靈芝屬三帖類成分之分佈模式與對肝功能及 HL-60 細胞之影響

Distribution and pattern of Triterpenoids in the Genus Ganoderma and the Physiological Activities on Hepato-protection and HL-60 Cell Line

中文摘要

靈芝屬內已命名者超過 50 種，其形態類似，不易以簡單方法鑑別之。本論文嘗試以簡便快速之 HPLC 及 TLC 方法分析源自於 CCRC 及農試所等不同國家之靈芝標本約 64 個；其學名分別爲：G. neo-japonicum, G. formosanum, G. australe, G. calidophilum, G. mastopolvm, G. webenanum, G. pfeifferi, G. resinaceum, G. lucidum, G. subamboiaefise var. laevisponim, G. boniense, G. tropicum, G. fornicatum, G. tsugae, G. curtisli, G. lobatum, G. mirabile, G. oerstedii 由其酒精萃取物中，以靈芝酸 B 及 C2 爲標準品，歸納成爲 18 種類型，此 18 種類型與臺灣省農試所值病系之形態及雜交可孕性試驗吻合。同時由於靈芝酸 B,C2 爲已知具肝臟保護作用之三帖類純化成分，利用 HPLC 分析而得知各種類型靈芝中靈芝酸 B,C2 含量情形；並以四氯化碳誘發小白鼠急性肝障礙之模式，以 GOT,GPT 及組織切片評估各種類型靈芝三帖類排除急性肝障礙之效果；結果顯示，相同劑量(30mg/kg 老鼠體重)之含三帖類萃取物投與小白鼠 3 劑後，以 G. tropicum 排除急性肝障礙效果最佳。另外，以人類急性前骨髓白血病細胞株(HL-60 Cell)爲體外治療血癌之模式，了解各種類型靈芝三帖類對於 HL-60 細胞分化作用及分裂作用之影響；在分化方面，進行血球功能特性包括吞噬作用，NBT 還原作用之評估，細胞表面抗原之偵測，及細胞形態之觀察；在分裂方面，測試靈芝三帖類對於細胞數，細胞存活率，增殖能力之影響，同時了解靈芝三帖類對於 HHL-60 細胞之細胞週期(Cell Cycle)之影響。首先利用流動細胞分析儀(Flow Cytometer)篩檢以 18 種類型靈芝酒精萃取物 50 μg/ml，0.5 μg/ml，0.005 μg/ml 處理 5 天後之細胞吞噬能力，同時以 NBT 還原作用確定各種類型靈芝酒精萃取物 50 μg/ml,5 μg/ml,0.5 μg/ml,0.5 μg/ml 處理 5 天後細胞分化情形；發現 G. weberianum (F, 50 μg/ml),G. lucidum (I,50 μg/ml), G. lucidum (K, 50 μg/ml), G. tsugae (O, 50 μg/ml),G.tsugae(P, 50 μg/ml), G. lobatum(Q, 5 μg/ml, 0.5 μg/ml),G. mirabile(R, 5 μg/ml,0.5 μg/ml)與未處理之細胞比較有顯著差異；再利用流動細胞分析儀偵測細胞表面 CD 標記 CD11b,CD14 百分比，以得知分化成熟之細胞百分比，結果顯示 G. weberianum(F, 50 μg/ml) 具有輕微促進 HL-60 細胞分化爲顆粒性白血球之效果($p<0.05$)：而 G. lucidum(K,50 μg/ml)，G. tsugae(0,50 μg/ml), G. lobatum (Q, 5 μg/ml, 0.5 μg/ml)具有輕微促進 HL-60 細胞分化爲單核球之效果($p<0.05$)。此外，G.resinaceum (G, 50 μg/ml), G. mirabile (R, 50 μg/ml)顯著降低細胞存

活率、增殖能力及細胞數目。探討 G. resinaceum(G, 50 μg/ml), G. lucidum (K, 50 μg/ml), G. tropicum (M, 50 μg/ml)對於 HL-60 細胞週期之影響，亦發現某些靈芝含三帖類酒精萃取物似乎其有細胞毒性 (Cytotoxic activity)。

英文摘要

To distinguish among the known over 50 recorded species in the genus Ganodenna by morphological characters of either mycelia or fruiting bodies usually leads to an ambigous result. In the present study, 64 strains of Ganoderma from Culture Collection and Research Center (CCRC) and the fruiting bodies cultivated by Taiwan Agricultural Research Institute (TARI) were used for analysis by HPLC and TLC. The taxon of the strains include: G. neo-japonicum, G. formosanum, G. australe, G. calidophilum, G. mastoporum, G. weberianum, G. pfeifferi, G. resinaceum, G. lucidum, G. subamboinense var. laevisporum, G. boniense, G. tropicum, G. fornicatum, G. tsugae, G. curtisii, G. lobatum, G. mirabile, and G. oerstedii. Ethanol extracts of these fruiting bodies were used for the analysis and ganoderic acid B and ganoderic acid C2, both known as hepato-protective triterpenoids, were employed as external standards for the analysis. From the patterns of HPLC and TLC, 18 groups (A to R) were classified with the contents of ganoderic acids B and C2 determined in each group and the result was in good agreement to that from inter-fertility test carried out by TARI.

Carbon tetrachloride (CCl4) induced liver damage in mice was used as an animal model to evaluate hepato-protective effect of the three doses of the 18 ethanolic extracts (total 30 mg/kg of mouse) orally administrated with an interval of 4 hours. It indicated that the extract of M group (G. tropicum) manifested the strongest effect by lowering SGOT and SGPT values and also showed prominent repair effect to the hepatocytes around central veins. HL-60 cell line, an acute leukemic cell culture was employed as an in vitro model for the effect of the triterpenoid containing extracts on cell differentiation and proliferation. The cell behaviours, including phagocytosis, NBT reduction, surface marker, change in morphology, cell growth curve, survival rate, cell proliferation and cell cycle of HL-60 , with retinoic add as positive control, were observed or analyzed by microscopy or flow cytometry. It revealed that in the treatment of G. weberianum (F, 50 μg/ml), G. lucidum (I, 50 κg/ml) G. tsugae (O, 50 κg/ml), G. tsugae (P, 50κg/ml), G. lobatum (Q, 0.5 &: 5 κg/ml) and G. mirabile (R, 0.5 & 5 κg/ml) showed significant enhancement of phagocytosis and NBT reduction. Further study on expression of myeloid-spedfic differentiate antigens (CD11b and CD14) indicated the extract of G. weberianum (F, 50 κg/ml) slightly induced HL-60 cells to differentiate to mature granulocyte and G. lucidum (K, 50 μg/ml), G. tsugae (O, 50 μg/ml), G. lobatum (Q, 0.5 & 5 μg/ml) also

mildly ($p<0.05$) induced HL-60 cell to differentiate to monocytes. In addition, the extracts of G. resinaceum (G, 50 µg/ml) and G. mirabile (R, 50 µg/ml) strongly suppressed the survival rate and proliferation. The extracts of G. resinaceum (G, 50 µg/ml), G. lucidum (K, 50 µg/ml) and G. tropicum (M, 50 µg/ml) severely changed cell behaviour during the stages of cell cycle. Certain compounds in the crude extracts are proposed to be responsible for cytotoxicity to the cell line.