ƒ ‹ F | Œfia‹ ˙'e›ß• |–M^D‹ ª¤s›p e ƒ¤"G‡ł§i

†o⁄ß⁄l⁄§§ ø‹ ¡'°ƒ¤⁄ ‹ ª¤s

›p e^ §¡G §O«‹ ›p e

›p e‰ ¸s„¡GNSC91-2320-B-038-042-

ıƒ · ¶¡¡G91 ƒ~08 ⁄°01 ⁄ Ø 92 ƒ~07 ⁄°31 ⁄Ø

ıƒ ‡ ƒ ¡G» O ¥´ ˙⁄j ˙¥ ˝ ^ § ‡N¤t

›p e¥ D ø⁄¡HG⁄ R^£

‡ł§i^ «‹¡G" °†‡ł§i

‡ B † zŁ¡fG¡¥»›p e¥ i ⁄ ‰ ¶‹ d,

⁄⁄ ¥` Œ 92 ƒ~11 ⁄°24 ⁄Ø

ƒ ‹ F | Œfia‹ ˙'e›ß• |, §U–M^D‹ ª¤s›p eƒ¤"G‡ł§i

¡ ¡ ¡ ¡ ¡ ¡ ¡ ¡ ¡ ¡ ¡ ¡ ¡¡ ¡ ¡ ¡

¡ †o⁄ß⁄l⁄§§ ø‹ ¡'°ƒ¤⁄ ‹ ª¤s ¡

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ıƒ · ¶¡¡G91 ƒ~08 ⁄°01 ⁄ ø 92 ƒ~07 ⁄°31 ⁄Ø

›p e¥ D ø⁄¡H⁄ R ^£

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¥»ƒ¤"G‡ł§i¥] ‹ A ¥ H Ʊ ⁄‡§"'¥ ¡G

¡… ›u Œ¥ ~ ¥ Xt‹'ª¸† ⁄–†ł§i⁄ ¥

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Abstract

Colorectal carcinoma is one of the leading causes of cancer deaths in Taiwan. Several previous studies demonstrated chemotherapy for colorectal carcinoma caused resistance and side effects on patients. Therefore, the new anticolorectal tumor drugs are going to develop from Chinese herbs. In this study, the cytotoxicity effects of the 50% ethanol extracts of Chinese herbs were screened by MTT assay in human colon adenocarcinoma cell lines (CoLo 205, HT-29). The cytotoxicity effects of 50% EtOH extracted-Pharbitidis Semen Atrum were exerted stronger in CoLo 205 cells than Chang normal liver cell (CNL). The cytotoxic principles of semen were isolated by bioassay-guided fractionation. The semen were extracted with methanol and chromatographed over Diaion HP-20 column with 70% aqueous acetone Moreover, the cytotoxicity effect of 70% acetone fraction (D-70A) showed stronger than the other fractions in CoLo 205 cells and IC_{50} was 17.38 μg/ml for 48h. Furthermore, the cytotoxic mechanisms of D-70A were measured by agarose gel electrophoresis, fluorescence flow- cytometry, Giemsa stain, Western blot analysis. The results showed that D-70A could induce apoptosis in CoLo 205 dells. Compound 1 was isolated from D-70A with RP-18 column chromatography. The IC_{50} value of compound 1 was 20.36 μg/ml and induced apoptosis in CoLo205 cells for 48h. Moreover, treatment with catalase prevented compound 1-induced cytotoxicity. In according to the results, we suggested that compound 1 might produce free radicals and induce apoptosis in CoLo 205 cells. However, the cytotoxic effect of bibberellin was lesser than D-70A in CoLo 205 cells. Therefore, we suggested the D-70A fraction extract is good for antitumor effects. In the future, compound 1 will be a biosubstance to control the quality of D-70A.

Keywords: Cytotoxicity, Apoptosis, Colorectal carcinoma, CoLo 205 cell, HT-29 cell, Pharbitidis Semen

† › M < r ' ° ¡ A † " G ¯ ª ¥ ¶ F ⁄ l ¡ B ¥ ° o ¡ B † o ⁄ ß ⁄ l „ ø ColoM¤ª ƒ †, ß–
205 " ‰z ø † M‹ ‡r'°j¡ A ¥ ¿ ¯¤k ›M (Chang Liver) † ⁄ p¡A¿ «...2<3ø›¿¡A¥B„ " ‰z
ø † ›MColo 205 ¤ ƒ ‡fi ¶ ¡ ⁄ ¸ fl¶q¤ªSSs ¤ S@¥a¸ ^ • »⁄¤ fi ‰ Ł k` DNA ⁄ ø ‹ q£¡¥´
¤ˇ¤ › ⁄ ' (apoptosis)¡A‹ G ¥ »Œ¿¯ † o ß⁄¶ i ƒS ø‹ ¡'°ƒ ¤ ⁄ ⁄´ ¡C

†"G»P Q‰

1.‹ ¡'°ƒ¤⁄ l´ ⁄ ´ [2-8]

¥ » ‹ ª ¤ s › " ¥ ¥ H ⁄ £ ƒ P ƒ ‡ • » · C œ †‹o¡'ß°⁄l¸¡A¶œƒ¡ S ¤ o"‰,‡z ø‹† ›Mœ"‹
S ¤ ø † › M ¥ ´ " Ł S @ ¥ ¸ ‡ – j ¡ A ‹ G – N ¥ H ⁄ ‹ ‹ •»'ƒ¸¶ i ƒ¯¡A ¤œ¡AƒA¥
ƒ ‡ ¤ t¸"I´ ⁄ ´ † o ⁄ ß ⁄ ŁSSs¡'°ø¤⁄ ¡C
¤ œ ⁄ h¡(AHEO¡ 20% MeOH¡ 40%MeOH¡ 60% MeOH¡ 80% MeOH¡ 70% Acetone¡
Acetone)¤ R£Diaion HP-20 " ‹ W¶ ƒ †°⁄ ¡A¤ ⁄⁄70% Acetone ¤ R£"«¤ª ƒ ‡ ‡ – j ⁄ S S ¤ S@¥¸¡AƒA
¥ H ¡B5¢M~100¢M¥ J⁄⁄¸ ‹¤ R£MCI CHP20P " ‹ W¡A¤ ⁄⁄95%¥ J¤ R£" ¥H$CH_3CN:H_2O$ =
80:20 ¤ R£RP-18 " ‹ W¶Al ´ ⁄ ˇ e¤ƒ X"«1¡ A ¤¥BButanol-Acetic acid-H_2O ⁄ ¸$CHCl_3$-MeOH- H_2O
¤ › ⁄ ƒP" "" † °‹¡ AHTLC ⁄ " R ‰'wfl´"«‰Ł¡A¥ « ¥¿¥S› ⁄ , Œ fi ˜ †SO ‡c¡C

2.„"⁄ ‡¡⁄¸‹ ¡'°ƒ¤⁄ ‹ ¡'° ¸·œ

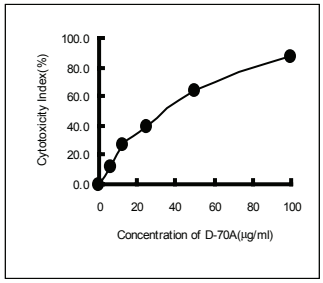
(1)¶ ´ † o ⁄ ß ⁄ l ¤ œ »⁄ † ›Mƒ"⁄'

¶ ´ † o ß⁄12.5 ~ 100 μg/ml ⁄ S O ¥⁄CoLo 205 † ›M¡ A ' ⁄ ƒP¤œ¶X¥HMTT
"k⁄ " R † ›M‹ r'°¡ C "Gfl2⁄pfi⁄"¡A¶ ´ † o ⁄ ß Ł „ ¤œ"M" " S ¤ ¤ ^ ŁŁ‡•N,q""fit
SO¡Aƒ ƒb"‡fi ¶¡U(24~48⁄pfi)« h S e † { @«' ª"ƒs«Y (dose-dependent) ""^ «Y¡C
^˜"«‡ Bz† ›M«Æ¡AS ¥¸ Giemsa ‹ ¥ "k¡ A ¥ œ ·ª•ŁŁ¸ ¡A o†{¤ † ›M⁄ S ¥ƒ
‰ Ł ƒ¥¿@` Y"" † {¶H(chromatin condensation)¡ F CoLo 205 † M¥H2.5 ~ 100 μg/ml ¿ @ ‡B
†z¡A i24⁄pfi «ÆAS ¥¸^ £ ‰ ƒq""a"⁄ "RAfbUV ¥œU˜ ¸¡A¥ i ‹ ¤† ›M⁄SDNA ladders
""† ¥˝¡AS Y† ›Mƒ‡DNA fragmentation ""† {¶H¡Ef A ´ · ¥‹ ‡y›f›M» ""˜ [·œ¥i¥H o†{¥ 12.5
~ 100 μg/ml ¿ @ ‡ Bz„L24⁄pfi ' ƒ¶¤ "" † ›M¡A¤ DNA ""⁄ SGfbG$_1$ phase «ef‡« 'œ
¯ª"Sub-G$_1$ peak † ¥˝¡Aƒ ¥BS e † { ¿ @ ¤° (Dose-dependence) ""^ «Y¡Aƒ]ƒ – ‰ ¶ fo
⁄ ß ⁄ ›⁄" ‰ z ‡ › M""⁄"„L { ⁄ •|ƒ‡¤ DNA ‡ Q ›‡ƒ¸¤´ _¡A– ‰ ¶ ´ † øß„lCoLo
205 † ›M¤ª ƒ‡† ›M‹ r'°¡A¥B» ⁄ " ‰ z ‡ › M¤ ¤V† ›M› ⁄ ' (apoptosis)¡C
¡]2¡^¥HDiaion " ‹ W⁄ ´ –o¤ ⁄S70% Acetone fraction¡]D-70A¡^» ⁄ † ›Mƒ"⁄'

70 % Acetone ¤ R£Diaion " W–oS¤ R£"«(D-70A)¥H6.25 ~ 100
μg/ml ⁄ S ¿ @ « ⁄SOLoJ205 † › M A 24 i⁄ p fi«ÆA¥HMTT "k⁄ "R† ›M‹ r ' ¡C¥i
– o ¤ S¤⁄ ƒS⁄ ¤Q† ›M¥ "Ł¿ @ ‹ 35.11μg/ml¡C

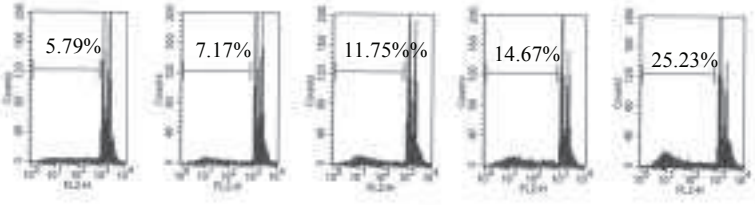
• CoLo 205 † › M[‡100μg/ml ¿ @ «⁄SD-70A i24⁄ p fi « Æ ¡ A ¥ ¥ Mœ ·¯ª•L^ƒ
„ ¤ † ›MSS¯"A ⁄ ˜ AƒA¤Giemsa stain ‹ ¥ « ÆAS Q ¥ ¥¿ƒV ª • L Ł˜¡[C †"Gª¥ ƒ††
›M» E ¶ ¸⁄¤p¯'Ø † £ ¥ "" ¡† A¶H¥gGiemsa ‹ ¥ «Æ« h ¥¤Ł ›M‹ ¥ ‰Ł¿@` Y" " † ¶H¡C

S ¥¸‹ ¥ƒ¡† ›M» "" ⁄ "RAi¥H o†{CoLo 205 † ›M⁄ S O ¥⁄6.25 ~ 50μg/ml ⁄SD-70A,
i12⁄¸24⁄ p fi « Æ A¤⁄SDNA ⁄ SƒSub-G$_1$ peak(M1)" ƒ¥¡A¥ B H @« ""…W
¥ [S† Dose-dependence ""– S¸(ƒ p˜1,2)¡C

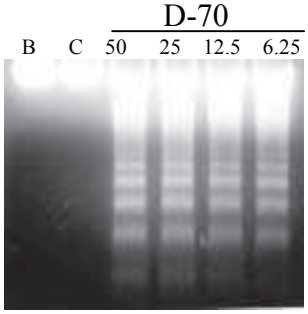


„˜1. ¶ ´ † øßÐ70A⁄ _CoLo 205 † › M ⁄ S ‹ r ' ° » ₽@« ""^ «Y¡C

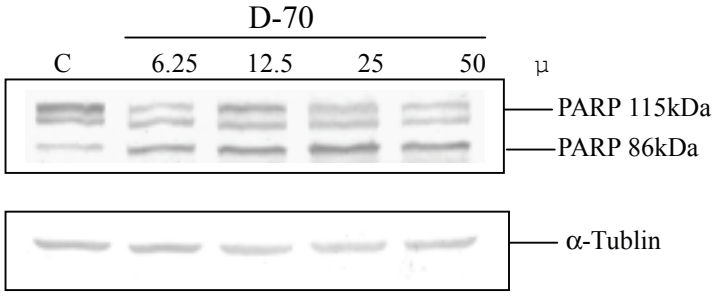
„˜2. ¶ ´ † øß-D-70A „ CoLo 205 † M⁄SDNA ⁄ S G » P ¿ @ ^ «Y¡C
¥H6.25 ~ 50μg/ml D-70A † BzCoLo 205 † M¡GD-70A † Bz12 ⁄ fi



CoLo205 † › M ⁄ S O¥ 6.25 J 50μg/ml ⁄ S ¶ ´ ϕ ⁄ D-70A i24 ⁄ p fi « Æ¡A CoLo 205 † ›M⁄S DNA ´ _ ^ ⁄ øq¡A¥B ¤ ª ƒ † ¿ @ «¤ ƒs'°¡C



„ˇ3. ¶ ´ † øßÐ 70A » ⁄ CoLo 205 † ›M⁄S DNA ´ ^ S ¥¿C



„ˇ4. D-70A ¥H Western blotting ⁄ Ł " k» PARP ⁄S¯ ⁄ ˘¡SC

• CoLo205 † ›M¥H 6.25 ~ 50μg/ml ⁄S D-70A i24 ⁄ p fi Æ¡A Western-blot ⁄S⁄Ł " k » · œ PARP " †{⁄S– S ¿¡A ϕ{ D-70A • | ¥ƒÐ PARP ‡ Q ›‡t¸{¶H¡Aƒ † £ ¥‹«¡Æ˘ ⁄S PARP (85kDa ⁄S† J ¥ Ł⁄ϕA¥qB H ¿ @ «"†"{¿W¥[«e ¤ ƒs–' S"¿"A¯ ¥ D-70A • |» ⁄ CoLo 205 † › M ¶ i ƒ› ⁄'¡C

• CoLo205 † ›M¥H 6.25 ~ 50μg/ml ⁄S D-70A i24 ⁄ p fi Æ¡A Western-blot ⁄S⁄Ł " k » · œ PARP " †{⁄S– S ¿¡A ϕ{ D-70A • | ¥ƒÐ PARP ‡ Q ›‡t¸{¶H¡Aƒ † £ ¥‹«¡Æ˘ ⁄S PARP (85kDa ⁄S† J ¥ Ł⁄ϕA¥qB H ¿ @ «"†"{¿W¥[«e ¤ ƒs–' S"¿"A¯ ¥ D-70A • |» ⁄ CoLo 205 † › M ¶ i ƒ› ⁄'¡C

(3)Compound 1 » ⁄ † ›Mƒ"⁄'

Compound 1 ¥H 3.13 ~ 100 μg/ml ¿ @ ⁄ S ΦB¢ CoLo 205 † ›M¡A' £ƒÐ ¶ ¡ œ¥X ÆH MTT "k⁄ "R† ›M‹r'°¡C Compound 1 S ¥¸24 ⁄ p fi «Æ¡A CoLo 205 † ›M¤ ª ƒ'œª⁄S† ›M ‹r'°¡Aƒ b S Cfl¶q 25 μg/ml ¥H·U⁄S¿ @)¡A H S ¥¿fi ¶¡… W[¡A‹r'°¥ • | H⁄S⁄ W ¡C¥ † " Go compound 1 ƒb 24, 48, 72 ⁄ p fi ¶¡´I¡AS ¤ ‰, ‡z ›Mƒ°⁄ ⁄S⁄ ›Q† ›M¥˝"Ł⁄S ¿ @ ⁄ S O‹ ¡A 61.27£g/ml, 20.36£g/ml, 13.88£g/ml ¡C

CoLo 205 † ›M⁄ S O¥ [⁄J 12.5 ~ 100μg/ml ⁄S compound 1 i48 ⁄ p fi « Æ¡A CoLo 205 † ›M⁄S DNA ´ _ ^ ⁄ øq¡A¥B ¤ ª ƒ † ¿ @ «¤ ƒs'°¡C

• CoLo 205 † ›M¥H compound 1 50μg/ml † B † z ¡ A ƒ P fi ¥ {J catalase « ⁄S (500~2000 U/ml) ¡A 24 ⁄ p fi « Æ S¥ MTT " k ⁄ "R catalase „ compound 1 ⁄ _ CoLo 205 † › M ‹ r ' ° " " … v T ¡C catalase fl † { ƒ † fi S compound 1 ' » ⁄S† › M ‹r'°¡C

(4) ‰

S ¥¸† › M r'°I ⁄ ´ ‹ ¡'°ƒ ¥ "" ⁄ Ł ¡¡A¥H (H_2O ¡ 20% MeOH ¡ 40% MeOH ¡ 60% MeOH ¡ 80% MeOH ¡ 70% Acetone ¡ Acetone) ¤ R£ Diaion HP-20 " ‹ W¡A¶ i ƒ† ⁄ ¡A¤ ⁄ ⁄ 70% Acetone ¤ R£ "« (D-70A) „ CoLo 205 † › M r† °–j¡AƒA¥H H_2O ¡B 5¢M~ 100¢M MeOH ¤ R£ ⁄ ¸ Acetone ¤ R£ MCI CHP20P " ‹ W¡A ¶G¯ ¥ 95% MeOH ¤ R£ "« S ¤ ø† ›M S @¥ ¸† –j¡AƒA ¥H $CH_3CN:H_2O$ = 80:20 ¤ R£ RP-18 column, fl ´ ⁄ ˘ o compound 1 ¡A¤ † £ ‡‹ 0.0014% ¡C

fi ‹ ¡'° I ⁄ ´ – ‰ ⁄S compound 1 „ CoLo 205 † ›M⁄S ‡°⁄æ D-70A ¤œ "« fit¡A¥B D-70A ⁄æ¥ „.g· £¤œ⁄S MeOH ¤œ "« S @¥ ¸–j¡A¯ ¥ ^ S , g† ¡⁄ ⁄ ´ fl ⁄˘⁄S† £ "« ¡A† ƒ v "¡B'° ‰ Ł ›'¡Afpfl S ¤ « …–ƒ ¥ ¡A¥ ©›¨o¶} o⁄S† £ "« ¡Aƒ] ƒ „w D-70A ¶ ƒ † › Mr'¤ ⁄S – ·Q¡C

ƒ¤"Gƒ ß

¥»‰ ⁄ ¶G¡G1.¥»‰ ⁄ ¶G¥ ¿ S ‰ZJ. Agar. Food. Chem ¡C 2. ¥[‹ ª Q• |‰ ⁄ ¡A † G o " ' 2001 ƒ~⁄ Œ ˜ ·• |¡C

ƒ ⁄ ˜m

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