extract treatment can increase the weight of the spleen, without changing the percentage of the NK cell and macrophage cell in the splenocytes; and that the Cistanche extract treatment can boost up the cytotoxicity of splenocytes *in vitro or in vivo*;

2. By applying the mouse macrophage cell line RAW264.7 cell as *in vitro* model, the addition of 100 μg/mL Cistanche extract can enhance the NO production and up regulate the nitric oxide synthase II expression and stimulate the phagocytosis in the cell culture model;

3. By applying the mouse epithelial cell line MODE-K cell as *in vitro* model, the addition of 50 μg/mL ECH can promote cell proliferation, deduce cell apoptosis, stimulate the TGF-β1 protein secretion, up regulate the TGF-β1 expression and enhance the mRNA expression. ECH can significantly stimulate cell proliferation and enhance cell survival by reducing cell apoptosis in the presence of H_2O_2 or the mixture of pro-inflammatory cytokines, while transforming growth factor expression was up regulated in a dose-dependent manner;

4. By applying the acute colitis mice model induced by 3% DSS, after 7 days of 600 μg/kg ECH treatment, we found the ECH suppressed the development of acute colitis, indicated by lowering disease activity index; and that ECH protected intestinal epithelium

from inflammatory injury but had less effect on inflammatory cellular infiltration. The beneficial effect of ECH treatment was associated with up-regulation of transforming growth factor as well as with an increase in the number of Ki-67^{+} proliferating cells in diseased colons;

5. In cultrued MODE-K cells, the addition of ECH enhanced *in vitro* wound healing that depended on TGF-β1 expression.

In conclusion, this study indicated that the oral administration of Cistanche extract reduces inflammatory hyperplastic polyps in the TGF-β1$^{+/-}$ Rag2$^{-/-}$ mice model for the first time, and further found the ECH is the major active component; that ECH was based on the up-regulation of TGF-β1 expression to play an important role in acceleration of epithelial cell proliferation, and ameliorated cell damage induced cell apoptosis by other factors; and that ECH could suppress the acute colitis in 3% DSS induced mice model, be associated with the up-regulation of TGF-β1 expression in the intestine epithelial cell, and maintain the structure and function of the colon mucous barrier.

Finally, we can do further research to discuss the application of ECH in the IBD and colon cancer associated with IBD, so that we can explore the related cytokines factors, chemokine factors, NK-KB and other factors associated with the inflammation, and it will be useful to imply the potential of ECH or its derivatives for clinically treating IBD and colorectal associated cancer.