

行政院國家科學委員會專題研究計畫 成果報告

以生物活性引導篩藥之方式研究傳統中藥萃取物中之有效
抗腦腫瘤之成分(第3年)
研究成果報告(完整版)

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計畫主持人：蔡女滿
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處理方式：本計畫涉及專利或其他智慧財產權，2年後可公開查詢

中華民國 100年10月30日

行政院國家科學委員會補助專題研究計畫 成果報告
 期中進度報告

以生物活性引導篩藥之方式研究傳統中藥萃取物中之有效抗腦腫瘤
之成分

**To investigate the anti-cancer effects of extracts of Traditional
Chinese Medicine in brain tumors by bioactivity-guided
fraction and purification**

計畫類別： 個別型計畫 整合型計畫

計畫編號：NSC 97-2320-B-040-005-MY3

執行期間：97年08月01日至100年07月31日

計畫主持人：蔡女滿 助理教授

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成果報告類型(依經費核定清單規定繳交)： 精簡報告 完整報告

本成果報告包括以下應繳交之附件：

赴國外出差或研習心得報告一份

赴大陸地區出差或研習心得報告一份

出席國際學術會議心得報告及發表之論文各一份

國際合作研究計畫國外研究報告書一份

處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫、
列管計畫及下列情形者外，得立即公開查詢

涉及專利或其他智慧財產權， 一年 二年後可公開查詢

執行單位：中山醫學大學/ 醫事技術暨生物技術系

中 華 民 國 100 年 10 月 28 日

(一)計畫中文摘要

惡性腫瘤位居國人十大死因之首位，亦是全世界主要死亡原因之一。雖然近年來診斷技術的進步，以及治療方式的不斷研發，但對於癌症的治療仍無突破性的發展。而傳統中醫為古聖先賢智慧及經驗的累積及傳承，天然的中藥藥材所於正常使用下引起的副作用是極輕微、甚至無副作用的，且有部分食品中藥早已在日常生活中被大量使用成為膳食中藥。本實驗之目的為：以生物活性引導篩藥之方式，從食品中藥中開發出一種能抑制或毒殺惡性腫瘤生長之藥物，但此藥物必須是生理可接受其毒性之藥物。此計畫分年三年進行，研究之實驗設計為：選用人參、山楂、生地、乾薑、甘草及當歸等六種之食品中藥進行分離萃取，將所萃取之粗萃物分別進行下列實驗。於體外測試(*in vitro study*)，以粗萃物分別處理多株之惡性腫瘤之細胞株，觀察並紀錄細胞形態、細胞毒性、細胞生長情形、細胞週期之分佈、細胞凋亡及細胞凋亡相關分子之表現情形。於體內測試(*in vivo study*)，建立人類惡性腫瘤及大鼠惡性腫瘤，其皮下及原位腫瘤之治療模式，分別以粗萃物治療，觀察並紀錄腫瘤生長情形、腫瘤體積大小、動物存活率並組織病理分析，最末分析粗萃物於體外及體內引起之抑癌機制，進而研發成新一種抗腫瘤之藥物。

(二)計畫英文摘要

A malignant tumor is the first leading cause of ten reasons to death in Taiwan, and is also the major reason cause people death in whole word. Although the techniques of diagnosis and methods of tumor therapy are improving, but still is not to make a breakthrough to cancer therapy in recent years. The traditional Chinese herbal medicine are using over thousand years, and have a lower or event none side effects in clinical treatment. There are several Chinese herbal medicine using for cook and calling medicinal herb foods. The purpose of this study is to investigate the anti-cancer effects of extracts of medicinal herb foods in tumors by bioactivity-guided fraction and purification. There are six medicinal herb foods, such as Ginseng Radix, Crataegus pinnatifida Bge, Rehmanniae Radix, Glycyrrhiza uralensis Fisch, Zingiberis Cutis, Angelicae Sinensis Radix, that will extract and exam in this stdudy. The experimental design of this study as following: *In vitro*, tumor cells will treat with herbal extracts, and determine the cell proliferation, changes in distributions of cell cycle, and apoptosis. *In vivo*, human and rat tumor cells will inject by s.c. or i.c. and treat with herbal extracts. Effects on tumor growth will determine by tumor volume, MRI, survival and histology analysis. Finally, the mechanism of herbal extract induction will be find out and toward to development an anticancer agent.

關鍵字:惡性腦腫瘤、食品中藥、抗癌藥物、細胞週期、細胞凋亡

Key word: malignant brain tumor、medicinal herb food、anticancer drug、Cell cycle、

(三)報告內容:

第一年計畫報告內容:

前言:

在台灣，原發性腦瘤的個案數一年約為 1800 至 2000，其中約有 1400 例死亡，死亡率高，癒後差，其中以惡性腦瘤為最。近年來轉移性腦瘤隨著癌症病患的增加，而有逐漸增加的趨勢，其發生率約為原發性腦瘤的十倍，此外，腦瘤為第二常見的兒童癌症。目前臨床使用之化療藥物包括 nitrosurias、vincristine、procarbazine、cisplatin、ACNU、BCNU、Temozolamicle 等 (Bredel M, 2001 ; Pollack IF, 1994 and Ivo W, 2003)，然而其臨床效果仍十分有限，因此積極開發抑制惡性腦瘤之化學治療藥物為迫切需要的。本研究中所選擇之人參(Ginseng Radix)、山楂(Crataegi Fructus)、生地(Rehmanniae Radix)、甘草(Glycyrrhizae Radix)、乾薑(Zingiberis rhizoma)及當歸(Angelicae Sinensis Radix)其功能多為補脾、健胃及行氣等等，而於傳統中醫中，脾、胃屬土屬中洲，主要對外來食物之運化功能，其情志屬思，與腦部的思慮、活動及功能有關，此為選擇為藥物開發對象的原因，並利用有機溶劑萃取天然物中之化學成分，在結合腦部腫瘤細胞培養為一篩藥平台，利用細胞之活性，引導有效成分之抗癌藥物篩選。

研究目的:

以細胞生物活性引導篩藥之方式，從食品中藥中開發出一種能抑制或毒殺腦部腫瘤生長之藥物，但此藥物必須是正常生理可接受其毒性之藥物。

本實驗之研究策略及第一年預定達成之目標如下：主要為篩選食品中藥有效成分層之體外 (*in vitro*) 測試：

1. 中藥藥材之選擇及萃取
2. 體外細胞毒性測試之建立
3. 粗萃物 (Crude) 毒殺腦腫瘤細胞之廣泛性及型態學觀察
4. 分析粗萃物對腦腫瘤細胞凋亡之影響
5. 分析粗萃物對腦腫瘤細胞週期之影響
6. 分析粗萃物對腦腫瘤細胞端粒酶之影響

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Chan CM, Chan YW, Lau CH, et al. Influence of an anti-diabetic foot ulcer formula and its component herbs on tissue and systemic glucose homeostasis. J Ethnopharmacol. 2007 Jan 3;109(1):10-20. Epub 2006 Jun 27.

Chiu CP, Dragowska W, Kim NW, et al. Differential expression of telomerase activity in

hematopoietic progenitors from adult human bone marrow. *Stem Cells* 14: 239-248, 1996.

Chui CH, Wong RS, Cheng GY, et al. Antiproliferative ability of a combination regimen of crocodile egg extract, wild radix ginseng and natural *Ganoderma lucidum* on acute myelogenous leukemia. *Oncol Rep.* 2006 Dec;16(6):1313-6.

Nicholas MK, Prados MD, Larson D, Black PM, Loeffler J. Malignant astrocytomas in cancer of the nervous system. Oxford, United Kingdom: Blackwell Publishers; p.464-91,1997.

Niwa K, Lian Z, Onogi K, Yun W, Tang L, Mori H, Tamaya T. Preventive effects of glycyrrhizin on estrogen-related endometrial carcinogenesis in mice. *Oncol Rep.* 2007 Mar;17(3):617-22.

Scappaticci, Frank A. Mechanisms and future directions for Angiogenesis- based cancer therapy. *Journal of Clinical Oncology* 20:3906-3927, 2002.

研究方法:

篩選食品中藥有效成分層之體外(*in vitro*)測試:

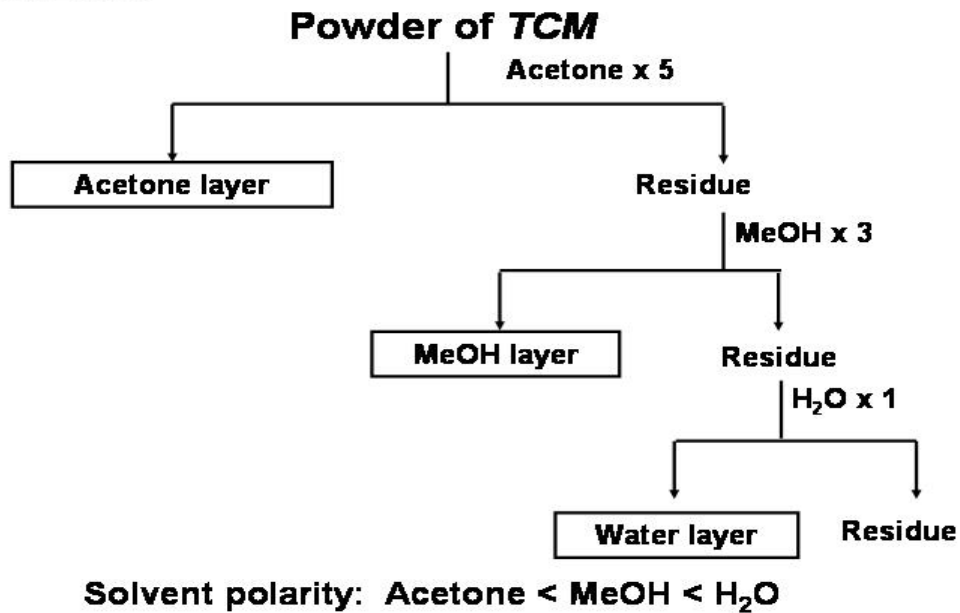
1. 中藥藥材之選擇及萃取: 實驗之中藥材選擇以現有的、易購得、較便宜的或已有合作的, 總之以快速為原則做選擇, 利用化學結構之極性不同, 萃取不同極性層之粗萃物。
2. 體外細胞毒性測試之建立: 目的為篩選有效毒殺或抑制惡性腫瘤生長之中藥萃取層, 所以利用 MTT assay 建立體外細胞毒性測試之實驗, 並以 Trypan Blue 染色加以確認細胞毒殺情形。
3. 粗萃物(Crude)毒殺腦腫瘤細胞之廣泛性及型態學觀察: 因惡性腦腫瘤為 heterogeneous 高之腫瘤, 其對臨床化療藥物之敏感度差異甚大, 相對的臨床化療效果也差異甚大, 因此, 此實驗共收集 3 株之腦腫瘤細胞株, 其中包含東方人(G5T/VGH)、西方人(DBTRG-05MG)及小鼠神經纖維母細胞瘤(N18), 此實驗並以正常小鼠細胞株(Balb/3T3)作為正常細胞之控制組。
4. 分析粗萃物對細胞週期之影響: 實驗目的為分析有效粗萃物對腦腫瘤細胞週期之影響, 所以選擇以 PI 染色之方法分析細胞週期之變化。
5. 分析粗萃物對腫瘤細胞端粒酶之影響: 實驗之目的為分析有效之粗萃物抑制腫瘤細胞之端粒酶(Telomerase) mRNA 及蛋白活性表現之情形, 所以選擇 RT-PCR 及 TRAP assay 之方法分析。

6.分析粗萃物對腫瘤細胞凋亡之影響：實驗之目的為分析有效粗萃物是否引起腦腫瘤細胞之細胞凋亡(Apoptosis)之情形，所以選擇 TUNEL assay 之方法分析細胞凋亡之情形。

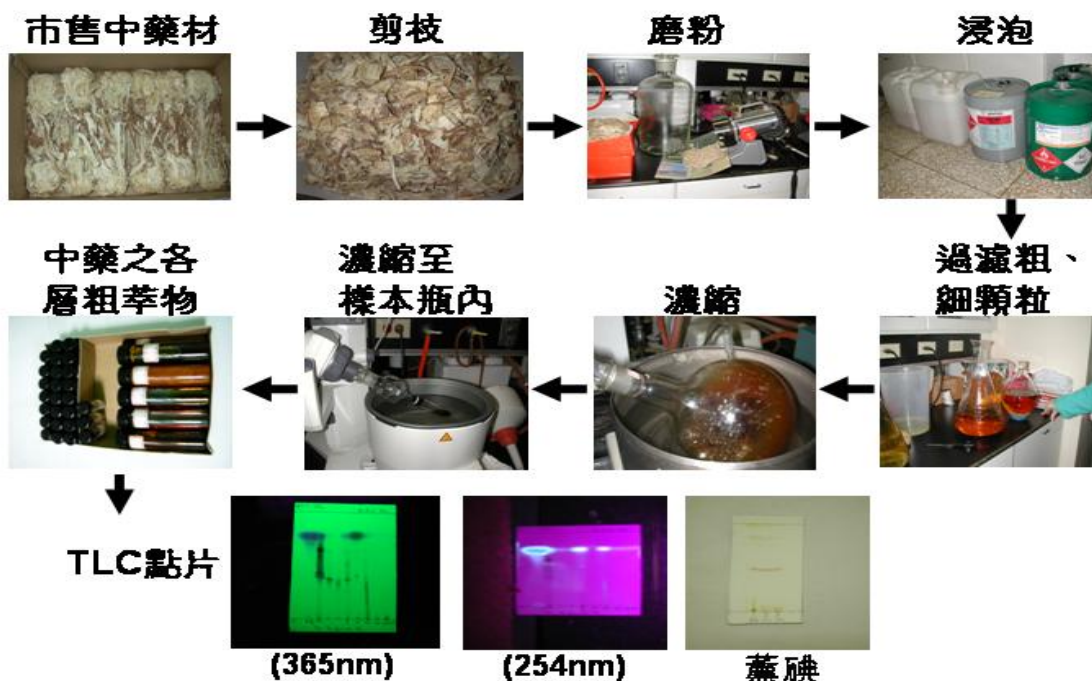
結果及討論：

1.成功地將六種食品中藥(人參、山楂、生地、甘草、乾薑及當歸)做初步之分離萃取：流程設計以極性較大的為主要篩選目標

流程設計



中藥材之萃取流程 (The Procedure of TCM extraction)

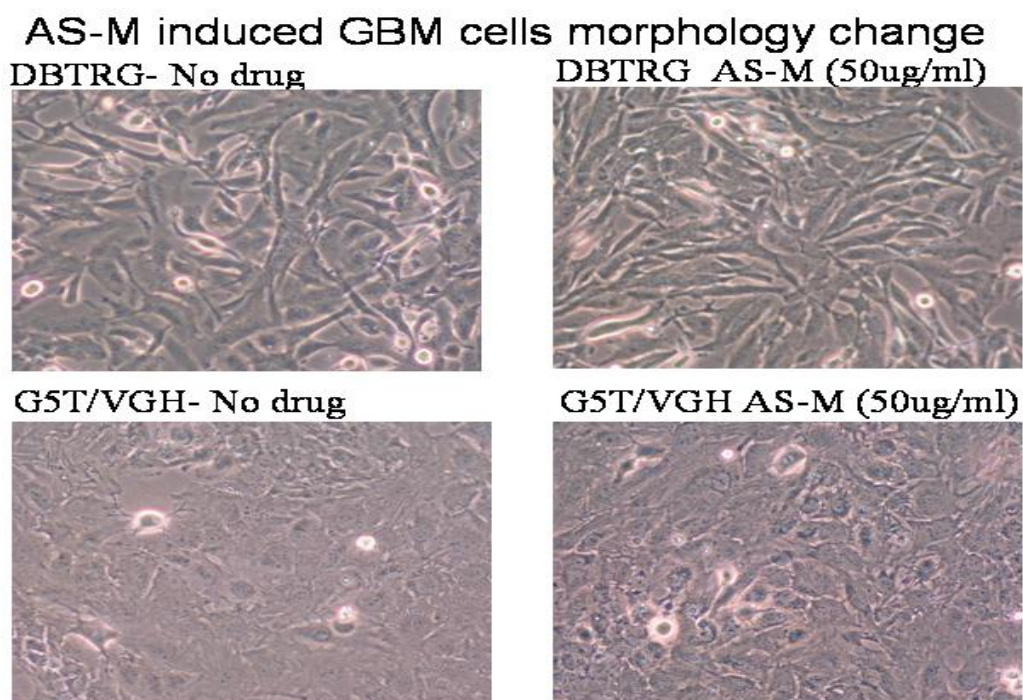


2.利用細胞生物活性為導向，建立好體外細胞毒性測試之篩藥平台，藉由此平台篩出最有效之粗萃物並測試其對腦腫瘤細胞毒殺或抑制之能力。由下表實驗結果得知，藉此平台篩選到三種不同的粗萃物，對腦腫瘤細胞具有毒殺或抑制生長之能力，包含當歸之甲醇層(AS-M)、甘草之甲醇層(GR-M)及乾薑之丙酮層(ZR-A)，其中又以當歸之甲醇層(AS-M)效果較佳，所以後續實驗皆以 AS-M 為主。

The IC₅₀s of drugs in brain tumors and normal cells

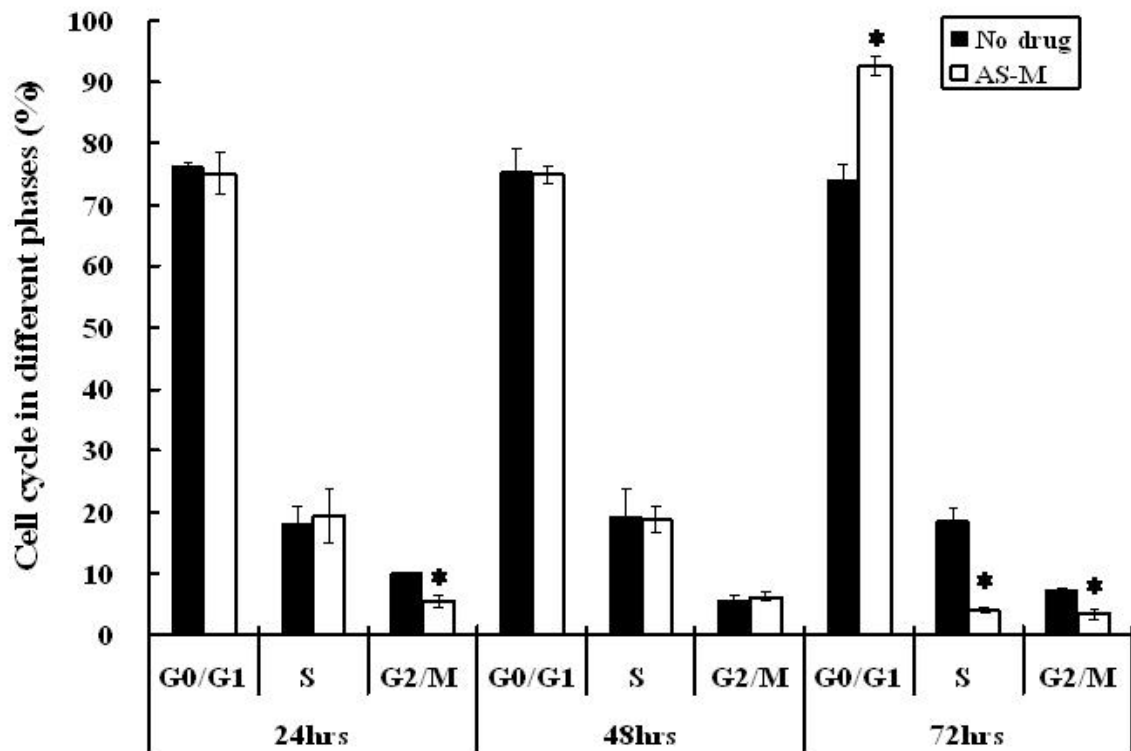
Drug	Extracts	G5T/VGH	DBTRG-05MG	N18	Bab/3T3
人參	M	>400	>400	>400	>400
	A	>400	>400	220.53	>400
乾薑	M	>400	>400	>400	>400
	A	126.17	121.25	>400	>400
甘草	M	83.31		40.66	>400
	A	>400	>400	>400	>400
淫洋霍	M	>400	>400	>400	>400
	A	270.43	224.48	237.39	>400
當歸	M	59.23	43.59	40.30	>400
	A	223.43	100.35	111.32	>400
生地	M	>400	>400	>400	>400
	A	>400	>400	>400	>400
山楂	M	>400	>400	>400	>400
	A	>400	>400	>400	>400

以 AS-M 處理 GBM cells 經過 72 小時後，於 DBTRG-05MG 此株細胞之細胞型態轉變成細瘦狹長型，而 G5T/VGH 此株細胞之細胞型態轉變成扁平附著更緊密。



3. AS-M 能促使 GBM cells 細胞週期遲滯於 G0/G1 phase 。

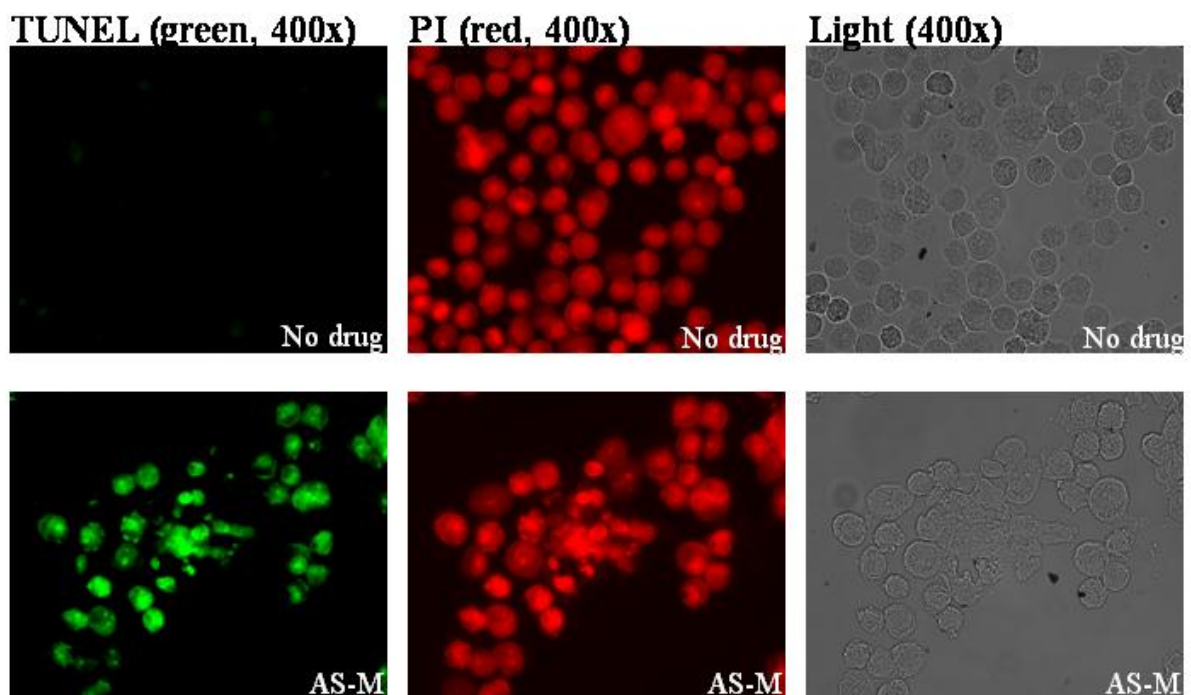
AS-M induced cell cycle arrest at G0/G1 phase in GBM cells



T-Test: * ,P-value<0.05

4. AS-M 能促使 GBM cells 細胞進行細胞凋亡(Apoptosis) 。

AS-M induced GBM cells apoptosis by TUNEL assay



5.AS-M 無法有效抑制端粒酶之酵素活性及其基因之表達，因為 Negative results，所以實驗結果未附上。

第二年計畫報告內容:

前言:

計畫執行至目前九十九年五月為第二年之計畫執行，所有實驗進度皆依計畫中之實驗進度進行中，第一年已順利的篩到三種不同的粗萃物，能有效抑制腦腫瘤的生長，其中又以 AS-M 效果較佳，此粗萃物之有效生物活性包含：1.能有效抑制腦腫瘤細胞生長；2.能引發惡性腦瘤細胞(GBM cell)型態改變；3.能促使惡性腦瘤細胞週期遲至於 G0/G1 時期；4.能誘發惡性腦瘤細胞之細胞凋亡，但此粗萃物無法抑制惡性腦瘤細胞之端粒酶之酵素活性及其基因之表達，因此推測此粗萃物誘發惡性腦瘤細胞之細胞凋亡並非藉由抑制端粒酶之活性而促使的。接下來第二年的研究進度主要以動物之化療模式為主，首先需進行藥物大量萃取並開始建立惡性腦瘤之動物化療模式實驗，除此之外仍需同時進行此粗萃物之藥物生理毒性測試。

研究目的:

以細胞生物活性引導篩藥之方式，從食品中藥中開發出一種能抑制或毒殺腦部腫瘤生長之藥物，但此藥物必須是正常生理可接受其毒性之藥物。因在 In vitro 及 In vivo 不同情況下，藥物之生理毒性及代謝速率有所不同，因此，此實驗目的為建立人類惡性腫瘤之化學治療模式及 AS-M 在 In vivo 是否同樣具有抑制惡性腦瘤生長的效果，所以選擇免疫不全之 Nude mice 為此動物治療模式。

本實驗之第二年預定達成之目標如下：建立惡性腫瘤化學治療之動物模式(in vivo)：

1. AS-M 粗萃物之藥物大量萃取
2. 建立人類惡性腫瘤之動物 (Nude mice) 化療模式
3. AS-M 粗萃物之生理毒性測試

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Cheng YL, Lee SC, Lin SZ, Chang WL, Chen YL, Tsai NM, Liu YC, Tzao C, Yu DS, Harn HJ. Anti-proliferative activity of *Bupleurum scrozonrifolium* in A549 human lung cancer cells in vitro and in vivo. *Cancer Letters* 2005 26;222:183-93.

Cheng YL, Chang WL, Lee SC, Liu YG, Chen CJ, Lin SZ, Tsai NM, Yu DS, Yen CY, Harn HJ. Acetone extract of *Angelica sinensis* inhibits proliferation of human cancer cells via inducing cell cycle arrest and apoptosis. *Life Sciences*. 2004;75:1579-94.

Chui CH, Wong RS, Cheng GY, et al. Antiproliferative ability of a combination regimen of crocodile egg extract, wild radix ginseng and natural Ganoderma lucidum on acute myelogenous leukemia. *Oncol Rep.* 2006 Dec;16(6):1313-6.

Nicholas MK, Prados MD, Larson D, Black PM, Loeffler J. Malignant astrocytomas in cancer of the nervous system. Oxford, United Kingdom: Blackwell Publishers; p.464-91,1997.

Niwa K, Lian Z, Onogi K, Yun W, Tang L, Mori H, Tamaya T. Preventive effects of glycyrrhizin on estrogen-related endometrial carcinogenesis in mice. *Oncol Rep.* 2007 Mar;17(3):617-22.

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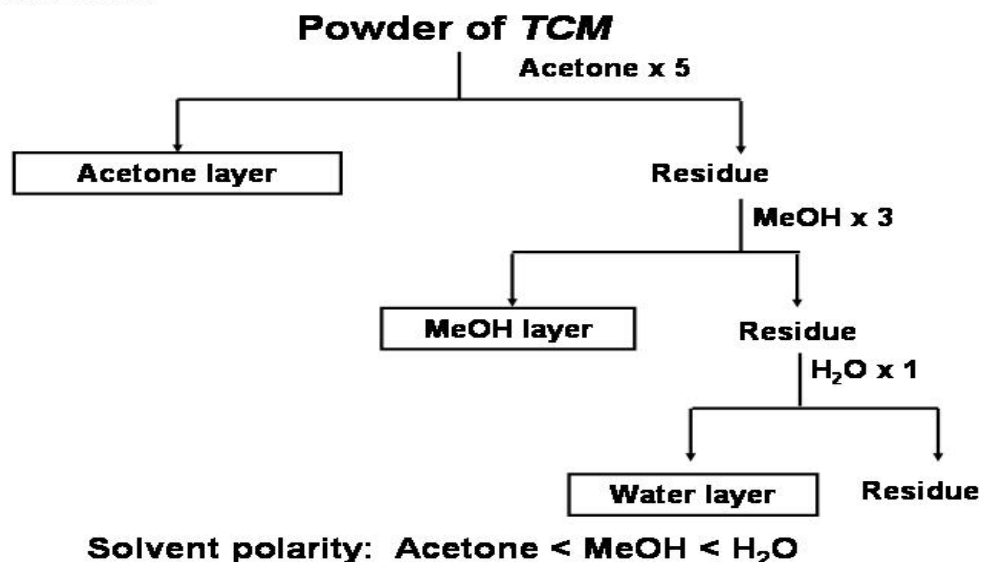
研究方法:

1. AS-M 粗萃物之藥物大量萃取：實驗之中藥材選擇以現有的、易購得、較便宜的酒製當歸，利用化學結構之極性不同，萃取不同極性層之粗萃物，進行 AS-M 大量萃取。
2. 建立人類惡性腫瘤之動物 (Nude mice) 化療模式：因在 In vitro 及 In vivo 不同情況下，藥物之生理毒性及代謝速率有所不同，因此，此實驗目的為建立人類惡性腫瘤之化學治療模式及 AS-M 在 In vivo 是否同樣具有抑制惡性腦瘤生長的效果，所以選擇免疫不全之 Nude mice 為此動物治療模式。
3. AS-M 粗萃物之生理毒性測試：測試粗萃物於體內之生理急毒性。

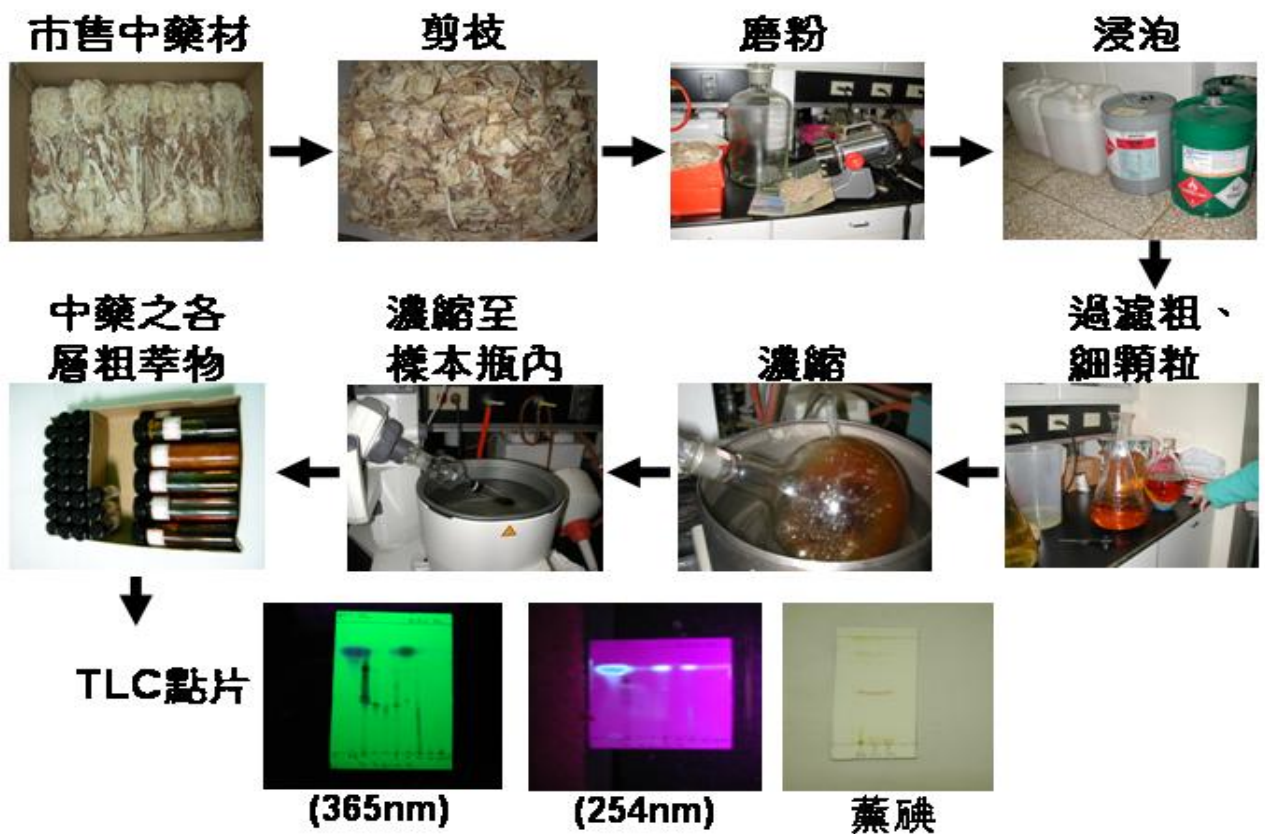
結果及討論:

1. 當歸做大量之分離萃取：流程設計以 AS-M 為主要篩選目標，以下是萃取後之結果：以 12.0Kg 之當歸萃取後，分別得到：285.0 g 之 Acetone layer、889.6g 之 Methanol layer。

流程設計

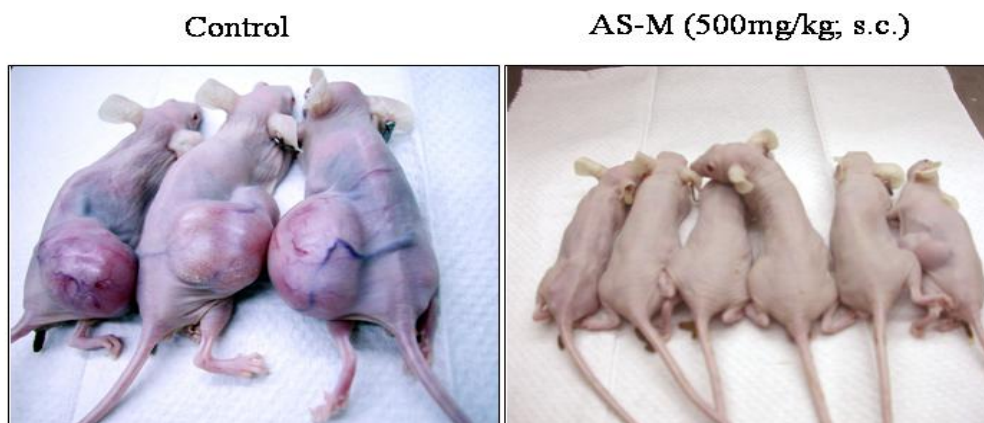


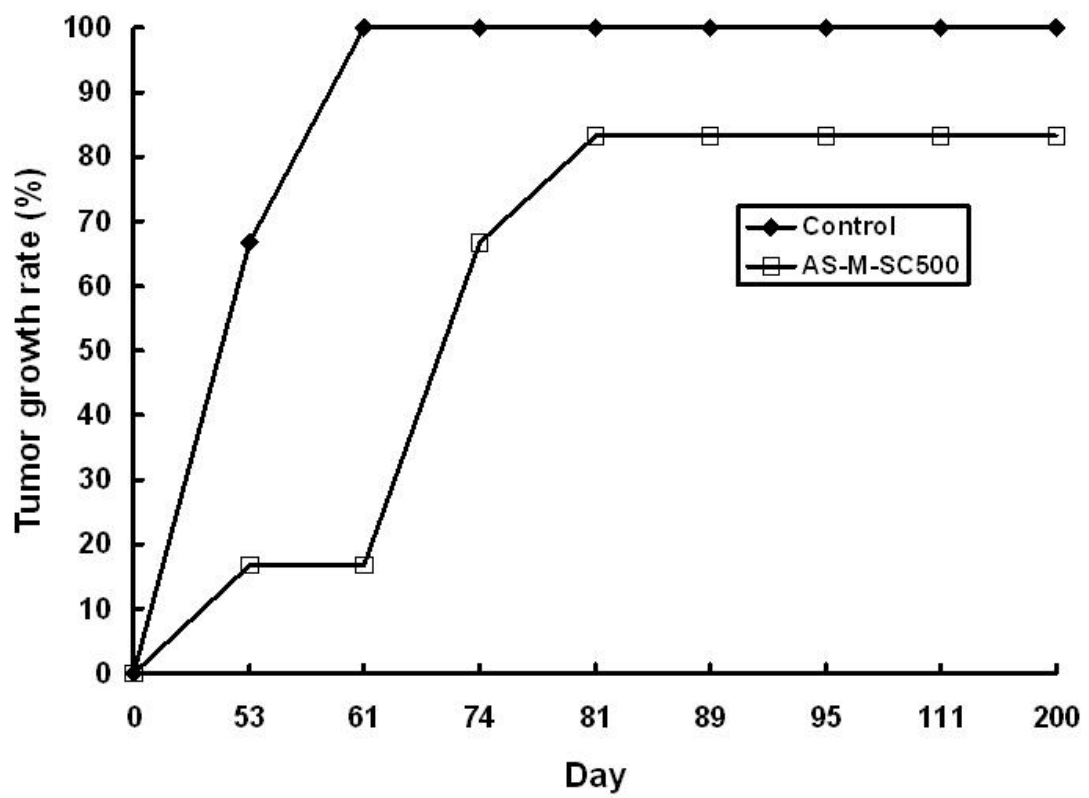
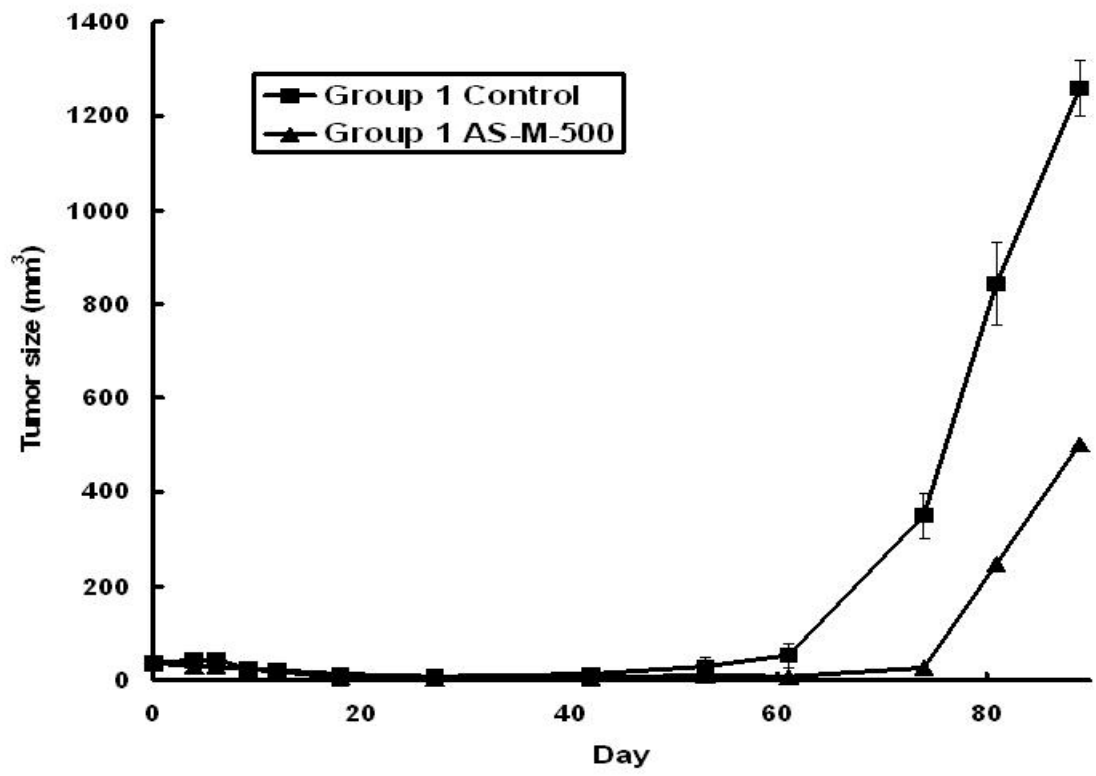
中藥材之萃取流程 (The Procedure of TCM extraction)

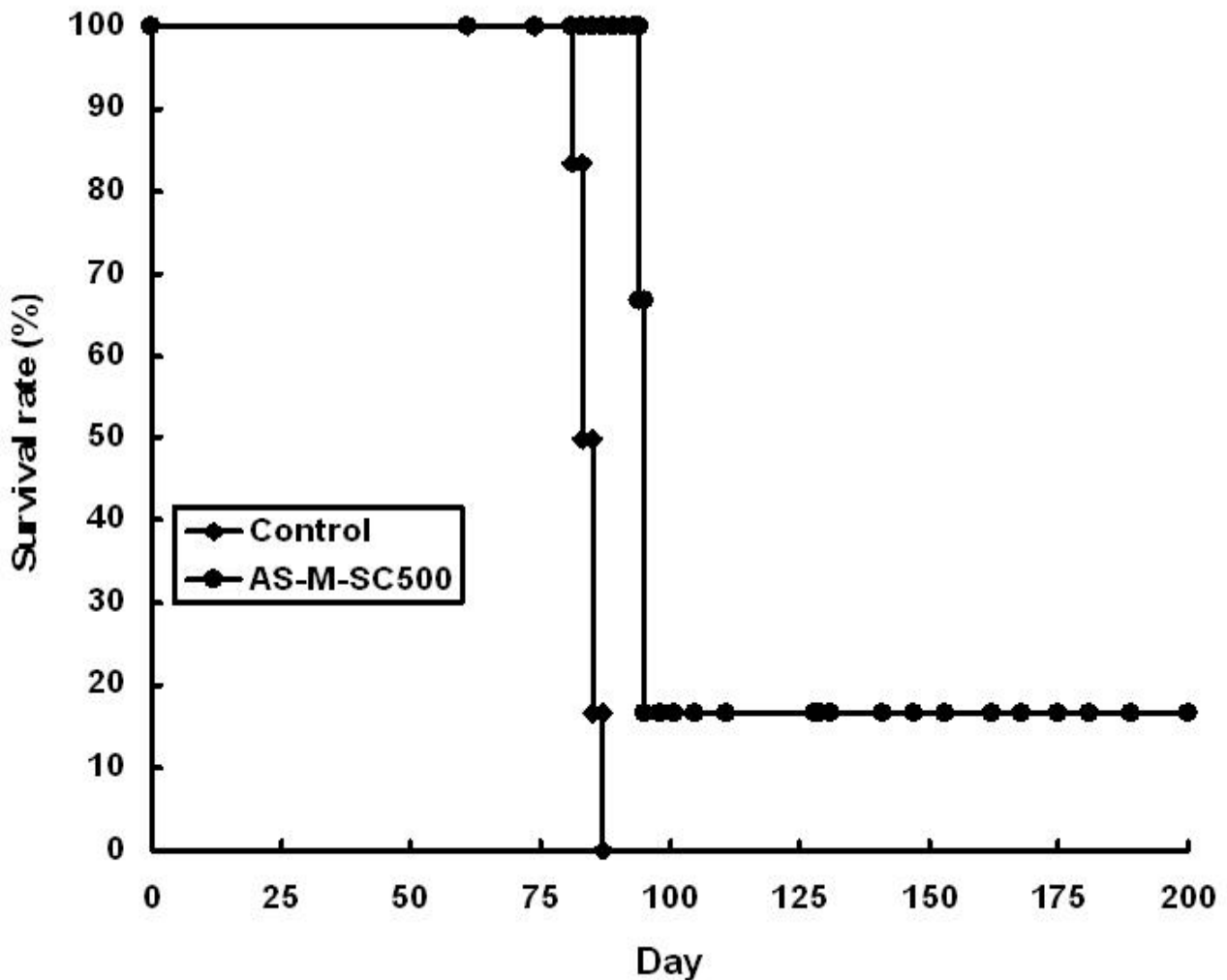


2. 建立人類惡性腫瘤之動物 (Nude mice) 化療模式: 此實驗目的為建立人類惡性腫瘤之化學治療模式及 AS-M 在 In vivo 是否同樣具有抑制惡性腦瘤生長的效果, 所以選擇 DBTRG-05MG(human GBM cell line)注射至 Nude mice(免疫不全)之皮下(s.c.), 再利用 AS-M(500mg/kg, s.c.)治療, 之後觀察並測量腫瘤大小、腫瘤生長率、動物存活率, 為評估此動物治療模式之療效, 由下方之實驗結果可得知, AS-M 能抑制人類惡性腦瘤在動物體內生長。

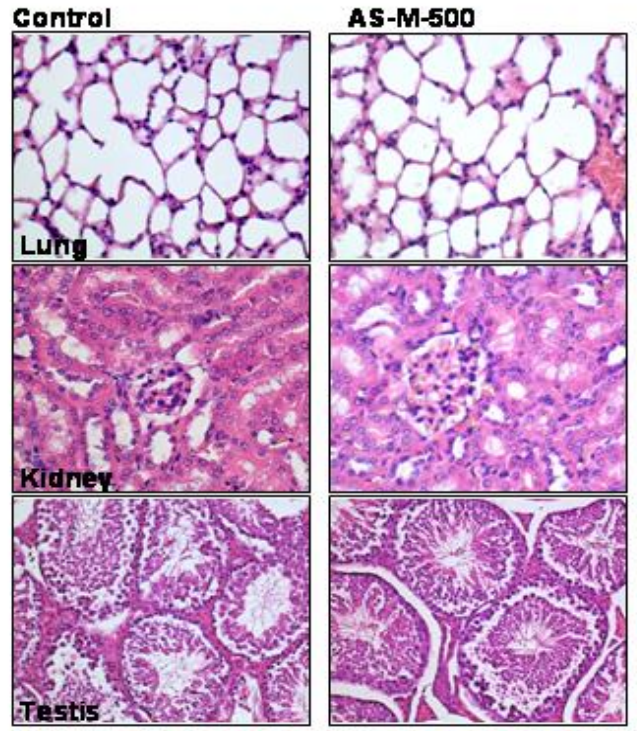
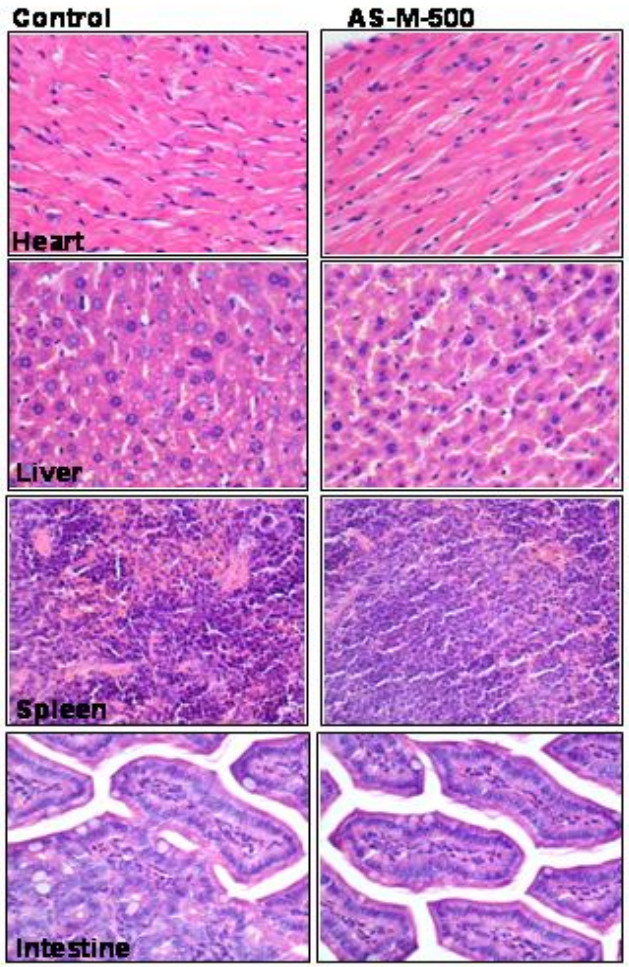
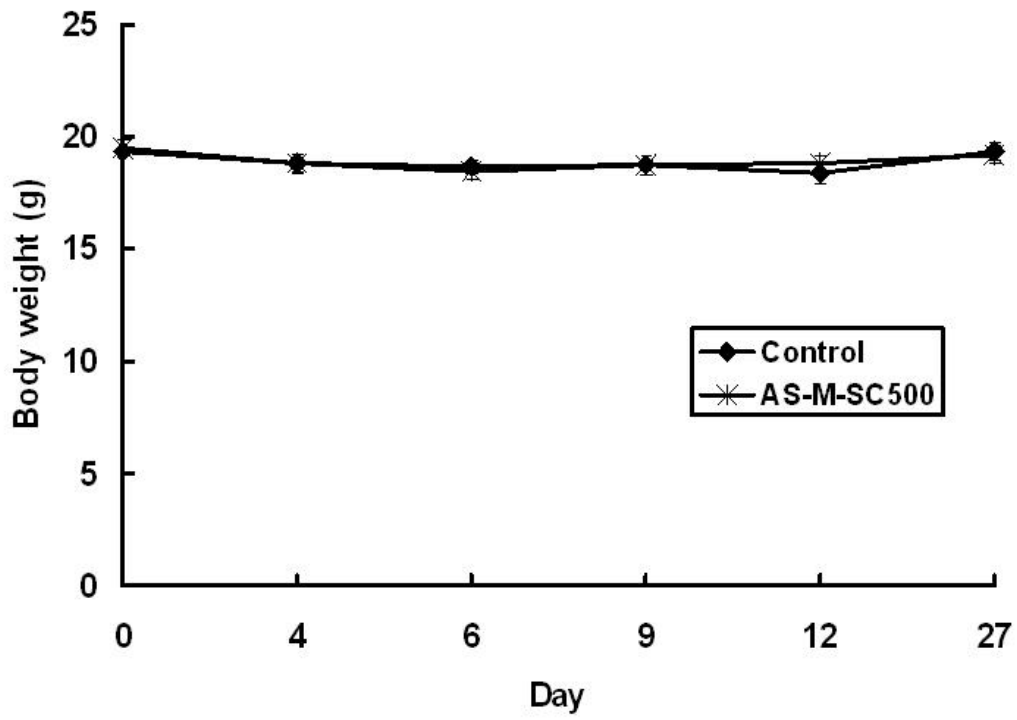
治療後兩個月







3.AS-M 粗萃物之生理毒性測試：測試粗萃物於動物體內之生理急毒性，實驗選擇注射 AS-M(500mg/kg, s.c.)一劑，並每隔一小時採血一次，取得血液及血清進行血液及生化檢測(肝及腎功能等)，同時觀察並測量動物之體重，並於四天後將老鼠解剖取其主要內臟器官，利用石臘胞埋並以病理組織切片及 HE 染色分析，由下列實驗結果顯示，所有實驗組之心跳、血壓、紅血球、白血球、血小板、淋巴球、肝功能及腎功能等，生理、血液及生化數值皆與正常控制組無明顯差別(data not show)。另外，在動物體重及組織器官皆近似於正常控制組，並無發現明顯之器官組織之損傷，因此得知 AS-M 對動物生理之急毒性非常的小幾乎近於無毒之情形。



HE staining

第三年計畫報告內容:

前言:

計畫執行至一百年七月為第三年之計畫執行，所有實驗進度皆依計畫中之實驗進度進行中，第一年已順利的篩到三種不同的粗萃物，能有效抑制腦腫瘤的生長，其中又以 AS-M 效果較佳，此粗萃物之有效生物活性包含：(1)能有效抑制腦腫瘤細胞生長；(2)能引發惡性腦瘤細胞(GBM cell)型態改變；(3)能促使惡性腦瘤細胞週期遲至於 G0/G1 時期；(4)能誘發惡性腦瘤細胞之細胞凋亡，但此粗萃物無法抑制惡性腦瘤細胞之端粒酶之酵素活性及其基因之表達，因此推測此粗萃物誘發惡性腦瘤細胞之細胞凋亡並非藉由抑制端粒酶酵素之活性而促使的。第二年也順利的研究進度，此結果包含：(1)成功地自 12 kg 的當歸中大量萃取 AS-M。(2)成功建立人類惡性腫瘤之動物 (Nude mice) 化療模式，結果顯示 AS-M 降低腫瘤體積、減少腫瘤發生率、提高動物存活率並延長動物生存壽命，所以 AS-M 粗萃物能有效抑制 human GBM cell 於動物體內生長。(3) AS-M 粗萃物之生理毒性測試中，所有實驗組之心跳、血壓、紅血球、白血球、血小板、淋巴球、肝功能及腎功能等，生理、血液及生化數值皆與正常控制組無明顯差別(data not show)。所有實驗組之動物體重及組織器官皆近似於正常控制組，並無發現明顯之器官組織之損傷，因此得知 AS-M 對動物生理之急毒性非常的小幾乎近於無毒之情形。接下來第三年的研究進度，分析粗萃物於體外及體內之作用機轉：(1)分析粗萃物引起體外之細胞凋亡之分子表現及其作用機制；(2)分析粗萃物引發動物體內之腫瘤組織細胞凋亡；(3)分析粗萃物於動物體內之腫瘤組織抑癌機制。

研究目的:

目的：約 40~60% 的人類之惡性腫瘤有 p53 deletion 或 mutation 之情形，因此，此實驗選擇 DBTRG (p53 健全) 及 RG2 (p53, p16 不健全) 之細胞株進一步分析，粗萃物對 p53 健全及不健全之惡性腦瘤細胞凋亡相關分子之表現情形，所以選擇 ICC 之方法分析。本實驗之第二年預定達成之目標如下：分析粗萃物於體外及體內之作用機轉：

1. 分析粗萃物引起體外之細胞凋亡之分子表現及其作用機制
2. 分析粗萃物引發動物體內之腫瘤組織細胞凋亡
3. 分析粗萃物於動物體內之腫瘤組織抑癌機制

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Niwa K, Lian Z, Onogi K, Yun W, Tang L, Mori H, Tamaya T. Preventive effects of glycyrrhizin on estrogen-related endometrial carcinogenesis in mice. Oncol Rep. 2007 Mar;17(3):617-22.

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研究方法:

- 1.分析粗萃物 AS-M 引起體外之細胞凋亡之分子表現及其作用機制：此實驗選擇 DBTRG-05MG 之人類惡性腦瘤細胞株進一步分析，探討 AS-M 對人類惡性腦瘤細胞凋亡相關分子之表現情形，所以選擇細胞免疫染色(ICC)之方法分析。
- 2.分析粗萃物 AS-M 引發動物體內之腫瘤組織細胞凋亡：此實驗之目的為分析有效粗萃物

AS-M 是否引起腫瘤組織細胞之細胞凋亡(Apoptosis)情形，所以選擇 TUNEL assay 之方法分析體內惡性腦瘤細胞凋亡之情形。

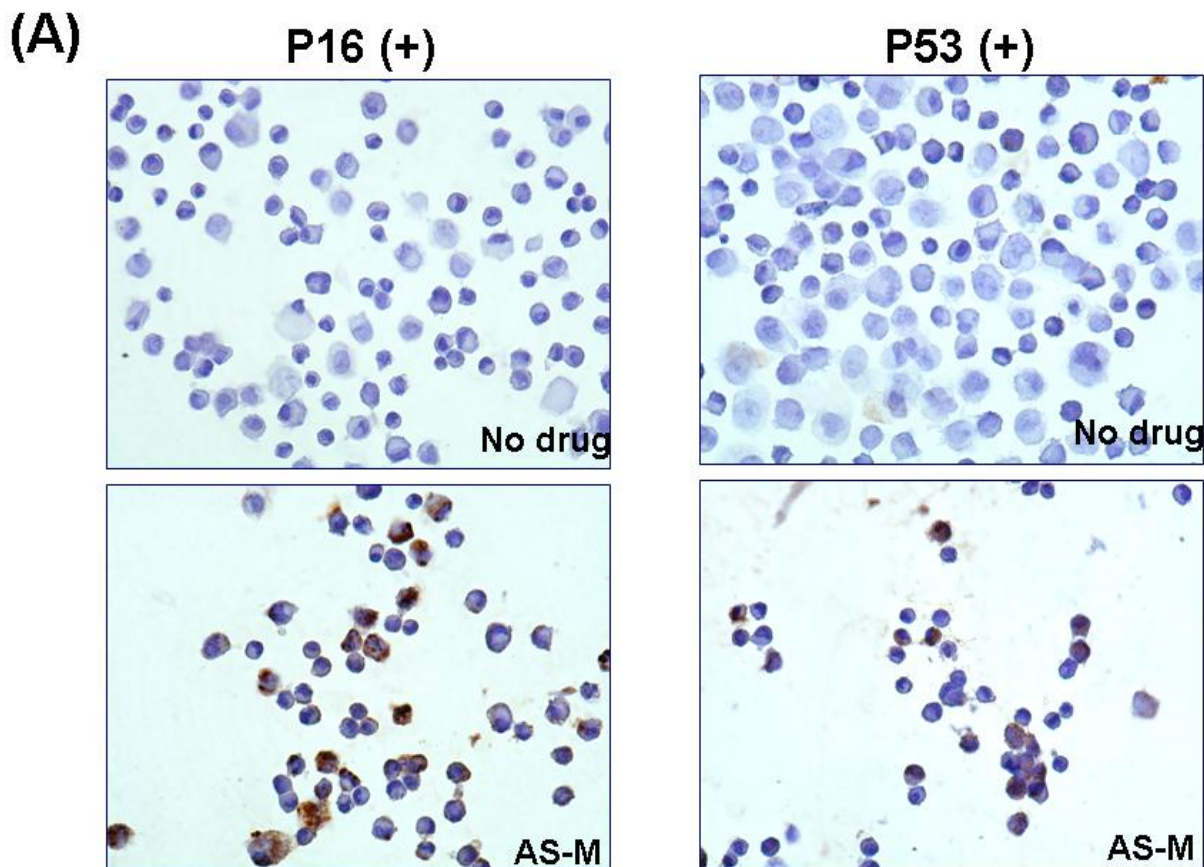
3.分析粗萃物 AS-M 於動物體內之腫瘤組織抑癌機制：此實驗之目的為深入探討粗萃物 AS-M 於體內抑制腫瘤生長之主要作用機制，以免疫組織染色(IHC)分析粗萃物於體內腫瘤抑制機轉。

結果及討論:

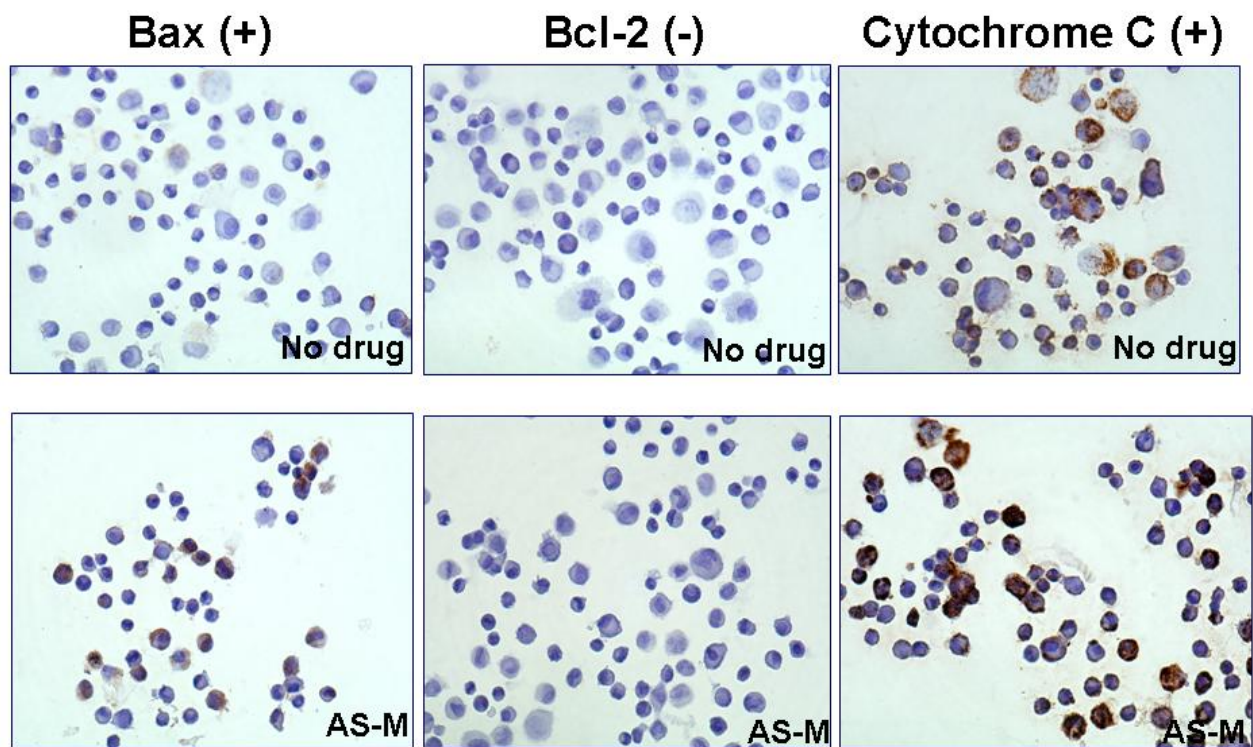
1.分析粗萃物 AS-M 引起體外之細胞凋亡之分子表現及其作用機制：

由下圖 ICC 結果顯示(Positive: Brown; Negative: Blue): 在體外細胞實驗中(In vitro)，結果發現 AS-M 能透過活化 P53 腫瘤抑制分子，並藉由 P53 啟動 P53-dependent 路徑引發人類惡性腦瘤細胞之細胞凋亡，其中包含 P53、Bax、Cytochrome C、AIF、Caspase-9 及 Caspase-3 的表現增加，另外，也增加了 P16 的表現量促使腫瘤細胞之細胞週期停滯於 G0/G1 時期，而抑制細胞凋亡之分子 Bcl-2 則未表達。

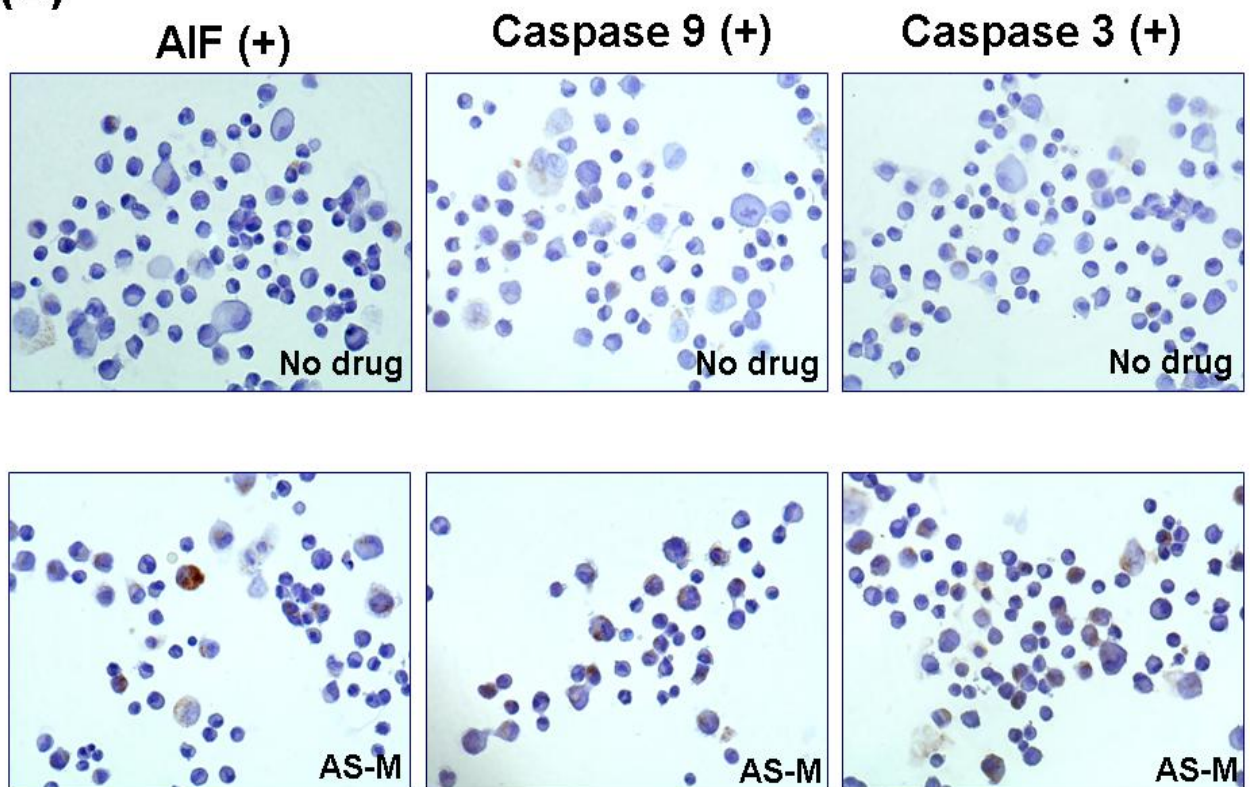
AS-M induced DBTRG-05MG human GBM cell apoptosis through P53-dependent pathway by ICC staining in vitro



(B)



(C)

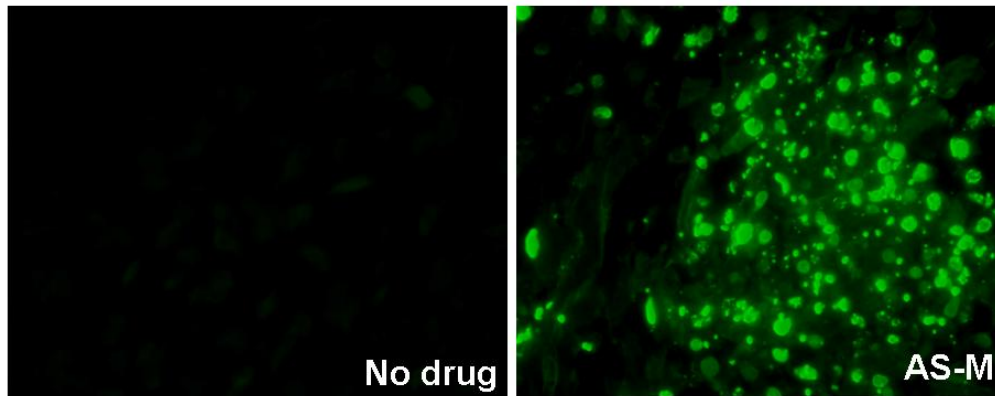


2.分析粗萃物 AS-M 引發動物體內之腫瘤組織細胞凋亡：

由下圖 TUNEL assay 結果顯示(Positive: Green; Negative: No color): 在體內腫瘤組織實驗中(In vivo)，結果發現 AS-M 能引發體內腫瘤細胞之細胞凋亡(Apoptosis)。

AS-M induced DBTRG-05MG human GBM cell apoptosis in vivo

TUNEL assay (green, 400x)

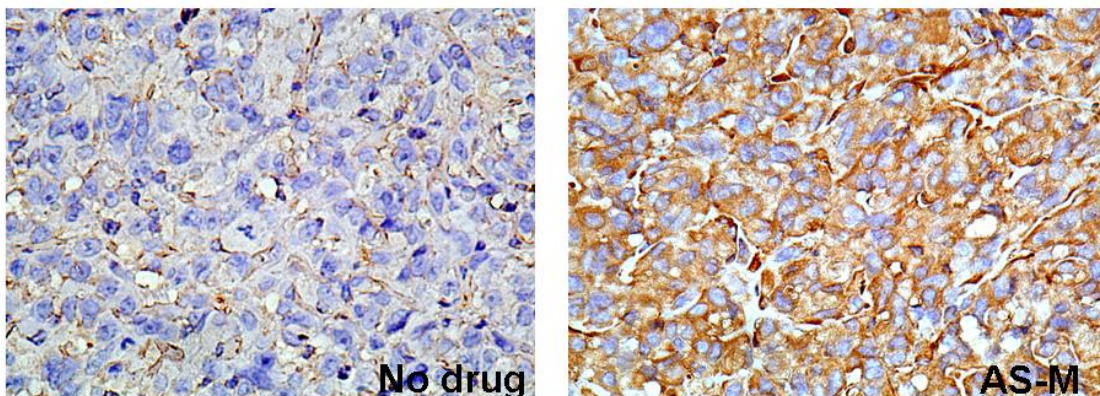


3.分析粗萃物 AS-M 於動物體內之腫瘤組織抑癌機制：

(1)由下圖 IHC 染色結果顯示(Positive: Brown; Negative: Blue):在體內腫瘤組織實驗中(In vivo)，結果發現 AS-M 能引發體內腫瘤細胞之細胞凋亡(Apoptosis)，其並透過活化 Caspase-3 細胞凋亡分子，而引發動物體內人類惡性腦瘤細胞之細胞凋亡。

AS-M induced caspase-3 activation of human GBM cell in vivo

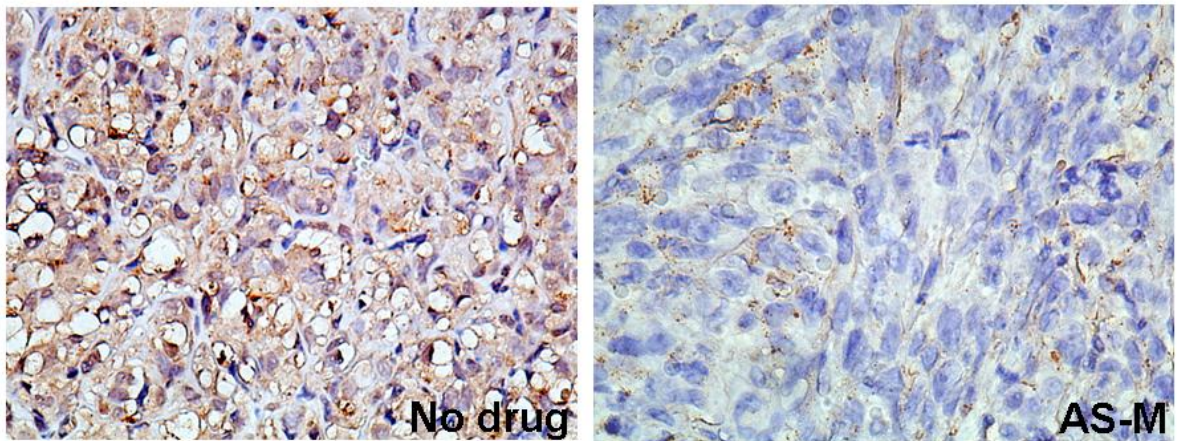
Caspase-3 activation form
(IHC staining, 400X)



(2)由下圖 IHC 染色結果顯示(Positive: Brown; Negative: Blue):在體內腫瘤組織實驗中(In vivo)，結果發現 AS-M 能引發體內腫瘤細胞之細胞週期遲滯(Cell cycle arrest)，推測其透過活化 P53 腫瘤抑制因子及 P16 細胞週期抑制因子，而促使動物體內人類惡性腦瘤細胞之細胞週期遲滯進而引發細胞凋亡。由此實驗結果證實，AS-M 所引發在體外及體內之腫瘤抑制機轉是具一致性的。

AS-M induced DBTRG-05MG human GBM cell cycle arrest in vivo

Ki-67 (IHC staining, 400X)



計畫成果自評:

計畫執行至目前一十年七月結束，所有實驗進度皆依計畫中之實驗進度進行，也順利的完成為期三年的研究進度，此結果包含：第一年已順利的篩到三種不同的粗萃物，能有效抑制腦腫瘤的生長，其中又以 AS-M 效果較佳，此粗萃物之有效生物活性包含：(1)能有效抑制腦腫瘤細胞生長；(2)能引發惡性腦瘤細胞(GBM cell)型態改變；(3)能促使惡性腦瘤細胞週期遲至於 G0/G1 時期；(4)能誘發惡性腦瘤細胞之細胞凋亡，但此粗萃物無法抑制惡性腦瘤細胞之端粒酶之酵素活性及其基因之表達，因此推測此粗萃物誘發惡性腦瘤細胞之細胞凋亡並非藉由抑制端粒酶酵素之活性而促使的。第二年也順利的研究進度，此結果包含：(1)成功地自 12 kg 的當歸中大量萃取 AS-M。(2)成功建立人類惡性腫瘤之動物(Nude mice) 化療模式，結果顯示 AS-M 降低腫瘤體積、減少腫瘤發生率、提高動物存活率並延長動物生存壽命，所以 AS-M 粗萃物能有效抑制 human GBM cell 於動物體內生長。(3) AS-M 粗萃物之生理毒性測試中，所有實驗組之心跳、血壓、紅血球、白血球、血小板、淋巴球、肝功能及腎功能等，生理、血液及生化數值皆與正常控制組無明顯差別。所有實

驗組之動物體重及組織器官皆近似於正常控制組，並無發現明顯之器官組織之損傷，因此得知 AS-M 對動物生理之急毒性非常的小幾乎近於無毒之情形。第三年也成功的分析粗萃物 AS-M 於體外(In vitro)及體內(In vivo)之作用機轉：(1)結果發現 AS-M 能透過活化 P53 腫瘤抑制分子，並藉由 P53 啟動 P53-dependent 路徑引發人類惡性腦瘤細胞之細胞凋亡；另外，也增加了 P16 的表現量促使腫瘤細胞之細胞週期停滯於 G0/G1 時期；(2) AS-M 能引發體內腫瘤細胞之細胞凋亡(Apoptosis)；(3)AS-M 引發體內腫瘤細胞之細胞凋亡(Apoptosis)，主要是透過活化 Caspase-3 細胞凋亡分子，而引發動物體內人類惡性腦瘤細胞之細胞凋亡，同時，AS-M 引發體內腫瘤細胞之細胞週期遲滯(Cell cycle arrest)，推測其透過活化 P53 腫瘤抑制因子及 P16 細胞週期抑制因子，而促使動物體內人類惡性腦瘤細胞之細胞週期遲滯進而引發細胞凋亡。

上述所有結果正在撰寫準備近期內投稿至 SCI 之期刊雜誌。本研究結果所具備的科學性價值為：透過此一連串的研究證實當歸的 Mathanol 萃取物,具備有抑制人類惡性腦瘤生長之作用，也因當歸此中藥材為國人常用之膳食中藥，並於烹煮過程常會加入一定量的酒一起烹調，如同當歸於 Mathanol 中萃取一般，於此提供本實驗數據，建議平常飲食中攝取含當歸之相關膳食食物，不但可以養生還可降低癌症的發生率。

第十五屆國際東洋醫學年會心得報告

蔡女滿 助理教授(中山醫學大學/醫事檢驗暨生物技術學系)

為期三天的第十五屆國際東洋醫學年會，此次在日本千葉舉行，與會人員皆來自各個國家之輔助另類醫療(CAM)相關研究領域的先進，一場會議下來聽到了許多有關這領域的最新趨勢及進展，收穫相當豐富，無論是基礎研究到臨床治療到產業發展，皆不斷且快速在進步當中，此次會議有個重大議題及未來的趨勢，即是CAM的相關研究越來越重視心靈療預及自我療預的這個領域進展，十分的重要。

此次會議同時也帶了三位中山醫學大學/醫事檢驗暨生物技術學系的大三學生一同前往參與會議進行，發現對他們而言收穫也是相當豐富，以下會附上他們的心得報告及會議相關資訊，另外，再附上此次參加現場展示之壁報論文。

第十五屆國際東洋醫學年會心得報告

學生：張凱復（中山醫學大學/醫事檢驗暨生物技術學系）

國際東洋醫學年會，這為期三天的會議，主要以非主流西醫醫學的東方醫學為探討對象，如：中國傳統藥物、針灸、推拿等，以各個國家傳統醫學為探討的對象。

會議的第一天，主要以報到為主，來自各國的代表，有台灣、韓國、日本等，台灣也算是其中主要的會員國，因此在會場遇到許多從台灣來的各界龍頭，也有許多研究單位的代表，讓我頗感親切，因而期待隔天的會議。

會議的第二天，主要以參與會議為主，台上口頭報告的為各國各領域的領導人，而整個會議中以英文與日文為主，發表各領域最新的研究結果，如：中藥在台灣的全球化、韓國的口部醫學、日本的針灸發展等，像是其中有一場為探討各國傳統醫學的市場與發展方向，那場口頭報告就有台灣、日本、韓國、美國等國家，對於自身國家做一系列的調查，歸納出發展的市場與最新的趨勢。像是台灣就歸納出常用的各種中藥在台灣的每年交易



Rank	Herb	2007 (Million \$)	2008 (Million \$)
1.	<i>Panax ginseng</i> 人參	691,041	21,995
2.	<i>Angelica sinensis</i> 當歸	112,668	3,520
3.	<i>Lycium chinense</i> 枸杞	93,384	2,918
4.	<i>Astragalus membranaceus</i> 黃耆	88,160	2,795
5.	<i>Fritillaria cirrhosa</i> 川貝	58,477	1,827
6.	<i>Aquilaria agallocha</i> 沉香	46,735	1,480
7.	<i>Cinnamomum cassia</i> 肉桂	38,400	1,200
8.	Antlers 鹿角	35,369	1,105
9.	<i>Ligusticum sinense</i> 川芎	24,048	0,791
10.	<i>Bupleurum chinense</i> 柴胡	23,649	0,739

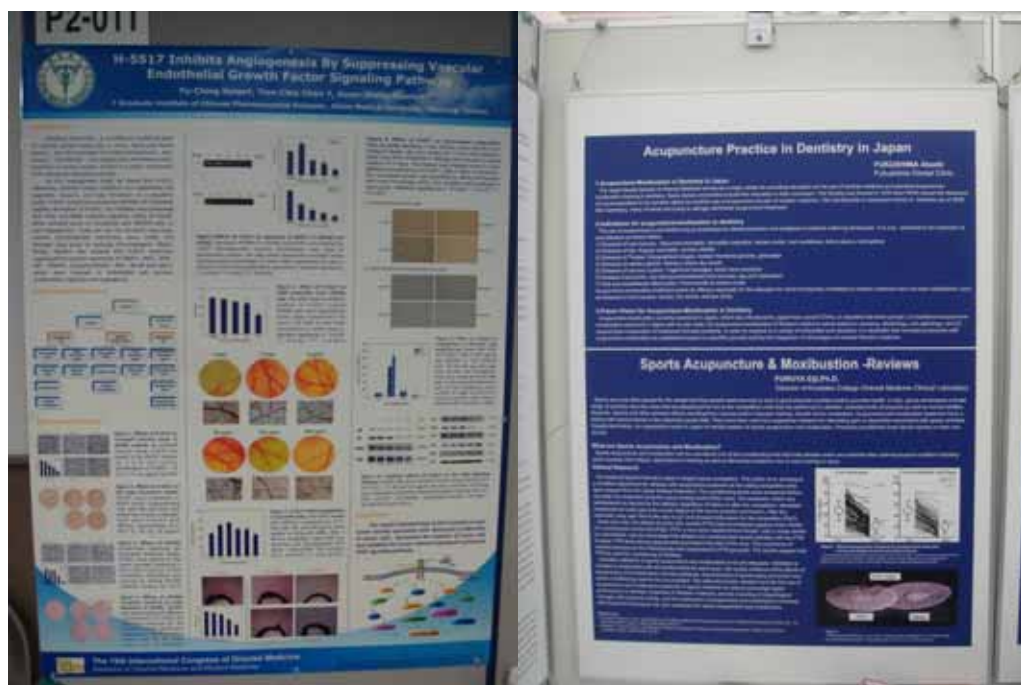
金額，第一名為人參 619041 millions NT、第二名為當歸 112668 millions NT 等二十種中藥材，而整個台灣每年在中藥材上的市場交易金額為 2.42 billions NT，而台灣每年外銷的中藥材交易金額為 0.635 billions NT，還有每年健保給付在中藥上 5.73 billions NT，而用在日常保健食品上的支出為 8.5 billions NT。從這個歸納可發現在台灣的中藥市場，以及中藥在台灣著重的使用方向，而讓各界提高對於台灣市場的了解。

會議的第三天，主要以張貼壁報論文為主，在張貼的過程中，發現許多來自台灣的研究單位，如：中山醫學大學、台北醫學大學、中國醫學大學等數間醫學研究單位。而各國研究的切入點不盡相同，像是日本、韓國大多以傳統醫學為根據，分析研究的結果，而台灣大多以西方醫學為切



入，如：動物實驗、細胞培養等方法來探討傳統醫學的研究。從實驗的材料而言，日本、韓國大多以針灸、口部醫學、推拿等非藥物的研究，而台灣大多以中藥為主要的研究。整個壁報論文的張貼，其實就像是個學術交流，每個實驗室都把自己的研究成果張貼出來，而讓有興

趣的人去了解，一方面提高作者研究結果的知名度，一方面提高各界一個研究的觀點。

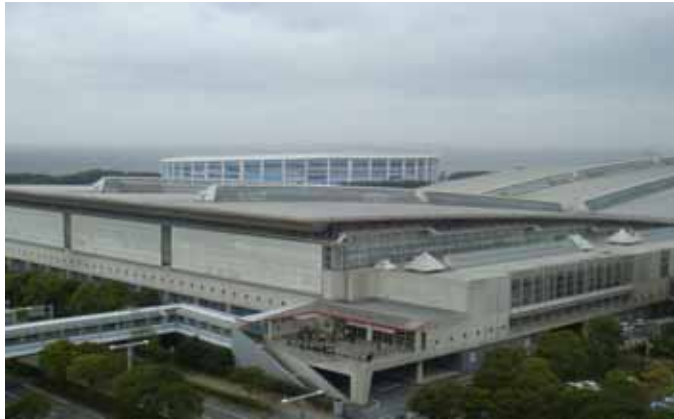


為期三天的會議，讓我覺得國際化的趨勢，每個國家或民族在研究的探討、論點、切入的觀點不盡相同，透過國際性的會議，讓彼此能交換彼此間的想法，促發更多的研究熱潮。而其中最基本的門檻，就是語文的能力，像在口頭報告時，演說者全程都以英文為主，因此英文的聽力格外重要，有基本的能力，才能與國際接軌。而從這個會議中，了解到各國傳統醫學的動向與市場，而提供自己對於未來該培養的能力與做一個國際人該具備的條件做一個準備。

第十五屆國際東洋醫學年會心得報告

學生：陳柏村（中山醫學大學/醫事檢驗暨生物技術學系）

去年暑假就開始準備投稿第 15 屆國際東洋醫學年會，而且還練習撰寫摘要；讓自己能夠更加了解實驗的內容和其特色。去年年底收到通知有通過壁報論文 (poster)，便開始整理數據和撰寫實驗的簡單流程、目的和結果，再進行壁報的排版；也多次的口頭訓練如何在外國人前簡單扼要的說明自己的實驗和回答相關問題。



(幕張國際會議中心)

這是我第一次參加國際型的學術會議，而且還是去到鄰近的日本。去參加國際型的會議可以多多充實學術上的知識，還可以訓練自己的英文能力。行前也做了許多的功課，如何從機場搭巴士到飯店還有飯店到幕張國際會議中心、該注意日本的氣候和國際上的禮儀。



(2/27 位於 201 室-第 1 場講說)

2 月 27 日會議期間有許多的演講場次，每場演講都事先安排好了。所以同一段有好幾場次的不同演講，內容都是一些最新的知識或是一些領域上的翹楚演講。雖然都想去聽，但是只能挑一個最有興趣的聽，聽完這個演講就馬上趕場，為了得到最新知識或是一些研究方向。在聽演講時，最大的問題就是語言能力的問題，要在短短時間內聽懂並且把整個內容有系統的連貫起來是有點難度的；每位演講者大多以英文演講，雖然會有各國不同的腔調，有時會一時無法會意或是聽不懂。對於第一次參加國際型的學術會議的我有點吃力，不過還是很認真的聆聽。如果真的聽不懂時，會議後便可以和教授或同學進行討論或請教。



(2/28 位於壁報論文會場)

2月28日會議期間是參加本次會議最重要的事，為張貼壁報論文 (poster) 的時間。在蔡女滿老師的指導下，我們總共張貼了四篇有關輔助另類醫療(Complementary and Alternative Medicine, CAM)的壁報論文(編號：P2-076~P2-079)。在壁報論文的會場面對陌生的環境和陌生的語言，就退縮了。而對於這樣的表現，感到非常的不好意思，也錯過了很好的學習方式；也不夠有自信去講說自己的實驗的內容，害怕解說不清楚和害怕聽不懂他人的提問。

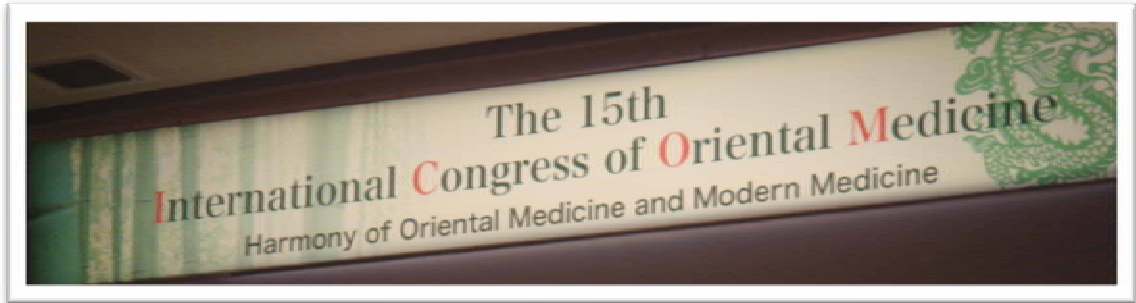


(學生陳柏村所發表的壁報論文)

今後會更加努力的補強英文的表達能力、理解能力和邏輯思考能力，並且加強專業知識的學習和充實。有這一次難得的經驗，使自己不再侷限於小小的台灣；反而使自己的國際觀打開，學習新知識不再受限於中文。也感謝 蔡女滿 老師的用心教導，讓我們有這麼一次的良好學習機會，也藉著這個機會來省思自己所不足的地方和如何加以改善。希望我能夠記取教訓，未來的我能盡自己最大的能力把每一件事完成，並且把所學的回饋於社會。

第十五屆國際東洋醫學年會心得報告

學生：陳盈如(中山醫學大學/醫事檢驗暨生物技術學系)



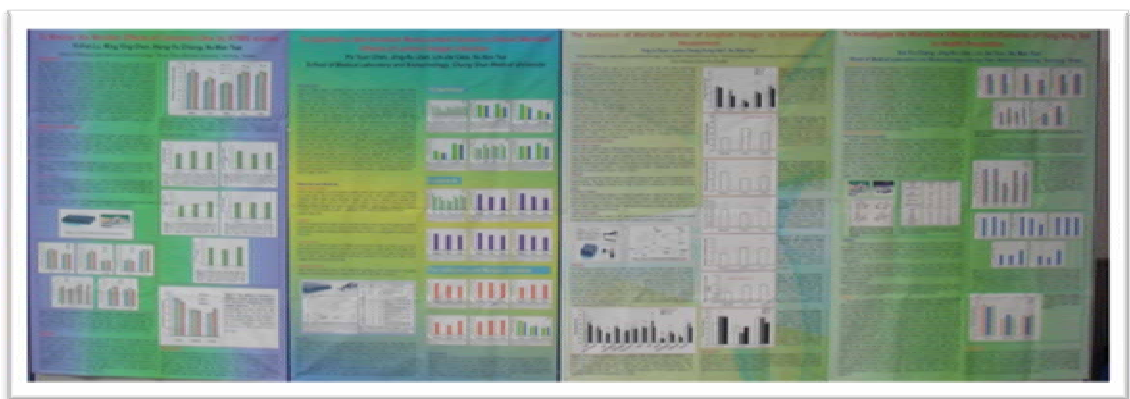
今年二月，獲得了難得的機會可以參加「第十五屆國際東洋醫學年會」。還記得去年，我們還是跟隨在老師和學姊們後頭見習的幼幼班，今年升級換做自己要參加學術會議，發表壁報論文。

第一次參加國際學術會議，除了學術的挑戰之外，更夾帶著語言的考驗。出發前心中百感交集，懷著期待，摻著緊張，伴著好奇，混著不安。我們一行人在蔡女滿老師的帶領下，前往日本東京。



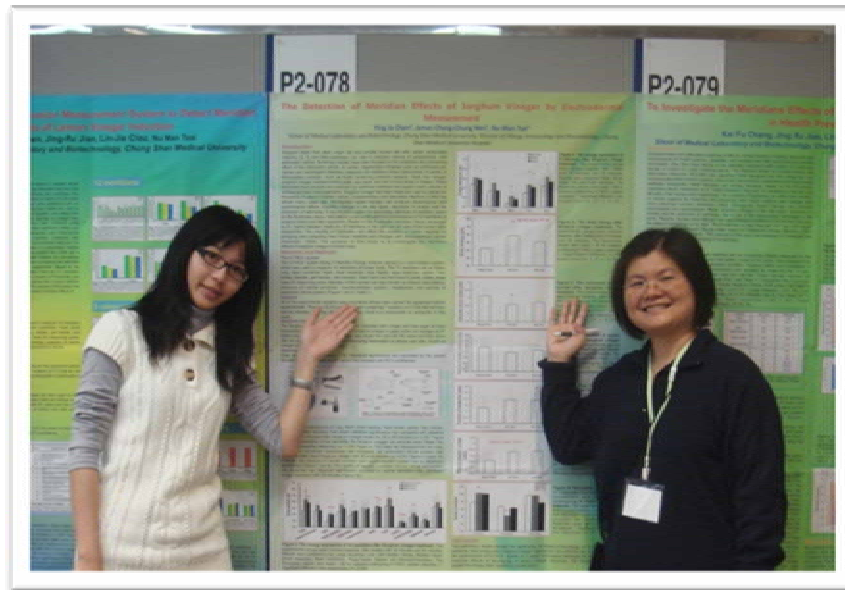
二月二十七日早晨，我們頂著日本 14°C 的低溫，抵達位於千葉

縣的國際會議廳。感受到日本人守時的優良傳統，每場演講都走在訂好的時間軸上。由於同一時間有不同場次的演講，雖然都想聽卻也分身乏術，只好有所取捨，有時甚至還得奔走於不同的會議室，為了能夠學到新知，我們也樂此不疲。像個認真的學生靜坐在會議室裡，聆聽台上的講者滔滔不絕的分享種種，首當其衝面臨的就是語言能力的問題。雖然每位前輩講的都是英文，但是對於在台灣鮮少練習英文聽力的我們，可以說是吃力至極。英文已經不是只分英倫腔和美國腔兩類而已，在亞洲國家，台灣、日本、韓國講英文的腔調都富含道地的口音，這是我始料未及的事。除了語言能力的衝擊之外，知識源源不絕的灌注也讓腦袋片刻不得閒，當學得的越多，就越感到自己無知。在輔助與另類醫療領域中，我是個剛入門的新生，專業知識的學習尚未到「懂」的階段，許多的專有名詞、學術用語、以及研究的思考邏



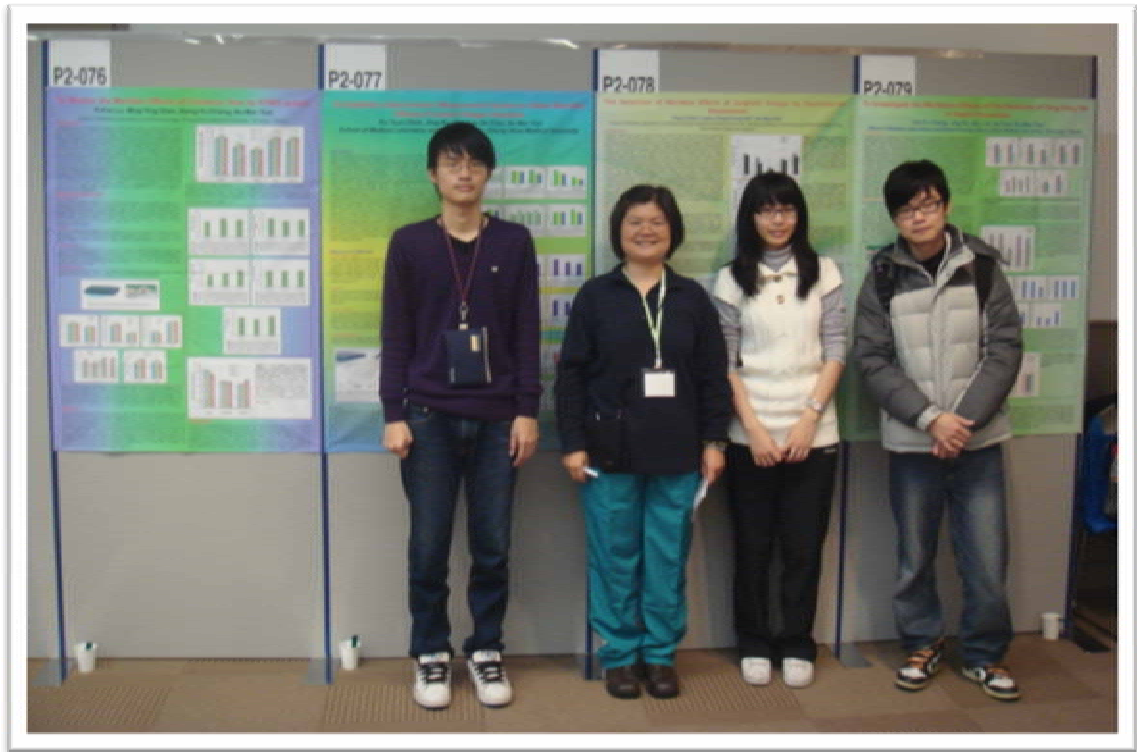
輯等等…，都是我要努力學習的方向。

二月二十八日上午，行事曆上記載張貼壁報論文的時刻，此次行程，我們團隊在蔡女滿老師的指導下，一共張貼了四篇的壁報論文。




雖然在台灣已經練習了一次又一次，到了現場面對陌生了環境陌生的語言仍舊卻步了。而對於今日的表現，除了慚愧之外，更覺得自己不夠具有學習的精神。在 Q&A 的時段中，不敢踏出第一步主動向人解釋自己的研究，害怕解釋不清也害怕聽不懂對方的問句。而這，即犯下學習最根本的錯誤。在回程中檢討，才發現自己錯過了學習的最佳機會，就算語言是個障礙，科學的本質依然存在，不應該因此而退縮不前，自我侷限。這樣不僅對不起自己，更加浪費了老師及社會給予的這個機會。

回到台灣，我會更加積極的練習外語聽說讀寫的能力，更加深入的去了解台灣這片土地，廣泛吸收科學的新知與新聞，練習對於科學邏輯思考的靈敏度，加強專業知識的學習，以及做人處事的進退和責任。



圖：左起依序為陳柏村、蔡女滿老師、陳盈如、張凱復

慶幸自己擁有這麼好的機緣，也感謝蔡女滿老師的用心，讓我們可以在生命的記錄本上，揮灑這豐富的經歷。此趟旅程，在時針像前撥一格的土地上，眼光也向前了一點，深感到自己還有許多地方需要好好學習，細細琢磨。見識到如何在語言不通的環境中自在優遊、學習國際禮儀、觀摩各區域學術研究的動向、了解自己在研究及學習時所欠缺的能力與態度、訓練邏輯思考的靈敏度等等…。「不懂不羞恥，不學方慚愧」，秉持著這樣的精神，希望在三月底的生物醫學年會中，會有更進步的表現。在學術的領域上繼續學習，也期勉有朝一日，能夠將所學回饋於社會。

日期: Thu, 14 Jan 2010 16:19:52 +0900 

寄件者: "[The 15th International Congress of Oriental Medicine Secretariat](#)"
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- [A00232 - A00392](#)
- [A00393 - A00483](#)

Registration No.	Poster No.	Session Theme	Presentation Date
A00339	P2-076	Traditional Herbal Medicine (Basic): Ryodoraku	Sun., 28, Feb.
A00340	P2-077	Traditional Herbal Medicine (Basic): Ryodoraku	Sun., 28, Feb.
A00341	P2-078	Traditional Herbal Medicine (Basic): Ryodoraku	Sun., 28, Feb.
A00342	P2-079	Traditional Herbal Medicine (Basic): Ryodoraku	Sun., 28, Feb

To Monitor the Meridian Effects of Cinnamon Dew by KYMS system

Nu-Man Tsai, Yi-Fon Lu, Ming Ying Chen, Hsing-Yu Chiang

School of Medical Laboratory and Biotechnology, Chung Shan Medical University, Taichung, Taiwan.

Introduction

Complementary and alternative medicines (CAM) which covering a wide range are away from modern western medicines. The National Center for CAM (NCCAM) classified CAM as 5 parts, including alternative medical system, mind-body interventions, biologically based therapy, manipulative and body-based methods, and energy therapies. The National Institute of Health statistics reports that according to a nationwide government surveys from 2007 to December 2008, approximately 40% of Asian adults use CAM. Many previous researches has shown that the prevalence of CAM use steadily increased, suggesting that more and more people start to accept alternative medicines in their life. In 1949, the Ryodoraku theorem has published by Dr. Yoshio Nakatani. This theorem is using to study the electrical conductance meridian. The KYMS system, an non-invasive system, is based on Ryodoraku theorem that can measure energy expression in 12 meridians of human body and evaluate the body healthy. The natural products are the most common therapies of CAM among adults (17.7%). In biologically based therapies, which involves functional foods are used widely. The cinnamon is a common herb in traditional Chinese medicine (TCM) that can dilate terminal blood vessel, advance blood circulation. In recent researches have proved that the cinnamon-derived impairs tumor growth. However, the energy regulation of cinnamon to human body is unclear. The purpose of this study is to determine the meridian effects of cinnamon dew in human body.

Materials and Methods

K.Y.M.S. system

The K.Y.M.S. system (Figure 1) is a non-invasive system, was used to measure 12 meridians of human body. The 12 meridians are as follow : lung, heart constrictor, heart, small intestines, triple heater, large intestines, spleen, liver, kidney, bladder, gall bladder and stomach. Each hand and foot have 6 points, totally, there have 24 measuring points. Besides, KYMS system can also monitor as follows : body energy, pressure of mental condition, muscle-skeleton system, metabolism, and activity of autonomic nerves system.

Subjects

All of the experimental subjects were volunteers whose were signed the agreement before experimented. They are 18-35 year-old people containing 21 subjects (n=21) that didn't have serious disease. Keeping relaxed and happy mood is a prerequisite to participate in this study.

Cinnamon dew

The *Cinnamomum osmophloeum* Kanehira is endemic species of Taiwan treated with distillation by high temperature and collected cinnamon dew in glass bottle and storage at RT. Before experiment, the Cinnamon dew was diluted six fold with RO water and then drank 300 ml each subject. Determination bio-energy expression at before and after cinnamon dew drank for 30 and 60 minutes.

Statistical analysis

Data showed as mean values \pm SE. Statistical significance had calculated by the paired Student's *t*-test to analysis and P values had indicated with 95 % confidence.

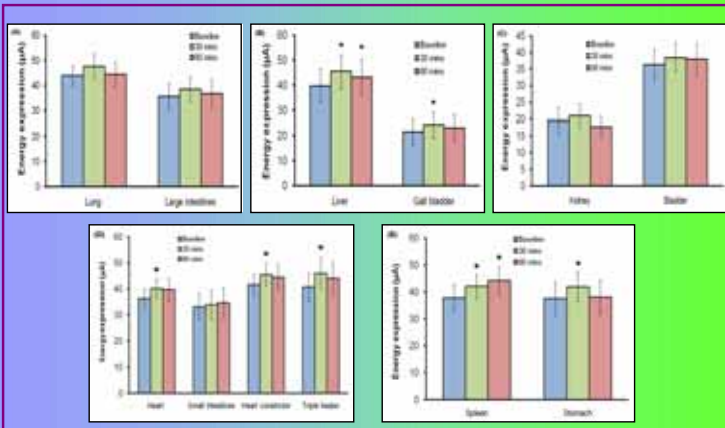


Figure 2. The energy expression of 12 meridians after cinnamon dew treatment. The experimental groups were involved baseline, after treated with 30 minutes and 60 minutes. (A) Lung and Large intestines ; (B) Liver and Gall bladder ; (C) Kidney and Bladder ; (D) Heart, Small intestines, Heart constrictor and Triple heater ; (E) Spleen and Stomach. The Ryodoraku values were mean \pm SE for 21 subjects undergoing K.Y.M.S. system detection. *: Significant different in the experiments ($P < 0.05$).

Results

The energy expression of liver, gall bladder, heart, heart constrictor, triple heater, spleen and stomach meridian had shown statistical significance ($p < 0.05$) that compared with baseline were evident increased after cinnamon dew treated with 30 minutes. (Figure 2. B,D,E) As the conception of five elements, the cinnamon dew can promote metal, wood, fire and earth properties effectively. (Fig. 3.) The data had displayed that the body energy had increased from 35.3 ± 3.566 to 38.7 ± 3.569 after drank cinnamon with 30 minutes. (Fig. 4.) However, the pressure of mental, muscle-skeleton system, metabolism and autonomic nerves system had no statistical significance ($p < 0.05$). (Fig. 5-8) The healthy of meridian in subject include normal, acceptable and abnormal had no statistical significance ($p < 0.05$) after cinnamon dew treatment with 30 minutes and 60 minutes. (Fig. 9.)

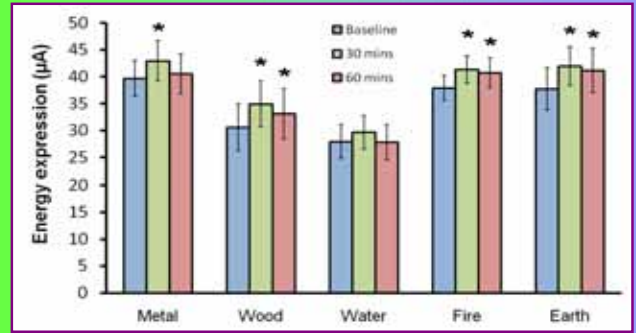


Figure 3. The energy expression of 5 elements after cinnamon dew treatment. The experimental groups were involved baseline, after treated with 30 minutes and 60 minutes. The Ryodoraku values were mean \pm SE for 21 subjects undergoing K.Y.M.S. system detection. *: Significant different in the experiments ($P < 0.05$).

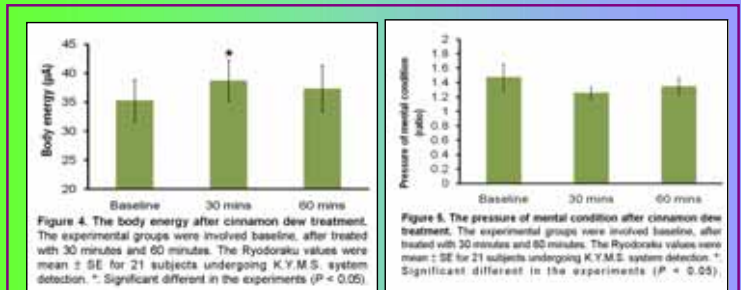


Figure 4. The body energy after cinnamon dew treatment. The experimental groups were involved baseline, after treated with 30 minutes and 60 minutes. The Ryodoraku values were mean \pm SE for 21 subjects undergoing K.Y.M.S. system detection. *: Significant different in the experiments ($P < 0.05$).

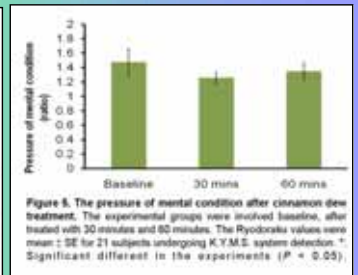


Figure 5. The pressure of mental condition after cinnamon dew treatment. The experimental groups were involved baseline, after treated with 30 minutes and 60 minutes. The Ryodoraku values were mean \pm SE for 21 subjects undergoing K.Y.M.S. system detection. *: Significant different in the experiments ($P < 0.05$).

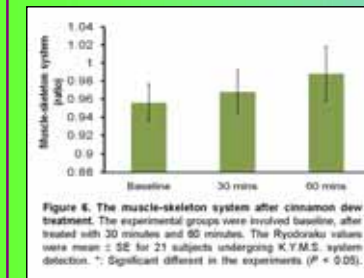


Figure 6. The muscle-skeleton system after cinnamon dew treatment. The experimental groups were involved baseline, after treated with 30 minutes and 60 minutes. The Ryodoraku values were mean \pm SE for 21 subjects undergoing K.Y.M.S. system detection. *: Significant different in the experiments ($P < 0.05$).

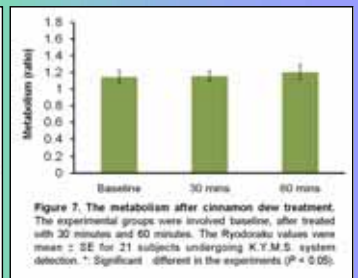


Figure 7. The metabolism after cinnamon dew treatment. The experimental groups were involved baseline, after treated with 30 minutes and 60 minutes. The Ryodoraku values were mean \pm SE for 21 subjects undergoing K.Y.M.S. system detection. *: Significant different in the experiments ($P < 0.05$).

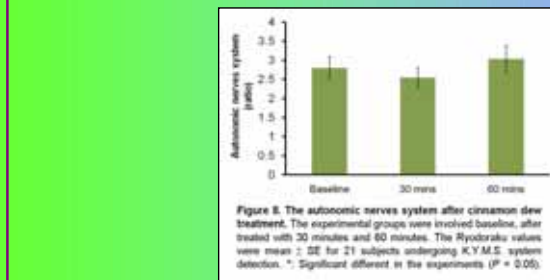


Figure 8. The autonomic nerves system after cinnamon dew treatment. The experimental groups were involved baseline, after treated with 30 minutes and 60 minutes. The Ryodoraku values were mean \pm SE for 21 subjects undergoing K.Y.M.S. system detection. *: Significant different in the experiments ($P < 0.05$).

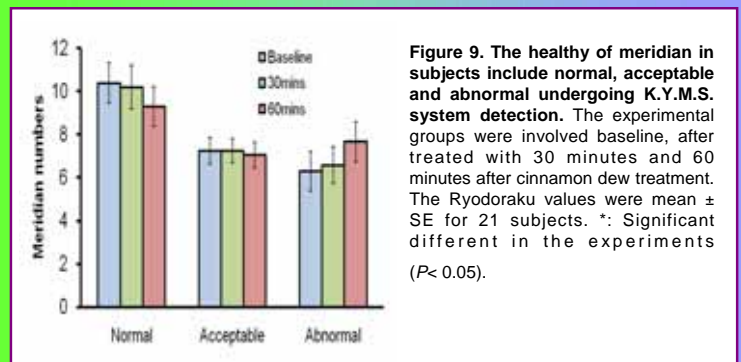


Figure 9. The healthy of meridian in subjects include normal, acceptable and abnormal undergoing K.Y.M.S. system detection. The experimental groups were involved baseline, after treated with 30 minutes and 60 minutes after cinnamon dew treatment. The Ryodoraku values were mean \pm SE for 21 subjects. *: Significant different in the experiments ($P < 0.05$).

Discussion

The data had displayed that the body energy had increased after drank cinnamon with 30 minutes which were represented the cinnamon dew can just only increased body energy in a short period. In liver, gall bladder, heart, heart constrictor, triple heater, spleen and stomach meridians were increased after cinnamon dew treatment with 30 minutes. According to traditional Chinese medicines (TCM), the cinnamon is useful to liver, heart, spleen and kidney meridians. This study proved that the traditional Chinese medicines are worthy and the cinnamon dew can effects some meridians and body energy in human being.

To Investigate the Meridians Effects of Fire Elements of Yang Xing Tea in Health Prevention

Nu Man Tsai, Kai Fu Chang, Jing Ru Jian, Lin Jie Ciou

School of Medical Laboratory and Biotechnology, Chung Shan Medical University, Taichung, Taiwan.

Introduction

Complementary and alternative medicine (CAM) is a broad domain of healing resources that encompasses all health systems, modalities, and practices and their accompanying theories and beliefs, other than those intrinsic to the politically dominant health system of a particular society or culture in a given historical period. The National Center for Complementary and Alternative Medicine (NCCAM) has classified CAM therapies into five categories that including alternative medical systems, mind-body interventions, manipulative and body-based methods, biologically based therapies and energy therapies. Based on the Ryodoraku (meridian) theory, developed by Dr. Yoshio Nakatani, there are 12 meridians on the right and left side of the human body respectively. The property of Ryodoraku can reflect the condition of certain organ(s) by analyzing and comparing their mutual relations and changes with micro-electrical current (Nakatani, 1956). Biologically based therapy can produce physiological and psychological effects, including changes in the vital signs, reductions in anxiety and the promotion of well-being. Tea is one of the most popular beverages in the world. Recently, studies show that tea has many biological functions in the human body, but the effect of bio-energy is not clear. The fire element of Yang Xing tea (YX-F tea) with natural energy, selection by Chinese Qigong healer based on Traditional Chinese Medicine (TCM) theory, was chose in this study. The purpose of this study had designed to investigate the meridian effects of YX-F tea drinking in human body.

Materials and Methods

The K.Y.M.S. system operation

Detection of computer software interface can be connection directly measured by the survey and measure of the hand and foot points, a total of 24 points, direct-measured by value, and automatic analysis of physical condition. Subjects settled and maintained a relaxed for testing. Electrical body conductivity had recorded by measuring the 12 main acupuncture channels bilaterally on the hand and the foot. These channels represent all functional systems of the human organism (Fig.1 and Table 1). Comparison the Ryodoraku points (Ryo points) and the Traditional Chinese Medicine Points (TCM points) has listed in Table1. The meridians are as follows: Lung (LU 9, H1), Large intestines (LI 5, H6), Liver (LV 3, F2), Gall bladder (GB 40, F5), Kidney (KI 4, F3), Bladder (BL 65, F4), Heart (HT 7, H3), Small intestines (SI 5, H4), Heart constrictor (HC 7, H2), Triple heater (TH 4, H5), Spleen (SP 3, F1), Stomach (ST 42, F6).

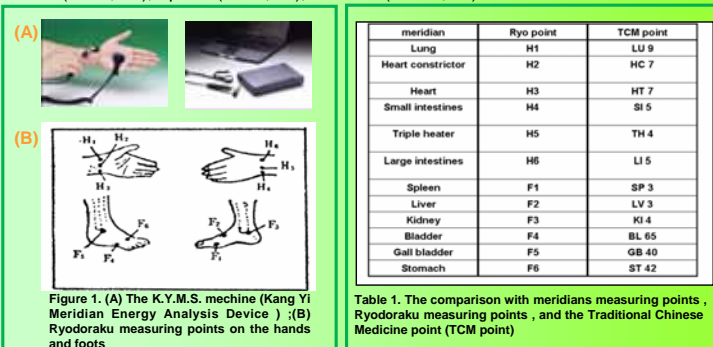


Table 1. The comparison with meridians measuring points , Ryodoraku measuring points , and the Traditional Chinese Medicine point (TCM point)

Subjects

The 26 subjects were volunteers and they know what they are drinking. Before detection, all subjects had to take off the watch, necklace, and cell phone. Because of those could interference K.Y. M.S. machine determination. The study designed to detect baseline and XY-F tea 400 ml drinking in 10 minutes with each subject for 30 and 60 minutes. Last, subjects had measured the bio-energy at pretest-posttest by K.Y. M.S. System.

Tea selection

The Yang Xing tea, selection by Chinese Qigong healer, with natural energy had purchased from Nature Return Cultural Enterprise Company (Taichung, Taiwan) and based on Traditional Chinese Medicine theory, which had wood (YX-W), fire (YX-F), earth (YX-E), metal (YX-M), and water (YX-W) elements. Finally, we chose the fire element of Yang Xing tea for this experiment.

Statistical analysis

Data showed as mean values \pm SE. Statistical significance had calculated by the paired Student's t-test for 30 minutes and unpaired Student's t-test for 60 minutes after YX-F-tea treatment. The P values that smaller than 0.05 ($P < 0.05$) were significance.

Results

The results showed the mean \pm SD of the Ryodoraku responses in 24 measuring points were significantly increased 12 meridians and 5 elements (which included Metal, Wood, Fire, Water element, and Earth elements) after YX-F-tea treatment ($P < 0.05$, Figure 2). The results showed that the bioenergy of bladder, small intestine, and spleen meridians had increased after YX-F-tea treatment for 30 minutes. On the other hand, we observed bioenergy of ten meridians including lung, large intestine, liver, gall bladder, kidney, heart, small intestine, heart constrictor, triple heater and stomach meridians were significantly decreased than baseline after YX-F-tea treatment for 60 minutes, and we verified the effects of YX-F-tea treatment were slowly and affect to whole body. The result shows that the YX-F-tea treatment for 30 minutes could make body energy transient to raise, and soon to decrease below baseline ($P < 0.05$). The ratio of the pressure of mental condition was the relationship between upper and lower. The baseline was much lighter, and after YX-F-tea treatment for 60 minutes, the ratio approached to one, as well as reached a balance ($P < 0.05$). The ratio of left and right muscle-skeleton system on baseline and after YX-F-tea treatment was similarly approach to one. That shows the YX-F-tea still maintain a balance ($P < 0.05$). The ratio of Yin and Yang metabolism increase after the YX-F-tea treatment, and the condition was also in a reasonable range, making the body to approach to a balance ($P < 0.05$). The ratio of the autonomic nerves system was no significantly between groups ($P < 0.05$). The healthy of meridian indicated balance of body system in subjects include normal, acceptable, and abnormal undergoing K.Y.M.S. system detection. There was significantly increased in number of acceptable meridians after tea treatment ($P < 0.05$, Figure 4).

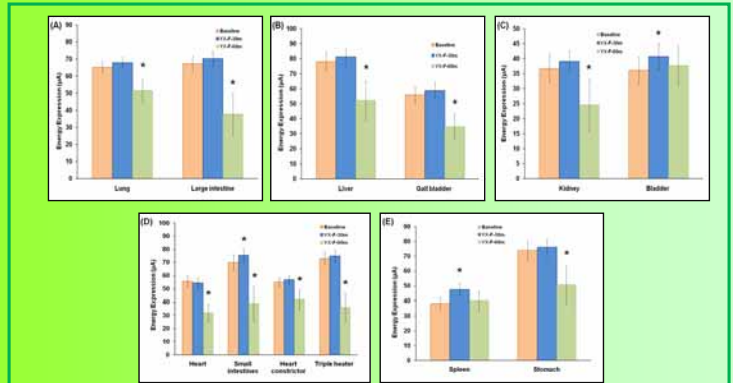


Figure 2. Energy expression of the 12 meridians before (baseline) and after YX-F-Tea treatment

The measurement groups were contained before and after YX-F-tea treatment for 30 and 60 minutes. (A) Metal include lung and large intestine meridians, (B) Wood include liver and gall bladder meridians, (C) Water include kidney and bladder meridians, (D) Fire include heart , small intestine, heart constrictor and triple heater meridians, (E) Earth include spleen and stomach. The Ryodoraku values were mean \pm SE for subject undergoing K.Y.M.S. system. *:Significant different compared with baseline ($P < 0.05$).

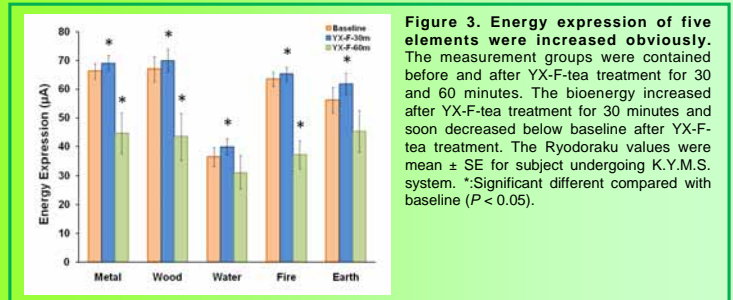


Figure 3. Energy expression of five elements were increased obviously.

The measurement groups were contained before and after YX-F-tea treatment for 30 and 60 minutes. The bioenergy increased after YX-F-tea treatment for 30 minutes and soon decreased below baseline after YX-F-tea treatment. The Ryodoraku values were mean \pm SE for subject undergoing K.Y.M.S. system. *:Significant different compared with baseline ($P < 0.05$).

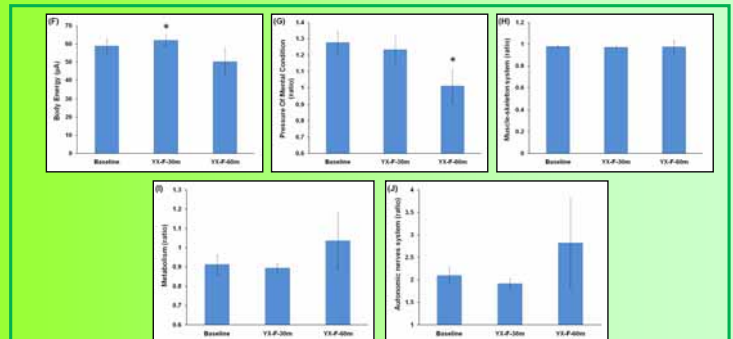


Figure 4. (G) The ratio of the pressure of mental condition was the relationship between upper and lower. The baseline was much lighter, and after YX-F-tea treatment for 60 minutes the ratio approached to 1, as well as reached a balance. (H) The ratio of left and right muscle-skeleton system on baseline and after YX-F-tea treatment was similarly approach to one. That shows the YX-F-tea still maintain a balance. (I) The ratio of Yin and Yang metabolism increase after the YX-F-tea treatment, and the condition was also in a reasonable range, making the body to approach to a balance. (J) The ratio of the autonomic nerves system was no significantly between groups.

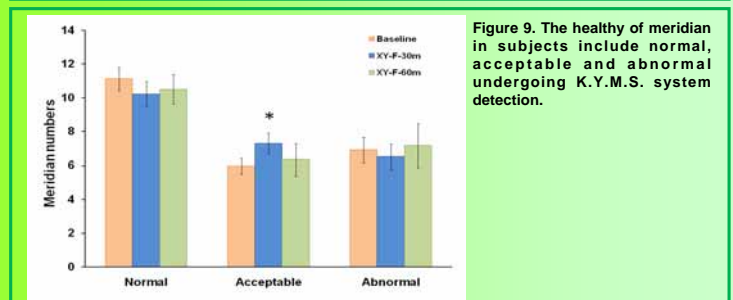


Figure 9. The healthy of meridian in subjects include normal, acceptable and abnormal undergoing K.Y.M.S. system detection.

Discussion

We observed the data those 12 meridians and 5 elements after YX-F tea treatment for 60 minutes were decrease, and let people to relax. The results showed the significantly increased of bio-energy of 12 meridians, 5 elements, body energy after XY-F tea treatment for 30 minutes. The balance of body system became healthier. In conclusions, the measurement of Ryodoraku is a useful noninvasive technique for the evaluation meridian effects of bio-energy of tea induction. The improved balance in the body and mind indicates that drinking XY-F tea has a potential to improve human health.

To Establish a Non-Invasion Measurement System to Detect Meridian Effects of Lemon Vinegar Induction

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Introduction

Lemon is widely used in medicine due to rich citric acid and Vitamin C content, which helps the body fight off colds. In addition, lemon juices have a liver stimulant and have the effect of anti-oxidation. It also can control irritable bowel syndrome and conditions like constipation and diarrhea. Lemon juice also has potassium, which is helpful for the brain and nerve cells. With some magnesium, it can help to treat some ailments such as asthma, colds, scurvy, fever, and heartburn. Lemon Vinegar has been made and used by people for thousands of years. Traces of it have been found in Egyptian urns dating from around 3000 BC. Many remedies and treatments have been ascribed to vinegar over millennia and in many different cultures. However, few have been verifiable using controlled medical trials and being effective to some degree have significant effects and carries the possibility of serious health risks. Complementary and Alternative Medicines(CAM) are defined as medical and health care systems, practices, and products that are not currently considered an integral part of conventional medicine it is viewed as an alternative if the patient decides to use it in place of a prescribed medical treatment. Many studies done in western countries have documented that CAM use is both common and varies among populations. A non-invasion system for bio-energy detection of meridian, based on the Ryodoraku theory, had used to measure the electrical conductance of 12 meridians of subjects before and after experiment. Based on the Ryodoraku (meridian) theory, developed by Dr. Yoshio Nakatani, there are 12 meridians on the right and left side of the human body respectively. The property of Ryodoraku can reflect the condition of certain organ(s) by analyzing and comparing their mutual relations and changes with micro-electrical current (Nakatani, 1956). The purpose of this study had designed to establish a non-invasion measurement system to detect meridian effects of lemon vinegar induction.

Materials and Methods

The K.Y.M.S. system

The K.Y.M.S. system is a non-invasive system, which was used to measure 12 meridians of human body. The 12 meridians are as follow: lung, heart constrictor, heart, small intestines, triple heater, large intestines, spleen, liver, kidney, bladder, gall bladder, and stomach. Each hand and foot have 6 points, totally, there have 24 measuring points. Besides, KYMS system can also monitor as follows: body energy, pressure of mental condition, muscle-skeleton system, metabolism, and activity of autonomic nerves system.(Figure 19).

Subjects

All of the experimental subjects were volunteers whose were signed the agreement before experimented. They are 20-35 year-old people containing 17 subjects (n=17) that did not have serious disease. Keeping relaxed and happy mood is a prerequisite to participate in this study.

Lemon vinegar

The Lemon is endemic species of Taiwan extracted with vinegar and less sugar at room temperature for one month and collected Lemon vinegar in glass bottle and storage at RT. Before experiment, the Lemon vinegar was diluted five fold with RO water and then drank 300 ml each subject. Determination bioenergy expression at before and after Lemon vinegar drank for 30 and 60 minutes.

Statistical analysis

Data showed as mean values ± SE. Statistical significance had calculated by the paired Student's t-test to analysis and P values had indicated with 95 % confidence.

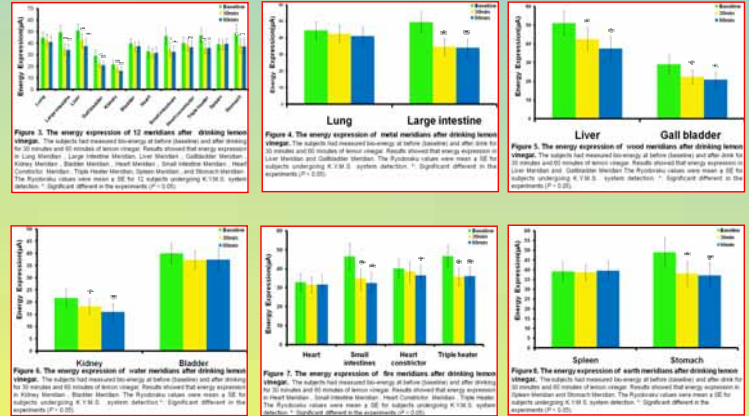
Meridians	Ryodoraku points	TCM points	Relative organ (tissue)
Lung	HL	LU9	Skin, hair, respiratory system, lung, nose
Pericardium	PC	PC7	Heart, blood vessels, spirit (pericardium)
Heart	HT	HT7	Brain, heart, tongue
Gall bladder	GB	GB43	Stomach, digestion, absorption/excretion, spirit (damp)
Triple energizer	TE	TE4	Lymphatic system, information (intracranially transmitted damp)
Large intestine	LI	LI4	Skin (hand and foot), nose, respiratory passages, digestion, the excretory system
Spleen	SP	SP9	Stomach, digest gland, digestion, central blood, endocrine system, immune system, muscle mass, spirit
Liver	LR	LR3	Genitals, endocrine system, spirit, liver, eye, urinary tract, head, stress and concentration
Kidney	KI	KI6	Adrenal glands, urinary tract, genital, feet, skeletal system, endocrine system, ear, spirit
Urinary bladder	UB	UB6	Urinary bladder, kidney, urinary tract, genital, head, hip, urinary system
Colonizer	CO	CO4	Stomach, head, nerve, spirit, ear
Bladder	BL	BL42	Stomach, endocrine, excretory glands, teeth and gums, spirit, digestion, hair, acutely transmitted disease

Table 1. Functional associated with each meridians: Corresponding the designation of Ryodoraku points, TCM measuring points, and functionally relative organ (and tissue) to each meridians. TCM: Traditional Chinese Medicine.

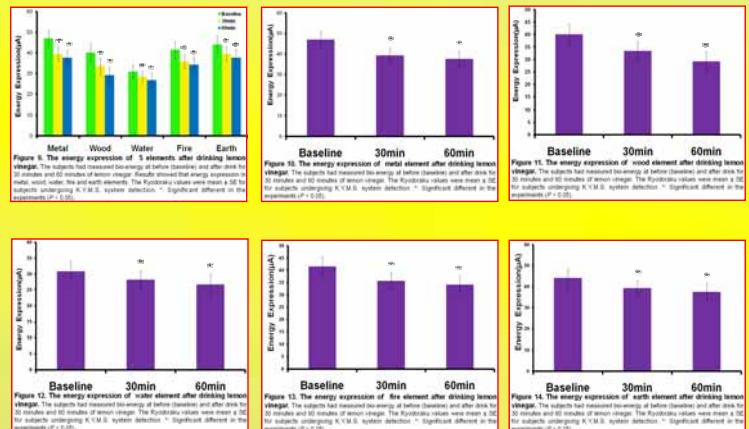
Results

The bioenergy expression of liver, gall bladder, heart, heart constrictor, triple heater, spleen and stomach meridian had shown statistical significance that compared with baseline were evident increased after lemon vinegar treated with 30 and 60 minutes ($p < 0.05$; Figure 1 to 6). As the conception of five elements, the lemon vinegar can promote metal, wood, fire and earth properties effectively ($p < 0.05$; Figure 7 to 12). The data had displayed that the body energy had decreased, but metabolism function had increased after drank lemon vinegar with 30 and 60 minutes ($p < 0.05$; Figure 13 and 16). However, the pressure of mental, muscle-skeleton system and autonomic nerves system had no statistical significance (Figure 14, 15 and 17). The healthy of meridian in subject include normal, acceptable and abnormal had no statistical significance after lemon vinegar treatment with 30 minutes and 60 minutes (Figure 18).

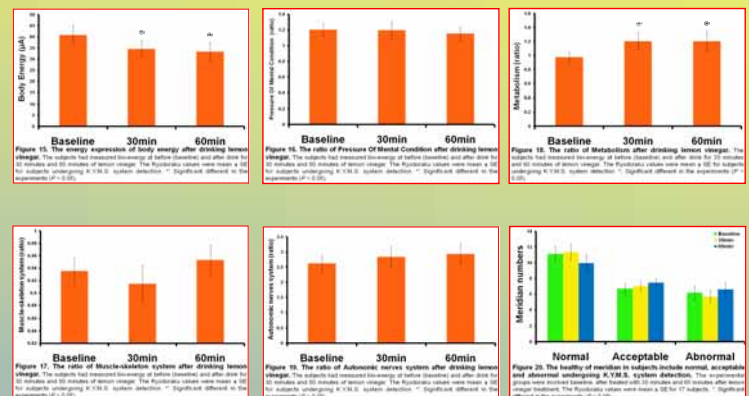
12 meridians



5 elements



Five indicators and Meridian numbers



Discussion

The preliminary results showed the significantly decreased of bio-energy of 12 meridians, 5 elements, body energy after lemon vinegar treatment for 30 and 60 minutes. The data had displayed that the body energy had decreased after drank lemon vinegar with 30 and 60 minutes. In conclusions, the measurement of K.Y.M.S. system is a useful noninvasive technique for the evaluation meridian effects of bio-energy of lemon vinegar induction. The lemon vinegar caused bio-energy down regulate in human body.

The Detection of Meridian Effects of Sorghum Vinegar by Electrodermal Measurement

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Introduction

Sorghum foods from plant origin not only provide human diet with certain antioxidant vitamins (C, E and beta-carotene), but also a complex mixture of polyphenols, with antioxidant activity. Numerous studies have been focused on the protective and preventing effect of this antioxidant activity on certain degenerative illnesses such as cardiovascular, cancer, and neurological diseases, cataracts and oxidative stress dysfunctions. A variety of fermentation products, such as foods containing probiotic bacteria, black rice vinegar, sorghum vinegar, soybean-barley paste, soy sauce, are sold in food stores in Taiwan. These fermented food products are produced by traditional methods that exploit mixed cultures of various non-toxic microorganisms. Sorghum Vinegar has been made and used by people for thousands of years in Eastern culture. Complementary and alternative Medicine (CAM) has shown many years ago. Biologically based therapy can produce physiological and psychological effects, including changes in the vital signs, reductions in anxiety and the promotion of well-being. A non-invasive system for bio-energy detection of meridian, based on the Ryodoraku theory, had used to measure the electrical conductance of 12 meridians of subjects before and after experiment. Based on the Ryodoraku (meridian) theory, developed by Dr. Yoshio Nakatani, there are 12 meridians on the right and left side of the human body respectively. The property of Ryodoraku can reflect the condition of certain organ(s) by analyzing and comparing their mutual relations and changes with micro-electrical current (Nakatani, 1956). The purpose of this study is to investigate the meridian effects of sorghum vinegar in human body.

Materials and Methods

The K.Y.M.S. system

The K.Y.M.S. system (Kang Yi Meridian Energy Analysis Device) is a non-invasive system, which was used to measure 12 meridians of human body. The 12 meridians are as follow: lung, heart constrictor, heart, small intestines, triple heater, large intestines, spleen, liver, kidney, bladder, gall bladder, and stomach. Each hand and foot have 6 points, totally, there have 24 measuring points. Besides, KYMS system can also monitor as follows: body energy, pressure of mental condition, muscle-skeleton system, metabolism, and activity of autonomic nerves system (Figure 1 and 2; Table 1).

Subjects

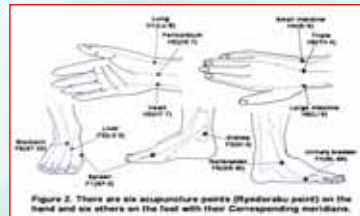
All of the experimental subjects were volunteers who were signed the agreement before experimented. They are 20-35 year-old people containing 7 subjects (n=7) that did not have serious disease. Keeping relaxed and happy mood is a prerequisite to participate in this study.

Sorghum vinegar

The Sorghum is endemic species of Taiwan extracted with vinegar and less sugar at room temperature for one month and collected Sorghum vinegar in glass bottle and storage at RT. Before experiment, the Sorghum vinegar was diluted five fold with RO water and then drank 300 ml each subject. Determination bioenergy expression at before and after Sorghum vinegar drank for 30 and 60 minutes.

Statistical analysis

Data showed as mean values \pm SE. Statistical significance had calculated by the paired Student's *t*-test to analysis and P values had indicated with 95 % confidence.



Results

The bioenergy expressions of lung, heart, small intestine, triple heater, spleen, liver, kidney, and gall bladder meridians had shown statistical significance that compared with baseline were evident increased after Sorghum vinegar treated for 30 or 60 minutes ($p < 0.05$; Figure 3). As the conception of five elements, the Sorghum vinegar can promote wood, Water, fire and earth properties effectively ($p < 0.05$; Figure 4). The data had displayed that the body energy and functional metabolism had increased, but autonomic nerves system had decreased after drank Sorghum vinegar with 30 or 60 minutes ($p < 0.05$; Figure 5, 6 and 16). However, the pressure of mental condition and muscle-skeleton system had no statistical significance (Figure 7 and 9). The healthy of meridian in subject include normal, acceptable and abnormal had statistical significance increased in normal meridians after Sorghum vinegar treatment with 30 minutes (Figure 10).

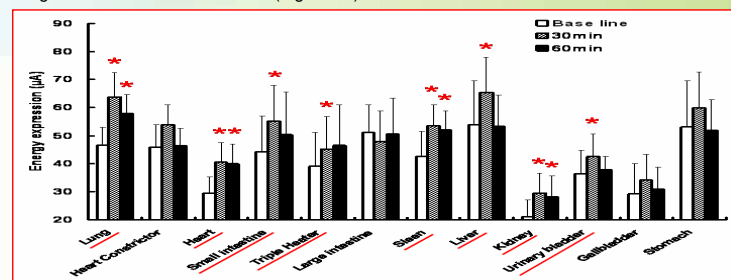


Figure 3. The energy expression of 12 meridians after Sorghum vinegar treatment. The experimental groups were involved baseline, after treated with 30 minutes and 60 minutes. Results were contained Lung, Large intestines, Liver, Gall bladder, Kidney, Bladder, Heart, Small intestines, Heart constrictor, Triple heater, Spleen and Stomach meridians. The Ryodoraku values were mean \pm SE for subjects undergoing K.Y.M.S. system detection. *: Significant different in the experiments ($P < 0.05$).

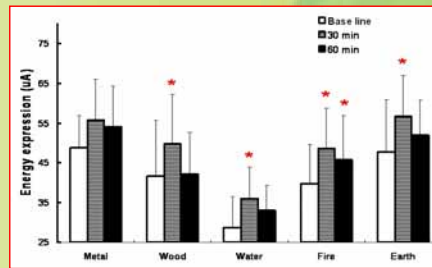


Figure 4. The energy expression of 5 elements after Sorghum vinegar treatment. The experimental groups were involved baseline, after treated with 30 minutes and 60 minutes. Results were contained Metal, Wood, Water, Fire and Earth elements. The Ryodoraku values were mean \pm SE for subjects undergoing K.Y.M.S. system detection. *: Significant different in the experiments ($P < 0.05$).

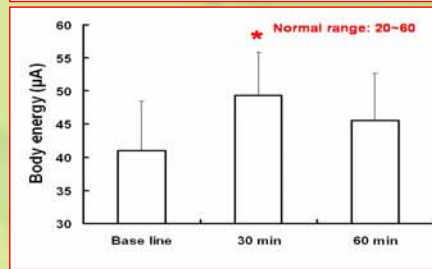


Figure 5. The body energy after Sorghum vinegar treatment. The experimental groups were involved baseline, after treated with 30 minutes and 60 minutes. The Ryodoraku values were mean \pm SE for subjects undergoing K.Y.M.S. system detection. *: Significant different in the experiments ($P < 0.05$).

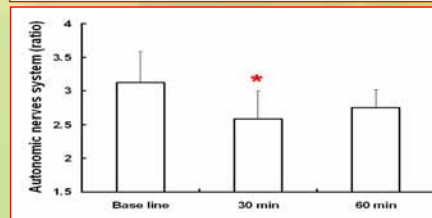


Figure 6. The autonomic nerves system after Sorghum vinegar treatment. The experimental groups were involved baseline, after treated with 30 minutes and 60 minutes. The Ryodoraku values were mean \pm SE for subjects undergoing K.Y.M.S. system detection. *: Significant different in the experiments ($P < 0.05$).

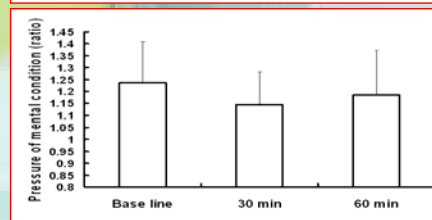


Figure 7. The pressure of mental condition after Sorghum vinegar treatment. The experimental groups were involved baseline, after treated with 30 minutes and 60 minutes. The Ryodoraku values were mean \pm SE for subjects undergoing K.Y.M.S. system detection. *: Significant different in the experiments ($P < 0.05$).

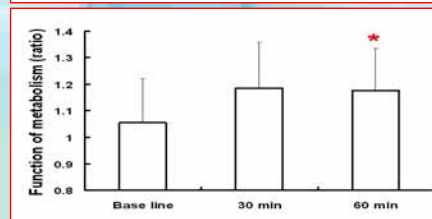


Figure 8. The functional of metabolism after Sorghum vinegar treatment. The experimental groups were involved baseline, after treated with 30 minutes and 60 minutes. The Ryodoraku values were mean \pm SE for subjects undergoing K.Y.M.S. system detection. *: Significant different in the experiments ($P < 0.05$).

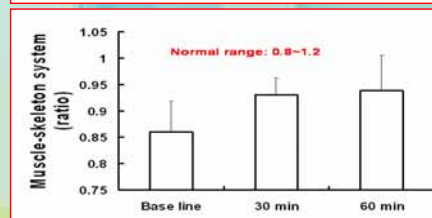


Figure 9. The muscle-skeleton system after Sorghum vinegar treatment. The experimental groups were involved baseline, after treated with 30 minutes and 60 minutes. The Ryodoraku values were mean \pm SE for subjects undergoing K.Y.M.S. system detection. *: Significant different in the experiments ($P < 0.05$).

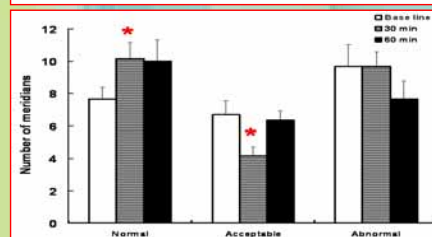


Figure 10. The healthy of meridian in subjects include normal, acceptable and abnormal undergoing K.Y.M.S. system detection. The experimental groups were involved baseline, after treated with 30 minutes and 60 minutes after cinnamon dew treatment. The Ryodoraku values were mean \pm SE for subjects. *: Significant different in the experiments ($P < 0.05$).

Discussion

The preliminary results showed the significantly increased of bio-energy of 12 meridians, 5 elements, body energy after Sorghum vinegar treatment for 30 or 60 minutes. In conclusions, the measurement of K.Y.M.S. system is a useful noninvasive technique for the evaluation meridian effects of bio-energy of lemon vinegar induction. The Sorghum vinegar caused bio-energy down regulate in human body.

國科會補助計畫衍生研發成果推廣資料表

日期:2011/10/30

國科會補助計畫	計畫名稱：以生物活性引導篩藥之方式研究傳統中藥萃取物中之有效抗腦腫瘤之成分
	計畫主持人：蔡女滿
	計畫編號：97-2320-B-040-005-MY3 學門領域：中醫藥
無研發成果推廣資料	

97 年度專題研究計畫研究成果彙整表

計畫主持人：蔡女滿		計畫編號：97-2320-B-040-005-MY3				計畫名稱：以生物活性引導篩藥之方式研究傳統中藥萃取物中之有效抗腦腫瘤之成分	
成果項目		量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）	
		實際已達成數（被接受或已發表）	預期總達成數（含實際已達成數）	本計畫實際貢獻百分比			
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	18	18	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	1	1	100%	人次	參與計畫人力主軸為五年一貫碩士生及臨時工（大專生）
		博士生	0	0	100%		
博士後研究員		0	0	100%			
專任助理		0	0	100%			
國外	論文著作	期刊論文	4	4	100%	篇	內含數個計畫共同成果
		研究報告/技術報告	0	0	100%		
		研討會論文	4	4	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	內含數個計畫共同成果
		已獲得件數	1	1	100%		
	技術移轉	件數	1	1	100%	件	
		權利金	250	250	100%	千元	內含數個計畫共同成果
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
博士後研究員		0	0	100%			
專任助理		0	0	100%			

<p>其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)</p>	<p>無</p>
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	成果項目	量化	名稱或內容性質簡述
科 教 處 計 畫 加 填 項 目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與(閱聽)人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

達成目標

未達成目標（請說明，以 100 字為限）

實驗失敗

因故實驗中斷

其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文： 已發表 未發表之文稿 撰寫中 無

專利： 已獲得 申請中 無

技轉： 已技轉 洽談中 無

其他：（以 100 字為限）

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）（以 500 字為限）

計畫執行至目前一百年七月結束，所有實驗進度皆依計畫中之實驗進度進行，也順利的完成為期三年的研究進度，此結果包含：第一年已順利的篩到三種不同的粗萃物，能有效抑制腦腫瘤的生長，其中又以 AS-M 效果較佳，此粗萃物之有效生物活性包含：(1)能有效抑制腦腫瘤細胞生長；(2)能引發惡性腦瘤細胞(GBM cell)型態改變；(3)能促使惡性腦瘤細胞週期遲至於 G0/G1 時期；(4)能誘發惡性腦瘤細胞之細胞凋亡，但此粗萃物無法抑制惡性腦瘤細胞之端粒酶之酵素活性及其基因之表達，因此推測此粗萃物誘發惡性腦瘤細胞之細胞凋亡並非藉由抑制端粒酶酵素之活性而促使的。第二年也順利的研究進度，此結果包含：(1)成功地自 12 kg 的當歸中大量萃取 AS-M。(2)成功建立人類惡性腫瘤之動物 (Nude mice) 化療模式，結果顯示 AS-M 降低腫瘤體積、減少腫瘤發生率、提高動物存活率並延長動物生存壽命，所以 AS-M 粗萃物能有效抑制 human GBM cell 於動物體內生長。(3) AS-M 粗萃物之生理毒性測試中，所有實驗組之心跳、血壓、紅血球、白血球、血小板、淋巴球、肝功能及腎功能等，生理、血液及生化數值皆與正常控制組無明顯差別。所有實驗組之動物體重及組織器官皆近似於正常控制組，並無發現明顯之器官組織之損傷，因此得知 AS-M 對動物生理之急毒性非常的小幾乎近於無毒之情形。第三年也成功的分析粗萃物 AS-M 於體外(In vitro)及體內(In vivo)之作用機轉：(1)結果發現 AS-M 能透過活化 P53 腫瘤抑制分子，並藉由 P53 啟動 P53-dependent 路徑引發人類惡性腦瘤細胞之細胞凋亡；另外，也增加了 P16 的表現量促使腫瘤細胞之細胞週期停滯於 G0/G1 時期；(2) AS-M 能引發體內腫瘤細胞之細胞凋亡(Apoptosis)；(3)AS-M 引發體內腫瘤細胞之細胞凋亡(Apoptosis)，

主要是透過活化 Caspase-3 細胞凋亡分子，而引發動物體內人類惡性腦瘤細胞之細胞凋亡，同時，AS-M 引發體內腫瘤細胞之細胞週期遲滯(Cell cycle arrest)，推測其其透過活化 P53 腫瘤抑制因子及 P16 細胞週期抑制因子，而促使動物體內人類惡性腦瘤細胞之細胞週期遲滯進而引發細胞凋亡。

上述所有結果正在撰寫準備近期內投稿至 SCI 之期刊雜誌。本研究結果所具備的科學性價值為：透過此一連串的研究證實當歸的 Methanol 萃取物，具備有抑制人類惡性腦瘤生長之作用，也因當歸此中藥材為國人常用之膳食中藥，並於烹煮過程常會加入一定量的酒一起烹調，如同當歸於 Methanol 中萃取一般，於此提供本實驗數據，建議平常飲食中攝取含當歸之相關膳食食物，不但可以養生還可降低癌症的發生率。