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论著

豹皮樟总黄酮体外对酒精性脂肪肝大鼠肝细胞脂肪变性的影响

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摘要 目的 探讨豹皮樟总黄酮(TFLC)对酒精性脂肪肝(AFL)大鼠肝细胞脂肪变性的影响。方法 大鼠自由饮用含乙醇饮料,起始浓度为5%,依次增加为10%, 15%, 20%, 25%, 30%和35%,每个浓度持续1周,第8周~第12周浓度为40%的方法制备大鼠AFL模型,原位二步灌流法分离AFL大鼠肝细胞,用细胞角蛋白18(CK18)抗体进行肝细胞鉴定。肝细胞培养16 h后加入TFLC 1,10和100 mg·L^{-1}或谷胱甘肽(GSH)10 mmol·L^{-1}孵育48 h。MTT法测定肝细胞存活,用试剂盒方法检测肝细胞丙氨酸转氨酶(ALT)和天冬氨酸转氨酶(AST)活性以及肝细胞内甘油三酯(TG)含量,免疫组化和逆转录PCR方法测定脂肪分化相关蛋白(ADRP)、过氧化物酶体增殖物激活受体γ(PPARγ)mRNA和蛋白表达的变化。结果 TFLC对正常大鼠肝细胞存活无明显影响。TFLC 100 mg·L^{-1}明显增加AFL大鼠肝细胞存活(P<0.05)。AFL大鼠肝细胞培养液ALT和AST活性分别为(63±12)和(74±19)U·L^{-1},TFLC 10和100 mg·L^{-1}分别将ALT活性降低到(42±12)和(44±8)U·L^{-1}(P<0.05),将AST活性降低到(46±14)和(47±8)U·L^{-1}(P<0.05)。AFL大鼠肝细胞内TG含量为(2.3±0.6)mmol·L^{-1},TFLC 10和100 mg·L^{-1}分别将AFL肝细胞内TG含量降低到(1.4±0.3)和(1.5±0.5)mmol·L^{-1}(P<0.05)。AFL肝细胞ADRP和PPARγ阳性表达细胞分别为(41±6)%和(37±10)%,TFLC 100 mg·L^{-1}治疗组ADRP和PPARγ阳性表达细胞分别降低为(26±6)%(P<0.01)和(25±5)%(P<0.05)。结论 TFLC可能通过改善肝细胞活性、减少肝细胞内TG生成、抑制ADRP表达及调控肝细胞PPARγ的表达,从而改善AFL大鼠肝细胞的脂肪变性。

关键词 脂肪肝,酒精性 豹皮樟总黄酮 肝细胞 脂肪分化相关蛋白 过氧化物酶体增殖物激活受体γ

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Effect of total flavonoids from *Litsea* coreana Leve on steatotic hepatocytes in rats with alcoholic fatty liver *in vitro*

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Abstract

OBJECTIVE To evaluate the effect of total flavonoid from *Litsea coreana* Leve (TFLC) on steatotic hepatocytes in rats with alcoholic fatty liver (AFL). **METHODS** A model of AFL in rats was established by intaking different doses of alcohol (concentration from 5% to 40%) over 12 weeks: 5% (*V/V*) for first week, then 10% alcohol for another week, and each week the alcohol concentration increased by 5% and finally 40% ethanol for four more weeks. Hepatocytes were isolated from rats by a modified two-step collagenase perfusion technique *in situ* and identified by cytokeratin 18 antibody. After 16 h of incubation, TFLC 1, 10 and 100 mg·L^{-1} or glutathione (GSH) 10 mmol·L^{-1} were added into the culture supernatant, respectively. The cell survival was determined with MTT assay. The activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the culture supernatant and contents of triglycerides (TG) in hepatocytes were measured using the kits. The expression of adipose differentiation-related protein (ADRP) and peroxisome proliferator-activated receptor γ(PPARγ) was determined by immunochemistry and RT-PCR, respectively. **RESULTS** TFLC could significantly improve the survival of AFL rat hepatocytes (P<0.05), but had no effect on the survival of normal hepatocytes. The activity of ALT and AST in AFL hepatocytes culture supernatant was (63±12) and (74±19) U·L^{-1}, and TFLC 10 and 100 mg·L^{-1} treatment markedly reduced the activity of ALT to 42±12 and (44 ±8)U·L^{-1} (P<0.05) , and AST to 46±14 and (47±8)U·L^{-1}(P<0.05), respectively. The content of TG in AFL rat hepatocytes was (2.3±0.6)mmol·L^{-1} and decreased to 1.4±0.3 and (1.5±0.5)mmol·L^{-1}(P<0.05) in TFLC 10 and 100 mg·L^{-1} treated groups. Furthermore, the expression of ADRP and PPARγ mRNA was significantly decreased in TFLC treated groups. The percentage of ADRP and PPARγ positive cells was (41±6)% and (37±10)% in AFL model group, which was also significantly decreased in TFLC 100 mg·L^{-1} treated groups to (26±6)% (P<0.01) and(25±5)%(P<0.05), respectively. **CONCLUSION** TFLC has a protective effect on steatotic hepatocytes from AFL rats. The mechanism may involve the inhibition of synthesis of TG by regulating the expression of ADRP and PPARγ.

Key words fatty liver alcoholic total flavonoids from *Litsea coreana* L. hepatocytes adipose

differentiation-related protein peroxisome proliferator-activated receptorγ

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