以前胡抽出物誘發大鼠脂肪細胞進行脂質分解之研究

Studies of Peucedanum praeruptorum Dunn Extract Induced Lipolysis in Rat Adipocytes

中文摘要

前胡(Peucedanum praeruptorum Dunn)爲繖形科(Umbelliferae)植物，具有降氣化痰、散風清熱的功效，主產於中國安徽、浙江、湖南、四川等地區。本研究之初篩試驗中，於前胡乾燥根部的甲醇抽出物顯示具有顯著誘發脂質分解的能力，因此本實驗的目的是自前胡乾燥根中，分離出具有相關活性的天然抽出物。

將前胡乾燥根的甲醇抽出物分別以正己烷、乙酸乙酯及水進行部份劃分，分別得到正己烷層抽出物、乙酸乙酯層抽出物及水層抽出物。再將最具脂質分解活性的正己烷層抽出物以 silica gel 層析管柱進行分離，劃分爲 Fr1~Fr24。其中將活性效果爲正己烷層 1.77 倍的分劃層 Fr22 以 C18 逆向層析管柱，由 10% 至 100% 甲醇濃度梯度沖提進行分離，獲得 Fr22-1~Fr22-9。取其中生物活性與 Fr22 相近的 Fr22-4 進一步以 C18 逆向高效能層析儀進行分離，分爲七個次分劃層 Fr22-4-3-1~ Fr22-4-3-7；而生物活性爲 Fr22 之 1.22 倍的 Fr22-7 則經由 Sephadex LH-20 層析管柱分離爲 Fr22-7-1~ Fr22-7-5。將最具活性的 Fr22-7-1 進而以 C18 逆向高效能層析儀分離，可得 Fr22-7-1-1~Fr22-7-1-4。

將各層抽出物進行誘發脂質分解測試，並以偵測大鼠脂肪細胞的甘油釋放量評估脂質分解程度。結果顯示以 Fr22-4-3-2 及 Fr22-7-1-3 抽出物活性分別爲空白對照組的 3.58 倍及 3.81 倍，爲最具活性效果的分劃層。而以 PKA、PKC 及 PKG 抑制劑分別處理脂肪細胞，結果發現 PKA 抑制劑(0.1 mM H89)具有顯著抑制前胡抽出物所誘導的脂質分解能力，故推測前胡抽出物是經由 PKA 的訊息傳遞路徑達到脂質分解的作用。

英文摘要

Peucedanum praeruptorurn Dunn (Umbelliferae) has been used for treatment of respiratory disease. Our preliminary lipolysis screening showed that the methanol extract of root tissues of P. praeruptorum Dunn exhibits high activity. Therefore, this study aims to identify the active fractions from the bioactive extract.

Three fractions, n-hexane extract, ethyl acetate extract, and aqueous extract, were obtained from sequentially partitioning fractionation of the methanol crude extract. Among these extracts, n-hexane extract contained ingredients with the most effective lipolysis potential, was followed by fractionation by silica gel column chromatography using gradient elution solvent: n-hexane to ethyl acetate, acetone and methanol respectively, collecting twenty four subfractions (Fr1~Fr24). The Fr22 subfraction with a 1.77-fold activity larger than n-hexane extract, was further purified

by RP-C18 column with 10% to 100% methanol gradient elution, and nine subfractions Fr22-1~Fr22-9 were obtained. The Fr22-4 subfraction, with nearly same activity as Fr22 subfraction, being applied in C18 RP-HPLC column followed by acetonitrile gradient separation. The Fr22-7 subfraction with a 1.22-fold activity larger than Fr22 subfraction, was further isolated by chromatography on Sephadex LH-20 by ethanol elution, and five subfractions Fr22-7-1~ Fr22-7-5 were collected. Among these subfractions, Fr22-7-1 showed the highest lipolysis activity comparison with others subfractions. Then Fr22-7-1 subtraction was applied onto a C18 RP-HPLC column, and four subfractions Fr22-7-1-1~Fr22-7-1-4 were obtained. Finally, the lipolysis activities and pathway of these fractions were evaluated. The lipolysis assay in the study was performed using primary adipocyte isolated from rat adipocyte tissue. The release of glycerol from rat adipocytes incubated with plant extracts were quantified as used as the criterion of lipolysis efficiency. Our results showed that markedly activity existed in the fractions of Fr22-4-3-2 (with a 3.58-fold activity larger than control) and Fr22-7-1-3 (with a 3.81-fold activity larger than control). And the extracts lipolysis effect was inhibited by 0.1 mM PKA inhibitor H89, but not by PKC and PKG inhibitors, respectively. That mean the P. praeruptorum Dunn extract directly stimulates lipolysis in rat adipocytes through activation of PKA pathway.