國立臺灣大學生物資源暨農學院食品科技研究所

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Doctoral Dissertation

天麻水萃物活性成分之抗憂鬱功效及其分子機制的探討

The Preventive Potential and Molecular Mechanism of Antidepressant-like Effects Exerted by the Active Compounds in Water Extract of *Gastrodia elata* Blume

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國立臺灣大學博士學位論文
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The Preventive Potential and Molecular Mechanism of Antidepressant-like Effects Exerted by the Active Compounds in Water Extract of *Gastrodia elata* Blume

本論文係陳威成君 (學號 D00641001) 在國立臺灣大學食品科技研究所完成之博士學位論文，於民國 105 年 05 月 09 日承下列考試委員審查通過及口試及格，特此證明

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胡宗明

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所長：呂廷璋

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誌謝

從輔大、嘉大到現在的臺大，我慢慢了解什麼叫作「做研究」，以前總覺得研究的一切都有正確答案，但事實並非如此，正確答案不會永遠是正確的。一開始來這裡很不適應自由研究風氣和沒有所謂的正確答案，研究的道路總是遇到比別人多的慘事 (儀器還沒開始使用就壞了、跟文賢學長一起花了一個月修理HPLC、口試前十天電腦壞了、口試前十分鐘另一台電腦突然故障.....等等)，雖然事情發生當下很不是滋味，但是現在卻成了印象深刻的回憶，也造就現在我面對危機能處之泰然。在開始攻讀博士班前後的幾年間，身邊多數親朋好友因神經退化性疾病而陷入人生低潮，有些人也因此離開人世，此時此刻我真的開始相信「健康」是人最幸福的資產，這也是我繼續攻讀博士班的動力。承蒙指導教授**沈立言**老師在傳統中醫與憂鬱症相關研究的雄厚基礎，我如小樹一般汲取豐富養分，從當初的懵懂到後來的老練，實際運用在日常生活中協助他人走出憂鬱陰影。

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志峰、Meghan、浤偉、歷屆碩博班學弟妹靜儀、惠君、巧憶、于淨、映竹、曉彤、云君、阿德、小康、庭郁、羿涵、毓恩、慧穎、偉豪、少鼎、小停、怡君、乃元、宜君、香蘭、冠伶、庭瑄、心愉、菀庭、家羽、柔安、蕙而、亭逸、逸婷在實驗技術、論文發表和獎學金申請的指導與協助，還有所上所有老師與職員周錦韻、陳碧雲、李美慧、周慶麟在任何生活大小事的幫忙。此外，還有我的鋼琴老師林金滿與吳瑞庭和在人生不同階段的認識的好朋友家瑋、sky、佳怡、瑋茹、朝裕、威庭、咨凱、伯維、立凡、大軟、小尾、子寬、河豚、鈞豪、仕暐、妤玥、尹妤、怡君、彥廷、邱導、羅婕、垂諭、阿謙、嘉義、咪咪與我分擔研究上的壓力。最後要謝謝我的父母 (陳桐榮先生和游薰伶女士)、女朋友英傑和所有家人的陪伴和鼓勵，讓我能平穩順利拿到博士學位。期許自己能繼續往神經退化研究的道路前進，並適時提供正確的資訊或方法來預防或改善病徵的惡化。

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中文摘要

重鬱症 (Major depressive disorder, MDD) 的問題普遍存在於世界各國。目前，全球約有超過 3.5 億的人口患有 MDD，世界衛生組織預測 MDD 在 2030 年將成為全球高負擔疾病的第一名。然而，現今西醫的治療方式，常會帶來許多病人無法接受的副作用，故從自然界尋找替代西藥的素材是迫切急需的課題。天麻 (*Gastrodia elata* Blume) 是中醫食療中用於作養心安神的藥材，近年研究發現具有改善多種神經退化性疾病的功效。本研究擬以天麻水萃物 (water extract of *G. elata* Bl, WGE) 之活性成分天麻苷 (gastrodin, GAS) 與天麻苷元 (4-hydroxybenzyl alcohol, HBA) 為素材，探討經強迫游泳試驗 (forced swimming test, FST) 誘導類憂鬱之大鼠是否藉由降低腦部單胺類系統與調節神經細胞骨架重組達到抗憂鬱效果。另外，進一步以 HPLC 建立 GAS 分析方法確效以利天麻原料的管理。在動物實驗方面，六週齡 Sprague Dawley (SD) 雄性大鼠經連續 21 天分別管灌餵食 WGE (500 mg/kg bw)、GAS (100 mg/kg bw) 和 HBA (100 mg/kg bw)，以 FST 誘導類憂鬱行為。在行為分析結果顯示 WGE、GAS 和 HBA 皆可顯著降低因絕望所導致的不泳動行為 (immobility time)。腦部單胺類濃度以 HPLC 進行分析，結果顯示三種樣品皆可降低腦部血清素和多巴胺的代謝速率。由於臨床研究發現憂鬱症患者除了與單胺類濃度異常具有高度關聯性外，神經可塑性 (neuronal plasticity) 亦扮演重要角色。因此，本研究後續以西方墨點法探討 WGE、GAS 和 HBA 對神經細胞骨架重組 (neuronal cytoskeletal remodeling) 相關路徑──Slit-Robo 之影響，結果顯示各處理組皆可顯著降低海馬迴中負調節因子 Slit1 和 RhoA 蛋白表現，同時提升正調節因子 CRMP2 和 PFN1 蛋白表現，進而提高神經可塑性。在細胞實驗方面，以人類寡樹突細胞瘤 (Human oligodendroglioma, Hs683) 驗證 Slit-Robo 路徑，此細胞株分別與 WGE (0.5 mg/ml)、GAS (50、100 和 200 μM) 和 HBA (50、100 和 200 μM) 共培養 24 小時，以西方墨點法分析證實 WGE、GAS 和 中高劑量之 HBA (100 和 200 μM)

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可直接降低 Slit1 蛋白表現，此現象呼應海馬迴西方墨點法的分析結果。化學分析實驗方面，以 GAS 確效之 HPLC 方法分析加工方式、萃取溶劑、產地及變種對 GAS 含量之差異，結果顯示熱水萃取之烏天麻 (*G. elata* f. *glauca* S. Chow) 切片可得到較紅天麻 (*G. elata* Bl. f. *elata*) 高的 GAS 含量。綜上所述，本研究證實 GAS 是 WGE 中抗憂鬱活性成分，藉由降低單胺類代謝與調節神經細胞骨架重組達到此功效，並建立 GAS 確效以利往後調節憂鬱情緒保健食品與抗憂鬱用藥開發之原料篩選。

關鍵字：憂鬱症、強迫游泳試驗、天麻、單胺類系統、神經細胞骨架重組

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Abstract

Major depression disorder (MDD) has generally been found in humans worldwide. More than 350 million people suffer from MDD. The World Health Organization (WHO) predicts that MDD will be the first global burden disease in 2030. Due to undesired side effects of depression treatment, patients with depression tend to avoid medical therapies. Therefore, it is important to discover an alternative therapy from natural sources. *Gastrodia elata* Blume (Tian-ma) is a common Chinese medicinal herb for mind tranquilizing. Recently, some studies have reported that *G. elata* Bl. could be applied to ameliorate various neurodegenerative disorders. The aim of this study is to investigate the active compounds, gastrodin (GAS) and 4-hydroxybenzyl alcohol (HBA) of the water extract of *G. elata* Bl. (WGE), on antidepressive-like activity in rats and explore monoamine metabolism and modulated cytoskeleton remodeling-related protein expression in the Slit-Robo pathway after forced swimming test (FST). Furthermore, the analysis method of GAS using HPLC was developed and validated for source of *G elata* Blume identification. *In vivo* studies, the male Sprague Dawley rats were oral gavage with 500 mg/kg bw of WGE, 100 mg/kg bw of GAS, and 100 mg/kg bw of HBA for 21 days. It was observed that the immobility time of WGE, GAS, and HBA groups were markedly decreased in FST. Thus the depressive-like behavior could be reversed. WGE, GAS, and HBA restored higher turnover rate of serotonin and dopamine compared to control group. Due to neuronal plasticity correlates with depression, Western blot analysis was applied to Slit-Robo pathway, the neuronal cytoskeletal remodeling related protein. It was found that WGE, GAS, and HBA significantly down-regulated Slit-Robo pathway in the hippocampus that promote neuroplasticity. In *vitro* studies, Human oligodendroglioma (Hs683) was co-culture with WGE (0.5 mg/ml), GAS (50, 100, and 200 μM), and HBA (50, 100, and 200 μM) for 24 hours. The result indicated that WGE, GAS (50,

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100, and 200 μM), and HBA (100 and 200 μM) notable down-regulated the protein expression of Slit1 directly. The validation method using HPLC was applied to investigate the quantities of GAS from preparation processes, sources and phenotypes. The results showed that the slices of *Gastrodia elata* f. *glauca* S. Chow under water extraction contained the quantity of GAS higher than *G. elata* Bl. f. *elata*. In conclusion, these results suggest that GAS provides for WGE exerting antidepressive-like effects through decreasing monoamine metabolism and down-regulating Slit-Robo pathway. Furthermore, the validation method can be used as the source of *G. elata* Bl. screening for health food development.

Keywords: depression, forced swimming test, *Gastrodia elata* Blume, monoamine system, neuronal cytoskeletal remodeling

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縮寫表

字母	縮寫	英文全名	中文全名
5	5-HIAA	5-hydroxyindoleacetic acid	5-羥基引朵醋酸
	5-HT	serotonin	血清素
A	ACN	acetonitrile	乙腈
B	BDNF	brain derived neurotrophin factor	腦衍生滋養因子
	BrdU	bromodeoxyuridine	溴化去氧尿苷
	BT	behavioral therapy	行為治療
C	CBT	cognitive-behavioral therapy	認知行為治療
	CMS	chronic mild stress	慢性壓力
	CMC	carboxymethyl cellulose	羧甲基纖維素
	CRHR1	corticotrophin receptor 1	促腎上腺皮質激素受器
	CRMP2	dihydropyrimidinase-related protein 2	
	CT	cognitive therapy	認知治療
	CTL	control group	控制組
D	DA	dopamine	多巴胺
	DOPAC	3, 4-dihydroxyphenylacetic acid	3,4-二羥基苯乙酸
	DCX	doublecortin	雙皮質素
	dPC12	differentiated PC12	已分化 PC12
E	ECD	electrochemical detector	電化學檢出器
	ECT	electroconvulsive therapy	電痙攣
	ESC	escitalopram	立普能
F	FST	forced swimming test	強迫游水試驗
G	GABA	γ-aminobutyric acid	γ-胺基丁酸
	GAPDH	glyceraldehyde 3-phosphate dehydrogenase	
	GAS	gastrodin	天麻苷
	GBY	*G. elata* Bl. f. *glauca* S. Chow in Yun-nan	雲南烏天麻
	GRH	*G. elata* Bl. f. *elata* in Hu-nan	湖南紅天麻
	GRY	*G. elata* Bl. f. *elata* in Yun-nan	雲南紅天麻
H	HB	4-hydroxybenzyl aldehyde	對羥基苯甲醛
	HBA	4-hydroxybenzyl alcohol	天麻苷元
	HPA	hypothalamus-pituitary-adrenal axis	下視丘一腦下垂體一腎上腺軸線

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	Hs683	Human oligodendroglioma	人類寡樹突細胞瘤
	HVA	homovanillic acid	高香草酸
I	ICD	International statistical classification of diseases and related health problems	疾病及有關健康問題國際統計分類
	IPT	interpersonal therapy	人際關係治療
L	LOD	limit of detection	最低檢測濃度
	LOQ	limit of quantification	最低定量濃度
	MAOA	monoamine oxidase A	單胺氧化酶 A
	MAOIs	monoamine oxidase inhibitors	單胺氧化酶抑制劑
	MCAO	middle cerebral artery occlusion	大腦動脈阻斷
	MDD	major depressive disorder	重鬱症
N	NC	normal group	正常組
	NE	norepinephrine	正腎上腺素
	nPC12	naive PC12	未分化 PC12
O	OPT	open field test	開放空間試驗
P	PC12	pheochromocytoma	大鼠腎上腺嗜鉻瘤
	PFN1	profilin1	
S	SNRIs	serotonin-norepinephrine reuptake inhibitors	血清素與正腎上腺素再回收抑制劑
	SRMs	serotonin receptor modulators	血清素受體調節劑
	SSRIs	selective serotonin reuptake inhibitors	選擇性血清素再回收抑制劑
R	RhoA	ras homologous member A	
T	TCAs	tricyclic antidepressants	三環類抗憂鬱藥
	TLC	thin layer chromatography	薄層層析
	TPH	tryptophan hydroxylase	色氨酸羥化酶
V	VAN	4- hydroxy-3-methoxybenzaldehyde	香草醛
W	WGE	water extract of *Gastrodia elata* Blume	天麻水萃物
	WHO	World Health Organization	世界衛生組織

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壹、前言

「念頭轉個彎，生命無限寬廣，讓我們陪伴您!」、「自殺解決不了問題，卻留給家人無比悲痛。請珍惜生命。再給自己一次機會!」以上的標語總會在自殺相關報導的結尾以跑馬燈呈現，也許一般人認為這些標語形容的很貼切，但是在憂鬱症患者眼中，精神障礙的不適感早已遠大過生命及親朋好友的影響力。目前，全球有超過 3.5 億人患有重鬱症 (major depressive disorder, MDD)，每年因憂鬱症自殺死亡的人口約有 100 萬人 (WHO, 2015)。世界衛生組織 (World Health Organization, WHO) 預測憂鬱症將在 2030 年高居全球十大負擔性疾病的第一名 (Mathers et al., 2008)。為了有效改善患者的憂鬱症狀，各種抗憂鬱用藥如雨後春筍般相繼問世，但有效治療率卻只有 20%，另外有大部分的患者在服用抗憂鬱用藥後出現更多身體的不適感，而使患者自行停藥甚至導致患者自殺。

天麻 (*Gastrodia elata* Blume) 之根部蒸煮或乾燥後常作為傳統中藥藥引，具有安神寧腦作用。研究報告指出，天麻具有抗氧化 (Yu et al., 2005)、抗發炎 (Hwang et al., 2009)、抗腫瘤 (Heo et al., 2007)、抗癲癇 (Hsieh et al., 2007)、改善學習記憶力 (Chen et al., 2011)、減緩神經退化 (Huang et al., 2011) 與抗憂鬱的效果 (Chen et al., 2008; Tsai et al., 2011)。本研究室先前研究亦發現天麻水萃物 (water extract of *Gastrodia elata* Bl., WGE) 可降低強迫游泳試驗中 (forced swimming test, FST) 大鼠腦中血清素與多巴胺的代謝，並提高神經可塑性達到抗憂鬱效果 (陳，2008；林，2012)。

本研究擬以 FST 評估 WGE 活性成分天麻苷 (gastrodin, GAS) 與天麻苷元 (4-hydroxybenzyl alcohol, HBA) 之抗憂鬱效果與機制。進一步以人類寡樹突細胞瘤 (Human oligodendroglioma, Hs683) 細胞株驗證 WGE、GAS 和 HBA 對 Slit-Robo 途徑之影響。另外，利用 HPLC 建立 GAS 的分析方法確效，比較天麻不同加工方式、來源和變種在 GAS 含量上的差異，以利調節憂鬱情緒保健食品與抗憂鬱用藥開發之原料篩選。

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貳、文獻回顧

一、憂鬱症

(一) 背景及概況

憂鬱症是常見的臨床疾病之一，根據估計大約有四分之一的人在一生當中的某個時期出現過憂鬱症的病癥。它比糖尿病或氣喘更常見，但未患有憂鬱症的人卻對它不甚了解 (Buckman and Charlish, 1999)。根據美國精神醫學會出版的心理疾病診斷及統計手冊 (Diagnostic and Statistical Manual of Mental Disorders, 5th edition, DSM-5) 及 世界衛生組織 (World Health Organization, WHO) 的國際疾病與健康問題統計分類 (The Tenth Revision of the International Statistical Classification of Diseases and Related Health Problem, ICD-10)，是以嚴重度 (severity)、是否有精神病特質 (psychoticfeature) 以及憂鬱病症或個體特徵 (melancholic or somatic features) 等作為憂鬱症分類基礎。由憂鬱症的嚴重度分為輕度、中度或重度；在發作類型可分為憂鬱發作、狂躁發作或混合發作。憂鬱症患者常見的症狀有睡眠障礙、體重明顯的變化、感到鬱悶、心情低落，對於自己開始失去信心，覺得自己是個毫無價值的人 (自卑感) 並對旁人造成困擾感到內疚 (自責感)，且開始焦躁不安。對於任何事情都提不起精神，影響自己在工作、學校和家庭的互動關係，最嚴重的情況就是患有自殺的念頭或行動。

世界衛生組織 (WHO) 在全球負擔性疾病 (The global burden of disease) 報告中，以失能調整生命年 (disability adjusted life years, DALYs) 做推測依據，憂鬱症將會在 2030 年時攀升至負擔性疾病第一名 (Mathers et al., 2008)。截至目前為止，全球 74 億人口中罹患憂鬱症佔 5%，每年約有 100 萬憂鬱症患者因此自殺。其中，15-29 歲佔憂鬱自殺年齡層的第二位 (WHO, 2015)。衛生福利部 103 年國人自殺率統計結果顯示，除了 93 年以外之各年齡層自殺死亡率均呈下降，但自殺死亡率仍有隨年齡增加而升高之趨勢，其中以 65 歲以上者之每 10 萬人口 31.3 人最高 (圖一)。不同年齡層的死亡統計顯示，自殺 (蓄意自我傷害) 死亡分別佔 15-24 歲和 25-44 歲年齡層之第二 (13.6%) 及第三名 (12.7%) (衛

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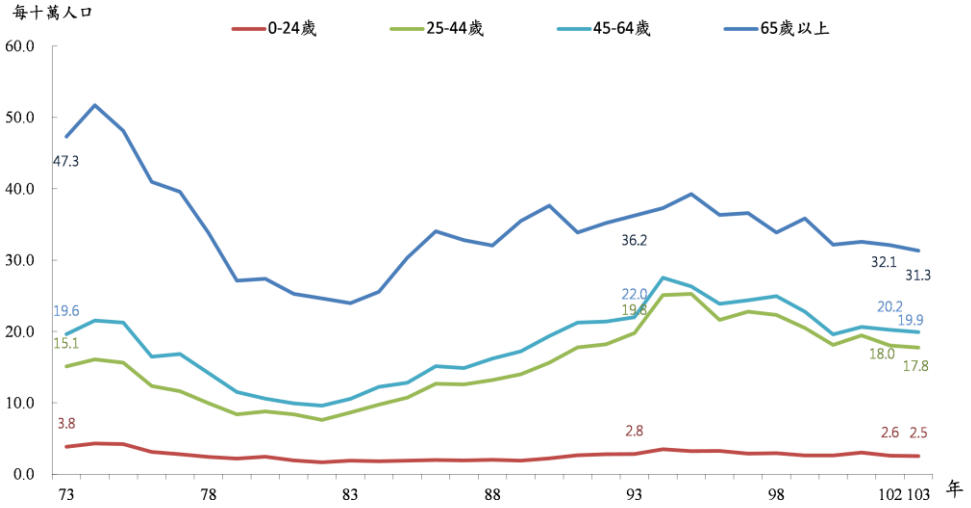
生福利部，2015)。追究其自殺原因，由表一顯示，「情感／人際關係」和「精神健康／物質濫用」分別佔據近五年自殺原因的第一和第二名 (衛生福利部，2012)。國人精神障礙比例逐年增加的趨勢也從 Fu 等人 (2013) 近十年的追蹤調查結果得到印證，從 1990 年的 11.5% 提升至 2010 年的 23.8%，平均每四人就有一人有精神障礙。對大部分民眾而言，心情不好或情緒低落是常見的，但長期處於情緒低落是不可忽視的，如能早期發現並接受治療，便能緩解病患遭受的痛苦、工作損失、家庭風波，甚至自殺。近年因經濟不景氣、社會壓力增加、飲食及生活型態改變，國人罹患憂鬱症的比例逐年升高，如何防治憂鬱症帶來社會的衝擊已成為全球值得重視的議題 (郭，2002)。

圖30　歷年自殺(蓄意自我傷害)死亡率-按年齡別分



(衛生福利部，2015)

圖一、歷年自殺死亡率-按年齡別分

Figure 1. People death for suicide from 1984 to 2014, categorized by age.

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表一、自殺原因佔率

Table 1. Account rate of suicide causes.

原因 / 年月	工作／經濟[註1]	不願說明或無法說明[註2]	生理疾病	兵役問題	其他	校園學生問題	情感／人際關係[註3]	精神健康／物質濫用[註4]
95 年	8.5%	0.1%	4.5%	0.0%	42.3%	0.6%	35.7%	24.8%
96 年	8.6%	0.6%	5.6%	0.4%	29.5%	0.7%	45.4%	33.0%
97 年	10.5%	8.0%	6.3%	0.4%	18.0%	0.8%	48.8%	37.5%
98 年	14.3%	0.1%	7.7%	0.3%	12.9%	0.7%	56.7%	40.7%
99 年	12.67%	0.03%	8.53%	0.39%	12.77%	0.74%	58.14%	40.35%
100 年	11.00%	0.10%	8.03%	0.27%	11.37%	0.84%	60.21%	38.35%

註 1：「工作／經濟」包含職場問題、失業、非失業經濟因素。

註 2：「不願說明或無法說明」包含個案（家屬）不願說明、個案因身體狀況無法說明。

註 3：「情感／人際關係」包含家人間情感因素、夫妻問題、感情因素、重大失落、同儕關係因素、其他人際關係因素。

註 4：「精神健康／物質濫用」包含憂鬱傾向、非憂鬱傾向精神心理健康、有憂鬱症病史、物質濫用（酒藥癮）。

(衛生福利部，2012)

(二) 憂鬱症類型

1. 典型憂鬱症 (classic depression)

又稱為單極憂鬱症 (unipolar depression)，特徵為意志消沉、情緒低落，無法從任何事情中得到樂趣，並認為自己一無是處，事情一出差錯就自責，夜晚難入眠或睡眠持續中斷，整天感到相當疲累、心神不寧，導致注意力不集中或記憶力衰退 (Buckman and Charlish, 1999)。

2. 躁鬱症 (manic depression)

又稱為雙極憂鬱症 (bipolar disorder)，從名稱上可以得知「躁」症跟「鬱」症是同時發生在憂鬱症患者上。當患者處在躁症時期通常精力旺盛，他們的想法可能出現瘋狂創意或說話速度很快卻無內容；鬱症時期就會出現典型憂鬱症的症狀 (Buckman and Charlish, 1999)。

3. 冬天憂鬱症 (winter blue)

季節性情緒障礙 (seasonal affective disorder, SAD) 通常發生在季節

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變換的時候，其中在秋季末和冬季初患者罹患率最高，由於秋冬日照減少，導致褪黑激素無法被光線抑制，進而擾亂生理時鐘。其症狀包括沮喪、疲累、脾氣暴躁、焦慮、悲傷、注意力和記性不佳，此外，這類型患者非常嗜睡 (Buckman and Charlish, 1999)。

4. 產後憂鬱症 (postnatal depression)

產後憂鬱通常會發生在第一次當媽媽的女性，一般在產後幾天症狀就會消失，但有時候產後憂鬱出現的時間比較晚，其發作症狀與典型憂鬱症相同。此外，媽媽可能對自己、嬰兒或兩者產生痛苦、恐懼或憤怒的情緒。暴躁、疲累和無法入眠是主要特徵 (Buckman and Charlish, 1999)。

5. 微笑型憂鬱症 (smiling depression)

有些憂鬱症患者會隱瞞自己憂鬱的心情來誤導他人的判斷，這類患者會表現心情很好的樣子，但卻可能在無預警的情況下自殺，由於不易判斷並提供必要協助，所以這類患者成了最可能自殺的人 (Buckman and Charlish, 1999)。

(三) 診斷方式

美國精神醫學會在 2013 年 5 月公佈第五版精神疾病診斷與統計手冊 (diagnostic and statistical manual of mental disorders, DSM-5)，並於 2014 年 10 月宣布精神疾病分類編碼全面採用 ICD-10-CM/PCS。MDD 診斷標準要求症狀必須包括以下五項 (或更多) 症狀在兩週中同時出現，造成先前功能改變；至少包含以下症狀之一 (1) 憂鬱情緒或 (2) 喪失興趣或愉悅感。

(1) 幾乎每天大多數時間都呈現憂鬱情緒；(2) 幾乎每天大多數時間，對於全部 (或幾乎全部) 的活動都喪失興趣或無法從中感到愉悅；(3) 顯著的體重減少或增加 (如在一個月之內體重變化超過 5%) 或幾乎每天都有食慾減低或增加的現象；(4) 幾乎每天都有失眠或嗜睡的現象；(5) 幾乎每天都呈現精神運動性激動或遲滯；(6) 幾乎每天都感到疲倦或沒有力氣；(7) 幾乎每天覺得自己沒有價值，或有過度或不合理的罪惡感；(8) 幾乎每天都感到思考或專注能力的下降，或變得猶豫不決；(9) 反覆出現輕生的念頭，或反覆出現沒有具體計劃的自殺意

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念，或已出現自殺企圖或已有特定的自殺計劃 (American Psychiatric Association, 2013；衛生福利部委託國立臺灣大學醫學院附設醫院精神醫學部，2015)。

(四) 憂鬱症病因之理論

1. 心理社會因素

一些研究指出不良的社會經驗與環境、身體疾病和性格等因素所產生的心理壓力，皆可能誘發憂鬱症狀的出現。Barnett 和 Gotlib (1988) 試圖以經驗區分心理社會因素與憂鬱症之間的關係，發現功能失調性態度 (dysfunctional attitudes)、人格 (personality)、社會支持 (social support)、婚姻窘迫 (marital distress) 和應對方式 (coping style) 等心理社會因素對於日後憂鬱症的發生具有高度關聯性。其中，有部分證據指出成人之人際功能紊亂可能是形成憂鬱之認知脆弱因子 (cognitive vulnerability to clinical depression) 的前因或後遺症。Fu 等人 (2013) 從 1990 至 2010 年間對國人非精神病之憂鬱、焦慮等精神健康狀況 (common mental disorders) 長期調查結果顯示，其盛行率由 1990 年的 11.5% 倍數增加到 2010 年的 23.8%，其主要原因與性別 (女性)、低教育程度、失業、健康狀況不佳等風險因子的影響有關。

2. 生物化學因素

(1) 神經傳導物質

神經傳導物質按照化合物種類分為 amine、neuropeptides、purines 和氣體，amine 類包括：單胺類 (monoamines) 和 amino acids (Breedlove and Watson, 2013)；neuropeptides 類包括：opioidpeptides 和一些神經肽類 (Breedlove and Watson, 2013)；purines 類包括：adenosine triphosphate (Wieraszko et al., 1989) 和 adenosine (Nishimura et al., 1990)；氣體類包括：nitric oxide (Snyder, 1992), carbon monoxide (Verma et al., 1993) 和 hydrogen sulfide (Gadalla and Snyder, 2010)，其中 monoamine 類又細分成 catecholamines 和 indoleamines，前者包含正腎上腺素 (norepinephrine, NE)、腎上腺素 (epinephrine) 和多巴胺 (dopamine, DA)，後者包含血清素 (serotonine, 5-HT) 和褪黑激素 (melatonin) (Bielecki et al., 2008;

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Breedlove and Watson, 2013)(表二)。

表二、神經傳導物質之分類

Table 2. Classification for neurotransmitters.

Family and subfamily	Transmitters	
Amines		
Monoamines	Catecholamines	Norepinephrine (NE)
		Epinephrine
		Dopamine (DA)
	Indoleamines	Serotonine (5-HT)
		Melatonin
Amino acids	Gamma-aminobutyric acid (GABA)	
	Glutamate	
	Glycine	
	Histamine	
Other amines	Aceylcholine (Ach), anandamide	
Trace amines	phenethylamine, N-methylphenethylamine, tyramine, 3-iodothyronamine, octopamine, tryptamine	
Neuropeptides		
Opioid peptides	Enkephalins	Met-enkephalin, Leu-enkephalin
	Endorphins	Beta-endorphin
	Dynorphins	Dynorphin A
Other neuropeptides	Oxytocin, Substance P, Cholecystokinin, Vasopressin, Neuropeptide Y, Hypothalamic releasing hormones	
Purines		
Adenosine triphosphate (ATP), adenosine		
Gases		
Nitric oxide, Carbon monoxide, Hydrogen sulfide		

四十幾年前有學者發現憂鬱症患者腦部單胺類神經傳導物質不足而提出單胺類假說 (Bunney and Davis, 1965; Schildkraut, 1965)，此假說促使各種提高神經突觸間單胺類濃度的抗憂鬱藥物如雨後春筍般相繼問世 (Hirschfeld, 2000)。由於

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單胺類物質與憂鬱情緒有極高的關聯性，單胺類假說成為現今研究憂鬱症的主流 (Van Praag, 1982; Hughes et al., 2004)，其中研究範圍包括：單胺類受器 (monoamine receptor) (Hsieh et al., 1998; Meyer et al., 2006; Guo et al., 2013; Wang et al., 2014)、單胺類合成酶 (monoamine-synthesizing enzyme) (Davis and Carlsson, 1973; Shin et al., 2011) 和單胺類氧化酶 (monoamine oxidase) (Riederer and Youdim, 1986) 等，其分別影響腦部單胺類的濃度。單胺類受器研究中，5-HT 受器目前至少有七種已被證實，透過刺激 5-HT1A 和 5-HT2B 受器與阻斷 (blockade) 5-HT2A/2C 受器可促進 5-HT 分泌至突觸間；刺激 5-HT4 受器可抑制 5-HT 轉運子 (transporter) 並維持 5-HT 神經元的電生理活動；阻斷 5-HT3、5-HT6 和 5-HT7 受器可間接抑制 5-HT 轉運子 (transporter) 並促使 5-HT 含量增加 (Artigas, 2013) 等受器的表現，改善憂鬱症患者腦部 5-HT 不足的現象。在 DA 受器研究方面目前有五種 DA 受器被證實，在臨床上發現憂鬱症患者的 DA2 受器密度顯著較正常人多並影響 DA 的正常傳導 (D'haenen and Bossuyt, 1994)。在動物實驗發現 SCH 23390 拮抗劑可阻斷 DA1 受器與 DA 的結合，進而抑制 DA1 受器回饋來維持 DA 在神經突觸間的濃度 (Cho et al., 1999)。此外，齧齒類在壓力與大腦衍生神經滋養因子 (brain-derived neurotrophic factor, BDNF) 的憂鬱症研究中顯示，壓力會造成 BDNF 濃度顯著降低並影響到 DA3 受器的基因的調控 (Sokoloff et al., 2002)。而在單胺類合成酶研究中，色氨酸羥化酶 (Tryptophan Hydroxylase, TPH) 是合成 5-HT 的關鍵酵素，目前發現有 TPH1 和 TPH2 兩種亞型 (isoforms)，在憂鬱症患者腦部發現此酵素活性較正常人高，因應腦部 5-HT 濃度不足的現象 (Boldrini et al., 2005)。酪胺酸羥化酶 (tyrosine hydroxylase) 是合成 DA 前驅物 L-dopa 的重要酵素 (Iuvone et al., 1978)。Baumann 等人 (1999) 發現非自殺的憂鬱症患者因有較低的正腎上腺活性，導致分泌 tyrosine hydroxylase 的神經細胞數量變少。單胺類氧化酶 (monoamine oxidase) 存在 MAOA 和 MAOB 兩種，MAOA 可代謝 5-HT、DA 和正腎上腺素 (norepinephrine)，而 MAOB 主要代謝 DA (Youdim and Weinstock,

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2004; Duncan et al., 2012)。在半世紀以前有學者發現憂鬱症患者的 MAO 活性顯著高於一般人，導致腦部神經傳導物質缺乏，因此單胺氧化酶抑制劑 (monoamine oxidase inhibitors, MAOIs) 被作臨床今常見的抗憂鬱用藥 (Sandler et al., 1975; Youdim et al., 2006)。

(2) 內分泌

下視丘—腦下垂體—腎上腺軸線 (hypothalamic-pituitary-adrenal axis, HPA axis) 是體內調節外界壓力的內分泌系統，當暴露在壓力環境下會促使糖皮質固醇 (glucocorticoid) 釋放至血液中應付壓力。人體內的糖皮質固醇稱作皮質醇 (cortisol)，在動物體內則稱皮質固酮 (corticosteron)，過去研究發現糖皮質固醇具有抑制發炎的作用 (Schleimer, 2004)，但是濃度過高反而會產生促發炎現象 (Sorrells and Sapolsky, 2007)。臨床研究發現憂鬱症患者血液中皮質醇含量較高，而此現象可能來自 HPA 過度活化所造成 (O'Toole and Johnson, 1997)，並影響神經新生 (Mirescu and Gould, 2006)。

3. 神經滋養因子 (neurotrophic factors) 和神經可塑性 (neuroplasticity)

臨床研究上發現腦部的神經滋養 (neurotrophic) 和細胞可塑性 (cellular plasticity) 與雙極憂鬱症患者具有高度關聯性，Soeiro-de-Souza 等人 (2012) 歸類出以下幾種可能的機制，包括：鈣離子失調、粒線體/內質網失調、神經元和神經膠細胞的萎縮/死亡和控制情緒腦區內的神經滋養/可塑性效應喪失。依序由小到大的層級來說，就是細胞內的離子層級、能量傳遞途徑分子層級、神經元/膠質的細胞層級和影響腦區等四個層級。鈣離子參與神經傳遞物質的合成、神經可塑性的活化/連結 (cascade) 和突觸鞏固 (consolidation)。細胞內的訊號傳導途徑像是 phosphatidylinositol-3-kinase/AKT、phospholipase C 和 extracellular regulated kinase/MAK 等受影響後，最終都會影響到粒線體等能量傳遞以及神經滋養效應，這些皆與神經可塑性和能量穩定有很大的關聯性 (Soeiro-de-Souza et al, 2012；臺灣生物精神醫學會雙極疾患小組，2014)。

4. 基因與環境

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從流行病學和家庭調查之數據顯示，基因與環境的交互作用對於精神疾病的產生具有舉無輕重的地位 (Cadoret et al., 1995; Kendler and Eaves, 1986)，此現象從 Caspi 等人的研究得到證實，此研究團隊發現血清素轉運子基因 5-HTTLPR 的啟動子區域會受到短的等位基因 (allele) 影響而降低功能，低落的 5-HTTLPR 功能與憂鬱症產生有關 (Caspi et al., 2003; Lesch et al., 1996)，但有趣的是當個體處於壓力環境下才會觸發短的等位基因表現，誘發個體產生憂鬱的症狀 (Caspi et al., 2003)。Heim 和 Binder (2012) 在促腎上腺皮質激素受器 (corticotrophin receptor 1, CRHR1) 基因與憂鬱症的相關研究中指出，幼童含有 CRHR1 同型合子 (homozygous) 之保護性單倍型 (haplotypes) 時，在年幼時期受到的虐待情形對於往後產生憂鬱症的機率顯著較低。在進一步研究中，Tyrka 等人 (2009) 發現暴露於早期創傷造成 HPA axis 功能失常的健康個體，其如果只攜帶 CRHR1 風險性單倍體 (risk haplotypes)，在內分泌刺激試驗中會增強皮質醇的分泌。

(五) 治療方式

1. 藥物治療 (antidepressant)

藥物治療是目前最常使用的方法，藥物作用機制為基於神經傳導物質失調假說所設計 (Torres et al., 2003)(圖二)。現行憂鬱症用藥依作用機制分為單胺氧化酶抑制劑 (monoamine oxidase inhibitors, MAOIs)、三環類抗憂鬱藥 (tricyclic antidepressants, TCAs)、選擇性血清素與正腎上腺素再回收抑制劑 (selective serotonin reuptake inhibitors, SSRIs)、血清素與正腎上腺素再回收抑制劑 (serotonin-norepinephrine reuptake inhibitors, SNRIs) 和血清素受體調節劑 (serotonin receptor modulators, SRMs)。過去研究顯示服用抗憂鬱藥物的患者確實能改善憂鬱症症狀，而在近年研究中發現，抗憂鬱藥物能增加神經可塑性與刺激海馬迴神經生成 (Malberg et al., 2000)。Piubelli 等人 (2011) 進一步利用蛋白質體學方式探討 SSRI 之藥物 escitalopram (ESC) 對於大鼠海馬迴與額葉皮質中抗憂鬱的機制，結果發現給予 ESC 後，與神經可塑性相關的蛋白質表現量有顯著上升。然而，憂鬱症用藥具有許多副作用，如：噁心、嘔吐、食慾異常、頭暈、

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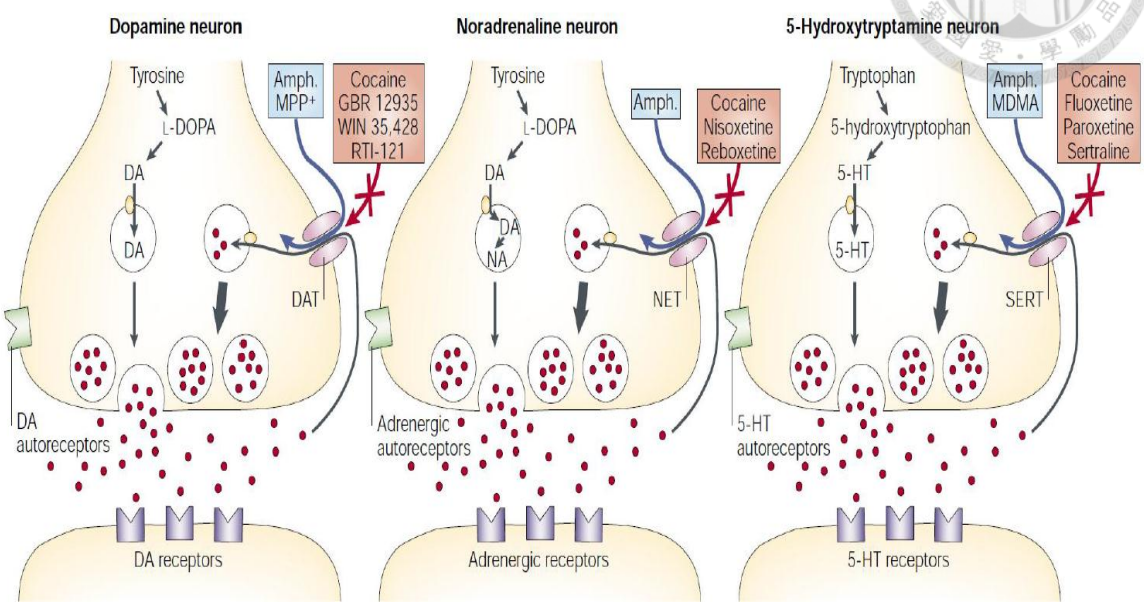
精神不集中與情緒異常波動等 (Khawam et al., 2006)，使得憂鬱症患者順從度降低，因此降低憂鬱症患者的治癒機會。



(Torres et al., 2003)

圖二、神經突觸末端之多巴胺、正腎上腺素以及血清素合成、釋放及回收路徑

Figure 2. Schematic representation of dopamine, noradrenaline, and 5-HT synaptic terminals. Amph, amphetamine; DA, dopamine; DAT, Dopamine transporter; L-DOPA, L-3,4-dihydroxyphenylalanine; 5-HT, 5-hydroxytryptamine; MPP+, 1-methyl-4-phenylpyridinium; MDMA, (+)-3,4-methylenedioxymethamphetamine; NA, noradrenaline; NET, noradrenaline transporter; SERT, 5-HT transporter.

2. 電痙攣治療 (electroconvulsive therapy, ECT)

電痙攣治療原理為在外部給予輕微電刺激，促進神經分泌更多神經傳導因子以治療憂鬱症。此治療方式常於藥物反應不佳或其他治療效果不彰時使用。李等人研究 24 位精神病患經 ECT 治療後的態度與經驗，約有 80% 的患者認為精神況狀有改善，但有 60% 的患者寧願吃藥也不願再接受 ECT 治療，顯示臺灣的部分精神病患者對於 ECT 的接受度不高 (李，2011) 。

3. 心理治療 (psychotherapy)

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心理治療是利用人際關係治療 (interpersonal therapy, IPT)、認知治療 (cognitive therapy, CT)、行為治療 (behavioral therapy, BT) 和認知行為治療 (cognitive-behavioral therapy, CBT) 等方式介入病患，目的除了改善憂鬱症病症，同時恢復病患家庭社會功能，減低其失能的嚴重性，此外也能防止疾病的惡化與復發。IPT 是 Klerman 等人在 1970 年代針對門診憂鬱症病人，所發展出來的心理治療 (Klerman et al., 1974)，至今已應用在輕鬱症、暴食症、憂鬱症復發、躁鬱症、物質濫用、社交恐懼症等等 (Klerman 1984; Weissman 2000)。目前 IPT 注重在病人的社交環境對其產生憂鬱相關的社交障礙 (Weissman 1998)。CT 是以糾正和改變患者適應不良性認知為重點的一類心理治療總稱，它的基礎是情緒障礙理論 (Beck, 1967)、心理學的實驗與臨床的研究 (Kovacs and Beck,1978)，以及界定清楚的治療技術 (Beck et al., 1979)，用來幫助病人減緩症狀並且學習有效的方式來處理病人所遭遇的困難。BT 亦稱矯正治療，此法源自行為主義理論，提出者包括俄國生理學家巴夫洛夫、美國心理學家華生及加斯金納 (牧之和單鵬，2012)。此方法被認為最強調科學化的一種治療方式，行為學派的治療者認為不適應習慣既然是學得的，當然也可以去除；而要成功地去除不適應習慣，最合理的方式是研究有關學習歷程的知識 (廖鳳池，1990)。

CBT 是 CT 和 BT 結合的產物，由於行為治療學者對於認知重組取向逐漸加以重視，及認知治療學者對於行為論在方法學上的優勢逐漸加以承認，而做了有目的的結合 (廖鳳池，1990)。Patelis-Siotis (2001) 認為，CBT 可以改變憂鬱患者的信念，學習適應性行為、改善情緒，促進憂鬱症患者的醫囑配合度，教導並有效預防憂鬱症復發。

4. 光照療法

光線可以抑制松果腺分泌褪黑激素，藉由調整生理時鐘來改善季節性情緒障礙患者的症狀 (Rosenthal, 1993; Eastman et al, 1998)。治療此類病患只需要一個燈箱，光照時間由三十分鐘到四、五小時不等，燈箱的強度隨著症狀嚴重度而定，對光照療法不論是白天或晚上皆有療效。光照治療跟服藥一樣，劑量太多或太少都沒

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有實質效果。一般而言，清晨光度最佳，黃昏次之，中午時間不建議嘗試。另外，睡前勿過度暴露於太過明亮的光線 (夜間褪黑激素分泌量會因此被抑制而造成失眠) (牧之和單鵬，2012)。

二、天麻

(一) 天麻的種類與分佈

天麻 (*Gastrodia elata* Blume) 是由一位荷蘭植物學家 Charles Ludwig de Blume (1796-1862) 所鑑別命名，他是荷蘭阿姆斯特丹國家植物標本館 (州立植物標本室) 主任，當時跟隨荷蘭東印度公司到亞洲南部與其殖民地-爪哇島進行探勘，包括菌種與植物的鑑別。天麻鑑別標本目前存放在荷蘭萊登國家標本館 (圖三)。

(荷蘭萊登國家標本館拍攝)

圖三、天麻標本

Figure 3. The specimen of *Gastrodia elata* Bl.

天麻別名又名赤箭、離母和鬼督郵等，為多年生草本蘭科植物，主要分布在熱帶、亞熱帶、溫帶以至寒溫帶山地，自西邊從馬達加斯加經斯里蘭卡、印度、喜馬拉雅山以南各國、東南亞諸國以至新幾內亞、澳大利亞、新西蘭、新喀里多

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尼亞、日本、朝鮮、中國，以及原蘇聯遠東地區均有分布，臺灣宜蘭、花蓮、台東、南投、嘉義山區海拔 1500 至 3000 公尺分布有少量的天麻 (吳，2016)。現今藥用天麻來源幾乎都來自中國大陸，周等人在 1987 年將天麻分為天麻 (*Gastrodia elata* Bl.)、原天麻 (*Gastrodia angusia* S.et S.C.Chen)、細天麻 (*Gastrodia gracilis* Bl.)、南天麻 (*Gastrodia javanica* Bl. *Lindl*.) 與疣天麻 (*Gastrodia tuberculata* F. Y. Liu et S. C. Chen) 等五個品種，其中天麻 (*Gastrodia elata* Bl.) 又有原變型之紅天麻 (*Gastrodia elata* f. *elata*)、綠天麻 (*Gastrodia elata* f. *viridis* Makino)、烏天麻 (*Gastrodia elata* f. *glauca* S. Chow)、黃天麻 (*Gastrodia elata* f. *flavida* S.Chow) 和松天麻 (*Gastrodia elata* f. *alba* S.Chow) 五個變型，前四種為藥用天麻的主要來源。由於野生天麻生長受到環境限制，加上中藥材市場供不應求，中國開始發展以人工方式栽種天麻，主要以紅天麻、烏天麻與烏紅天麻之雜交種為主栽種最廣。紅天麻因生長快與環境適應力強的特性，其種植區域遍及中國大江南北 (周等人，1987)。目前，雲南昭通市是人工栽種天麻的主要產地，根據當地財經新聞報導 2011 年至 2014 年，全市累計完成天麻種植 24.09 萬畝、產量 7455 萬公斤，累計種植產值達 109.23 億元人民幣，去年全市天麻種植面積預計達到 7 萬畝，實現商品麻產量 2100 萬公斤，總種植產值 27 億元人民幣，預估 2020 年，全市天麻種植面積達到 15 萬畝，種植業產值達到 35 億元人民幣，加工產值突破 70 億元人民幣 (徐，2015)。中華人民共和國衛生部為了防止天麻市場的價格被有心人士漫天喊價，將天麻分成四個等級，其等級標準如下：

一等天麻乾貨：呈長橢圓形，扁縮彎曲；去淨粗栓皮，表面黃白色，有橫環紋，頂端有殘留莖基或紅黃色的枯芽，末端有圓盤狀的凹臍形疤痕；質堅實、半透明，斷面角質、牙白色；味甘微辛；每千克 26 支以內，無空心、枯炕、雜質、蟲蛀、黴變。

二等天麻乾貨：呈長橢圓形，扁縮彎曲；去淨粗栓皮，表面黃白色，具橫環紋，頂端有殘留莖基或紅黃色的枯芽，末端有圓盤狀的凹臍形疤痕；質堅實，半透明，

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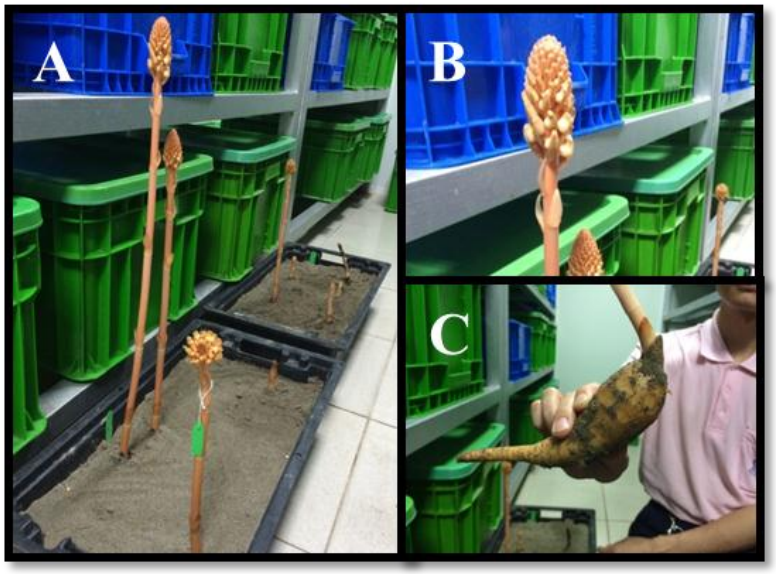
斷面角質、牙白色；味甘微辛；每千克 46 支以內，無空心、枯炕、雜質、蟲蛀、黴變。

三等天麻乾貨：呈長橢圓形，扁縮彎曲；去淨栓皮，表面黃白色，具橫環紋，頂端有殘留莖基或紅黃色的芽，末端有圓盤狀的凹臍形疤痕；質堅實、半透明，斷面角質、牙白色或棕黃色稍有空心；味甘微辛；每千克 90 支以內，大小均勻，無枯炕、雜質、蟲蛀、黴變。

四等天麻乾貨：每千克 90 支以外；凡不符合一、二、三等的碎塊、空心及未去皮者均屬此等；無蘆莖、雜質、蟲蛀、黴變 (中華人民共和國衛生部，1984)。

天麻為傳統著名中藥材具有極高的保健及藥用功效，由於價格昂貴且臺灣尚未普及栽種，藥材多購自中國大陸，品質參差不齊令人堪憂，有鑒於此行政院農業委員會桃園區農業改良場於近年開始投入天麻研究 (圖四)，以期能自給自足 (行政院農委會，2011)。農委會桃園區農業改良場以 7 至 8 坪大小的冷房種植



圖四、天麻外觀圖 (A) 花 (B) 和塊莖 (C)

Figure 4. Photographs of *Gastrodia elata* Bl. (A), flowers (B), and tuber (C).

有機天麻的副研究員葉志新表示，在國內平地或低海拔的地區，可以冷房種植天麻，將天麻植栽種如同菇寮的冷房內，全年溫度控制在 25 ^{o}C 以下，濕度維持在 60% 至 80% 之間，由於冷房成本較高，冷房規模要達到 100 平方公尺以上才符合經濟效益，臺灣每年從中國進口 6、7 萬公斤的天麻，所需要的種植面

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積約須 3 公頃，才足以因應國人的需求量 (吳，2016)。

(二) 天麻活性相關研究

1. 對中樞神經系統的作用

(1) 抗癲癇

根據自由基誘發神經損傷之體外及活體試驗模式顯示，天麻萃取物及主要化合物具有神經保護之功效 (Liu and Mori, 1992; Hsieh et al., 1999; Hsieh et al., 2001)。天麻甲醇萃取物可改善由低含量 γ-aminobutyric acid (GABA) 和高含量 glutamate 所誘發的神經興奮毒素 (excitotoxin)，有效減緩癲癇的症狀 (Huh et al., 1995; Ha et al., 2000)。Kim 等人在動物癲癇模式中發現，天麻甲醇萃取物具有保護小鼠海馬迴免於 kainic acid 誘導的神經傷害，應用於自發性癲癇動物模式中也得到相似的實驗結果(Kim et al., 2001; Kim et al., 2003)。天麻抗癲癇主要的路徑是經由 c-Jun N-terminal Kinase 訊息傳導路徑來調節 activator protein 1 表現，達到治療癲癇的效果 (Hsieh et al., 2007)。

(2) 抗憂鬱

本實驗室先前在 FST 誘導類憂鬱的動物模式中給予大鼠天麻水萃物，發現可顯著降低老鼠在水中不泳動的時間，並顯著增加腦部 5-HT 和 DA 含量 (Chen et al., 2008; Chen et al., 2009)。此外，進一步以基因體學與蛋白質體學分析天麻水萃物對大鼠在 FST 模式抗憂鬱效果的機制，在基因體學分析發現與軸突新生 (axongenesis)、神經新生 (neurogenesis)、神經系統發展和多巴胺分泌最具相關性 (陳，2011)；在蛋白質體學分析發現與神經細胞骨架重組 (neuronal cytoskeleton remodeling) 成正相關 (林，2012)。不只天麻水萃物具有抗憂鬱效果，Zhou 等人在 FST 和尾部懸吊 (tail suspending test, TST) 誘導憂鬱之動物模式中，餵食小鼠天麻乙醇萃取物後發現也可顯著降低老鼠不泳動及不掙扎的時間。

(3) 神經保護

一些抗氧化劑和自由基清除劑已經被證實可抑制腦部缺血和神經興奮毒素所導致的神經損傷 (Block et al., 1995; O'Neill et al., 1997; Lee et al., 2000)。利用

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血清缺乏、組織缺氧、過氧化氫、氯化鉀或 1-methyl-4-phenylpyridinium 誘導 rat pheochromocytoma (PC12)、human dopaminergic (SH-SY5Y)、primary neurons 造成損傷，經天麻甲醇或乙醇萃取物處理後皆可提高細胞的存活率 (An et al., 2010; Huang et al., 2004; Kam et al., 2011; Kim et al., 2007b; Zeng et al., 2006)。

(4) 改善記憶衰退

阿茲海默症主要是由不可溶的 amyloid β-peptide 不正常的累積在腦部造成神經細胞功能異常或死亡 (Glenner and Wang, 1984)。*In vitro* 方面，有文獻指出天麻甲醇萃取物可以降低神經或類神經細胞經 CT105 (一種 amyloid β-protein 前驅蛋白) 所誘導的記憶衰退 (Kim et al., 2007a)；*In vivo* 方面，天麻也具有改善經金屬鋁所誘導神經細胞與記憶退化之動物模式 (Shuchang et al., 2008)。

2. 對心血管系統的作用

有文獻指出天麻酚類成分可藉由抑制 collagen、epinephrine、sodium arachidonate 和 9, 11-dideoxy-11α, 9α-epoxymethanoprostagrandin F2α 等凝血因子，以達抗凝血的效果 (Pyo et al., 2004)。人類靜脈內皮細胞 (umbilical vein endothelial cells) 是藉由凝血系統 (coagulation) 和纖溶作用 (fibrinolysis) 來調節血小板凝集，Hwang 等人 (2009) 將此細胞株利用天麻乙醇萃取物處理後，結果發現可降低 tumor necrosis factor (TNF)-α 所導致的動脈粥狀硬化症發生。大腦動脈阻斷 (middle cerebral artery occlusion, MCAO) 的動物模式主要是模擬病患缺血性腦中風，大鼠手術前餵食天麻的活性成分 HBA 和 GAS，具有明顯降低梗塞程度與水腫體積的作用 (Kam et al., 2011; Yu et al., 2010)。

3. 抗發炎作用

天麻之乙醇萃取物或分離之酚類化合物如：4-hydroxybenzaldehyde、HBA、benzyl alcohol 和 vanillyl 能有效治療由醋酸和花生四烯酸引起的發炎模式 (Lee et al., 2006; Ahn et al., 2007)，推測是利用抑制 cyclooxygenase (COX) 和氧化壓力來達到抗發炎功效 (Ahn et al., 2007; Hwang et al., 2009; Lee et al., 2006)。

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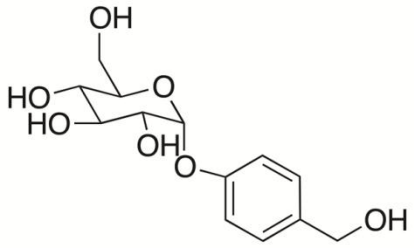
三、天麻苷 (gastrodin, GAS)

(一) 一般性質

IUPAC 名稱為 p-hydroxymethylphenyl-b-D-glucopyranoside，化學式為 $C_{13}H_{18}O_7$，分子量為 286.28，熔點 154-156^{o}C，可溶於甲醇和乙醇，結構式如圖五所示。



圖五、天麻苷結構式

Figure 5. The structure of gastrodin.

(二) 天麻苷來源與生理活性

天麻苷 (GAS) 為天麻中的一種主要活性成分，其毒性低和功效佳的特性多用於治療暈眩、頭痛、失眠、神經痛、神經衰弱和癲癇等中樞神經失調的症狀 (Liu et al., 1988; Wu et al., 1996; Hsieh et al., 1997; Ojimann et al., 2006; Zeng et al., 2006)。目前，GAS 多應用於探討改善癲癇與缺血性腦中風之研究上。癲癇中有三種降解 γ-Aminobutyric acid (GABA) 的酵素 (GABA-transaminase、succinic semialdehyde dehydrogenase 和 succinic semialdehyde reductase)，當酵素活性失控時會使大量的 GABA 降解並傷害大腦(An et al., 2003)。An 等人 (2003) 連續管灌餵食沙鼠 60 mg/kg bw GAS 一週後，發現 GAS 無法藉由提升 glutamate decarboxylase 的表現來增加 GABA 的含量，也無法降低 GABA transporter 1 減緩 GABA 過度的回收，但可抑制 GABA-transaminase、succinic semialdehyde dehydrogenase 和 succinic semialdehyde reductase 此三種酵素的表現，防止 GABA 大量的降解。Choi 認為腦部局部缺血 (ischemia) 會增加細胞間 glutamate 的濃度，造成神經細胞的 glutamate 受器過度活化，進而導致 N-methyl-D-aspartate 通道增進鈣和鈉離子流入細胞內 (influx)，使神經膜電位去

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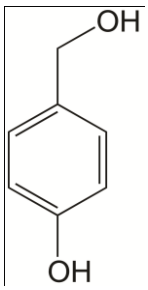
極化，因此推測神經中過多的鈣離子是腦部局部缺血主要造成神經死亡的主要機制 (Choi, 1985; 1988)。腦部局部缺血初期傷害會偵測到 nNOS 和 eNOS 的存在，之後會藉由 iNOS 產生大量的 NO (Iadecola, 1997)。Zeng 等人 (2006) 給予初代海馬迴細胞 15 μg/ml 天麻苷後，其可經由降低鈣離子和 NO 的濃度來提高神經細胞的存活率。在 MCAO 動物模式誘導大鼠產生短暫腦內缺血結果發現，天麻苷可降低腦部缺血所造成的神經傷害 (Ren et al., 1992; Zeng et al., 2006; Zeng et al., 2007)。此外，天麻苷可具有改善高血壓造成的細胞死亡率 (Wang et al., 1994)。

四、天麻苷元 (4-hydroxybenzyl alcohol, HBA)

(一) 一般性質

IUPAC 名稱為 4-hydroxybenzyl alcohol，化學式為 $C_7H_8O_2$，分子量為 122，熔點 116-117^oC，可溶於甲醇和氯仿，結構式如圖六所示。



圖六、天麻苷元結構式

Figure 6. The structure of 4-hydroxybenzyl alcohol.

(二) 天麻苷元來源與生理活性

天麻苷元 (HBA) 廣泛存在於植物中 (Kobayashi et al 2003; Huang et al 2004; Cai et al 2008)，為天麻中其中一種主要的酚類化合物，可由 vanillin 和 4-hydroxybenzaldeyde 合成 (Ha et al., 2001; Jung et al., 2006)。在氧化壓力相關疾病研究如：心血管疾病、中風和癌症上扮演重要的角色。目前在治療神經相關疾病 (neuronal disorders) 的研究如下：抗癲癇 (Liu and Mori, 1993)、抗焦慮 (Jung et al., 2006)、防止腦部缺血性中風所導致的神經損傷 (Yu et al., 2005; Kim et al.,

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2007b; Yu et al., 2010) 和改善由 cycloheximide 誘導 memory consolidation 障礙之失憶問題 (Hsieh et al., 1998)。天麻中活性成分 HBA 具有保護腦內缺血所造成的細胞凋亡。Kam 等人及 Yu 等人利用 MCAO 誘導大鼠產生短暫腦內缺血，於誘導前 30 分鐘給予 HBA 對神經細胞保護之可能途徑進行研究。研究結果發現預先給予 HBA 經由抑制 caspase-3 和增強 Bcl-2 表現，並且活化 *PDI*、*Nrf2* 和 *BDNF*、*GDNF*、*MBP* 及 *NGF* 等神經滋養基因大量表現來協助神經細胞對抗氧化壓力所造成的傷害 (Kam et al., 2011; Yu et al., 2010)。而 Tsai 等人 (2011) 在 PC12 細胞的缺血模式中，結果發現天麻層析物可經 adenosine 受器的接收，由 cAMP/PKA/CREB 途徑提升 cAMP response element-binding protein (CREB) 表現來抑制細胞凋亡的發生。

五、憂鬱症細胞模式

(一) 大白鼠腎上腺髓質嗜鉻細胞瘤

大白鼠腎上腺髓質嗜鉻細胞瘤 (rat adrenal medulla pheochromocytoma, PC12) 是 Greene 和 Tischler 首次在 1976 年成功選殖出的細胞株，此細胞與交感神經 (sympathetic neurons) 同源，培養過程可經由神經成長因子 (nerve growth factor, NGF) 的添加來誘導未分化 PC12 (naive PC12, nPC12) 的細胞分化作用，使其神經突觸外生與分枝產生，各分支的中、末端上儲存之兒茶酚胺類 (catecholamine) 神經傳導物質 (如：多巴胺 (DA) 及正腎上腺素 (NE)) 之小液泡 (vesicles)、分解酵素及相關蛋白質的鈣/鈣調素依賴蛋白激酶二 (calcium/camodulin-dependent protein kinase II) 和單胺氧化酶 (monoamine oxidase) 等，細胞膜上富含糖皮質固醇 (glucocorticoid) 和 N-methyl-D-aspartate (NMDA) 的受器，明顯地表現出成熟交感神經細胞特性，我們稱之為已分化的 PC12 (differentiated PC12, dPC12)。在研究上 nPC12 和 dPC12 細胞株皆可做為模擬神經細胞的細胞模式，廣泛應用於神經相關研究，如：缺血性中風造成的神經損傷 (Leiser et al., 2012)、癲癇 (Sirerol-Piquer et al., 2006)、阿茲罕默症

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(Hamaguchi et al., 2010)、漸凍症 (Ghadge et al., 1997)、憂鬱症 (林，2010) 等相關的神經研究。

(二) 人類寡樹突細胞瘤

大多數原發性腦腫瘤是從神經膠質細胞衍生而來，因此被統稱為神經膠質瘤。其中，惡性神經膠質瘤是成人最常見的一種腦腫瘤 (Griscelli et al., 2000)。人類寡樹突細胞瘤 (Human oligodendroglioma, Hs683) 屬於低度惡性 (low-grade) 腫瘤，最早是從一位 76 歲男性白人的左顳葉分離至體外培養，此細胞株有微絨毛 (microvilli) 但沒有橋粒 (desomosomes)，目前廣泛應用於抑癌的相關研究。Songc 和 Moon (2006) 在人類神經膠質瘤的研究中，發現 glial cell-derived neurotrophic factor (GDNF) 是活化 mitogen-activated protein kinases (MAPKs) (包括：c-Jun N-terminal protein kinase (JNK)、extracellular signal-regulated kinases (ERKs) 和 p38 MAPK) 並促使 Hs683 移行 (migration) 的關鍵因子，增進往後對於神經膠質瘤惡性進展的了解。Leucine-rich glioma inactivated (*LGI*) 和 *SLIT* 基因有一個富含白氨酸的重複序列位於蛋白質 N-末端，其具有受器功能或與胞外基質相互作用。*LGI* 和 *SLIT* 基因家族成員與特定癌症具有密切關係，並限制正常細胞中的生理表現。Rossi 等人 (2005) 在此研究中以數種腫瘤細胞株探討這些基因在其細胞內的表現程度與分佈，以利釐清各基因的功能。其中，包括 Hs683 在內的神經膠質瘤皆有少量 *SLIT1* 基因表現。

六、憂鬱症動物模式

McKinney 和 Bunney (1969) 依據憂鬱症其病因、生物化學，症狀條件和治療情況制定了一套評估動物憂鬱症模式是否有效的標準。此標準包括：表面效度 (face validity)、建構效度 (construct validity) 和預測效度 (predictive validity)。表面效度涉及心理性測驗和其個別的項目有關之 (視覺) 特徵或印象，其定義為以一個評量受測者的角度，對於評量和其評量的項目與其曾經深思熟慮過的情境、處境或情況之相關程度。表面效度不是依據心理學家或專家的判斷，而是接受評

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量的受測者，其對於評量的領域皆具有相當純真的想法；建構是抽象的概念，如同個人的個性、態度、動機等，皆屬於無形特徵，只能觀察與這些建構有關的行為、徵兆或表現。建構效度通常是數組指標或項目所構成，實際評量 (測驗、量表) 工具能測量理論上某建構、概念或特質的程度，故建構效度可以視為研究探索之理論構念或理論概念與實際評量項目之間的契合程度，以統計方法估算獲得數值；預測效度其效標分數在評量實施後一段時間內可以取得，期望可以使用實際評量分數預測效標分數，屬於一種對未來行為的預測準確程度之指標。Willner (1988) 利用 McKinney 和 Bunney 制定的標準評估當今十八種動物憂鬱症模式，其中以大鼠顱內自我電刺激 (intracranial self-stimulation)、慢性壓力 (chronic stress)、習得無助 (learned helplessness) 與靈長類動物模型分離模式 (the primate separation model) 具有確實誘導憂鬱的效果。即使如此，並非所有憂鬱症患者的誘因與症狀皆一致，所以動物模式通常只能觀察其誘發後的行為是否與人類憂鬱症相符 (Gronli et al., 2004)。目前，誘發憂鬱症的動物實驗分為行為絕望模式和藥物相互作用模式，常見模式種類如下列幾種：

(一) 行為絕望模式

1. 強迫游泳實驗 (forced swimming test, FST)

強迫游泳試驗 (FST) 由 Porsolt 等人 (1977) 提出，在此模式中老鼠會強迫游泳並習得無論如何掙扎都無法逃脫此環境，會呈現放棄狀態，這狀態稱之為行為絕望。方法為將大鼠或小鼠被迫侷限在透明圓柱桶裡游泳，老鼠會在水裡掙扎試圖逃脫，隨後會放棄掙扎而漂浮在水面上處於靜止不動的狀態。不動時間愈長顯示老鼠愈絕望。Porsolt 以此模式篩選抗憂鬱藥物，發現只有抗憂鬱藥物能夠降低老鼠的不泳動時間，而抗焦慮、抗精神病用藥皆無作用 (Porsolt et al., 1979)。過去的研究證實此模式會降低神經傳導物質 (West, 1990)、造成神經損傷 (Llorens-Martin et al., 2007)，因此，FST 為目前最廣於篩選抗憂鬱藥物效力的平台之一。

2. 尾部懸吊試驗 (tail suspension test, TST)

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尾部懸吊試驗 (TST) 是由 Steru 等人於 1985 所提出，此模式小鼠尾巴會被吊起並倒掛懸於半空中，小鼠為掙脫而掙扎扭動，但數分鐘之後其會變得無助而不再掙脫扭動，呈現失望之現象。此現象與 FST 中大鼠因絕望而靜止不動相似。

3. 習得無助模式 (learned helplessness, LH)

習得無助模式 (LH) 是由 Seligman 和 Maier (1967) 所提出，動物遭受無法逃逸之電擊後，將其放在可以逃逸的電擊環境下，呈現逃逸行為障礙或自發活動減少等等。方法為將大鼠放在電擊箱並給予電擊，電擊後形成逃避行為缺欠，可持續數日甚至數週，可利用這段時間評估藥物的作用。

4. 慢性壓力試驗 (chornic mild stress, CMS)

慢性壓力試驗 (CMS) 是由 Katz (1981) 所提出，在此模式下每天給予動物不同的壓力，如：禁食、禁水、潮濕環境、日夜週期顛倒和冰水中游泳等，並持續數週，經長期處於這些壓力環境之下，動物被誘發類似人類憂鬱症的症狀，如運動能力、社會交往能力和探索行為能力下降等 (Willner, 1997)，並使正向回饋與獎賞機制 (reward system) 受損，減低食慾與對糖水的嗜好 (Pucilowski et al., 1993; Gronli et al., 2005)。目前，CMS 被認為是一個較符合人類憂鬱症發生原因的模式，因此廣泛被用於憂鬱症研究。

5. 慢性社交挫敗壓力模式 (chronic social defeat stress, CSDS)

慢性社交挫敗壓力模式 (CSDS) 最早是由 Kudryavtseva 等人 (1991) 建立，並經 Berton 和 Nestler (2006) 修正。主要模擬人類因經歷社交失敗後所產生的壓力，對其情緒產生憂鬱、孤獨、焦慮和社交退縮等負面影響。因此，此模式相較於 CMS，其壓力來源並非只有人為的環境壓力，還有動物間社交過程遭遇挫敗所引起的社會壓力，藉由此模式反映人類憂鬱症病程 (Hollis et al., 2014)。方法是將欲誘導之小鼠作為侵入者，放入另一隻年紀、體型較強勢小鼠所居住之籠子中，兩者進行 5 分鐘肢體接觸，體型劣勢的小鼠經歷挫敗並且產生服從行為 (submissive behavior)，如：仰臥姿勢 (supine posture)、頻繁的發出叫聲、逃跑

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(escape) 及凍結行為 (freezing behavior) (Kabbaj et al., 2001; Yu et al., 2011)。肢體接觸後以透明具有孔洞的隔板分離進行 24 小時感官接觸，同樣的流程重複持續進行十天。動物經歷社交挫敗後會產生社交迴避 (social avoidance) 以及興趣喪失 (anhedonia) 等類似人類憂鬱症的現象 (Krishnan et al., 2007)。

6. 嗅球切除術 (olfactory bulbectomy model)

嗅球切除術是 Marks 等人 (1971) 依據 Lindley (1930) 發現大鼠嗅覺喪失會造成學習及行為表現障礙的觀念所建立，如：自發性活動增加、探索行為喪失和進食和性行為改變等 (Kelly et al., 1997; Zueger et al., 2005)。Van Riezen 等人 (1976, 1977) 發現此模式可用於新抗憂鬱用藥的開發。因此，將此模式憂鬱症認定是憂鬱症誘導模式 (Wren et al., 1977)。方法是將大鼠嗅球切除，經 14 天左右的恢復期。然後進行 OFT 試驗觀察 3 分鐘內大鼠活動情況。具體指標多以運動量為主 (如：移動距離、時間和速度等)。

(二) 藥物相互作用模式

1. 蛇根鹼拮抗 (reserpine reversal)

Reserpine (商品名：Raudixin、Serpalan 和 Serpasil) 是一個吲哚生物鹼 (indole alkaloid)，早期用於高血壓的控制和精神病症狀緩解，但其會抑制單胺神經傳導物質在突觸神經末梢囊泡之貯存，促使神經末梢的單胺類被排空耗盡。長期的單胺儲存減低會導致腦內和周邊的 NE、DA、5-HT 耗竭，誘發類憂鬱的異常生理和行為反應 (Holzbauer, M., Vogt, M. 1956; Carlsson et al., 1957)。

2. 高劑量 apomorphine 拮抗法

Apomorphine 是一種催吐劑，藉由刺激腦中的 D1、D2 受體及化學受器觸發區 (Chemoreceptor trigger zone)，活化髓質嘔吐中樞引起想吐反應。低劑量 apomorphine 會透過 D1、D2 受體引起體溫下降 (hyperthermia)；高劑量的 apomorphine 的降溫作用是由 NE 神經系統參與。因此，可透過動物失溫與否評估抗憂鬱藥功效 (Lapin and Samsonova, 1968; Puech et al., 1981)。

3. 育亨賓增強實驗 (yohimbine potentiation model)

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Yohimbine 是一種 MAOI，具有壯陽與興奮劑功效。Yohimbine也是 α2 受器的拮抗劑，當與 NE 神經元突觸前 α2 受體結合後，阻斷 NE 的負回饋造成 NE 大量釋放 (Quinton, 1963)。Yohimbine 與三環類抗憂鬱用藥、paragyline MAOIs 及各種新的「非典型」的抗抑鬱藥，包括：iprindole、mianserin，nomifensine、buproprion、viloxazine 和 zimelidine 等並用時，對動物具有致死性的殺傷力 (Malick, 1981; Willner, 1984)。

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參、研究目的與實驗架構

本實驗主要目的分為以下兩類:

一、探討天麻苷和天麻苷元抗憂鬱的功效及機制

本實驗室先前成果

1. 利用 HPLC 分析天麻水萃物的組成分，在 220 nm 波長下的分析結果顯示，主要成分為天麻苷和天麻苷元。

2. 在 FST 誘導動物類憂鬱實驗結果顯示，天麻水萃物可降低腦部單胺類神經傳導物質的代謝轉換率來達到具有抗憂鬱的功效。

3. 基因體和蛋白質體學研究顯示，天麻水萃物抗憂鬱的效果是透過調控神經細胞骨架重組中之 Slit-Robo 路徑。

提出假說:

此兩種成分天麻苷和天麻苷元提供天麻水萃物抗憂鬱的功效。

二、探討不同加工處理、萃取方法、來源和變種天麻間，其天麻苷含量的變化

目前現況

1. 天麻苷是臺灣中藥典鑑別天麻的指標成分，但是薄層層析 (thin layer chromatography, TLC) 的分析方法易因人為因素造成誤差；HPLC 之分析方法因其移動相沒有以梯度方式沖提，降低定量天麻中天麻苷的準確性。

提出假說:

天麻中的天麻苷含量會受到不同加工方式、來源和變種而有差異。

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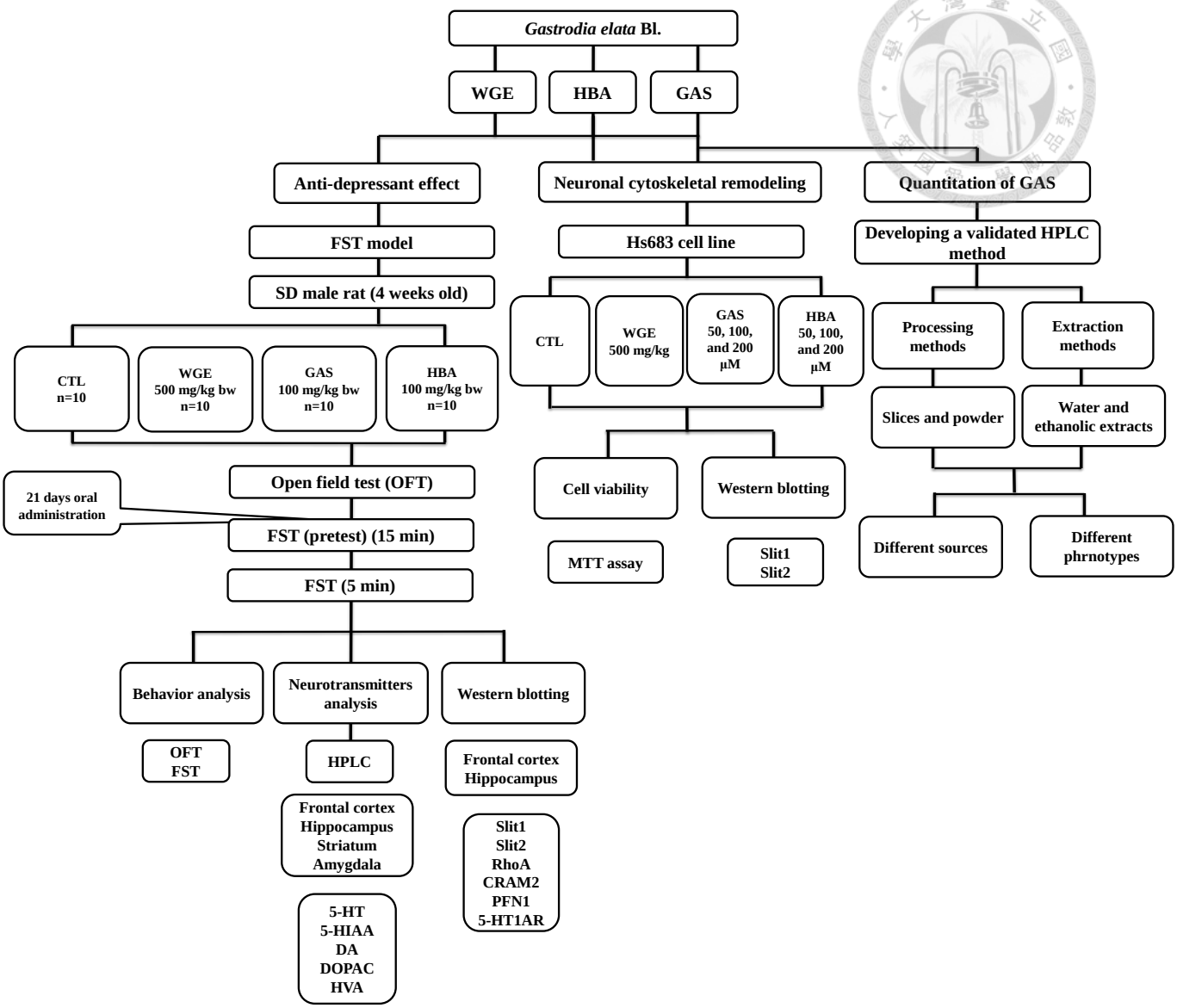

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肆、實驗材料與方法

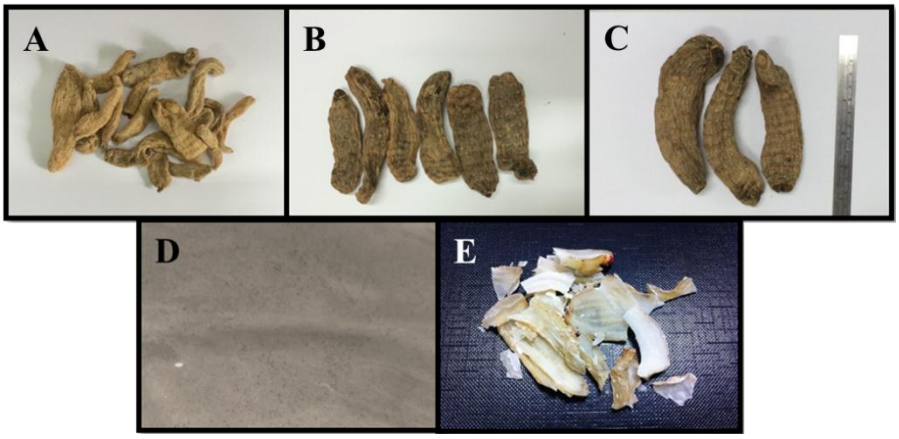
一、實驗材料

(一) 天麻樣品

1.天麻樣品由雲南中醫學院中藥材優良種苗繁育中心周志宏副研究員提供，產地來源如下 (圖七)：

- 雲南紅天麻 (*Gastrodia elata* Bl. f. *elata* in Yun-nan, GRY)：昆明菊花村中藥材市場 (該市場是國內批准的 17 家合法中藥材經營市場)，中國大陸
- 雲南烏天麻 (*Gastrodia elata* Bl. f. *glauca* S. Chow in Yun-nan, GBY)：雲南昭通，中國大陸
- 湖南紅天麻 (*Gastrodia elata* Bl. f. *elata* in Hu-nan, GRH)：湖南，中國大陸
- 紅天麻粉末 (powder) 和切片 (slices)：雲南昭通，中國大陸

2. 天麻水萃物：柯達製藥有限股份公司，桃園，臺灣



圖七、雲南紅天麻 (A)、雲南烏天麻 (B)、湖南紅天麻 (C)、紅天麻粉末 (D) 和紅天麻切片 (E)

Figure 7. *Gastrodia elata* Bl. f. *elata* in Yun-nan (A), *Gastrodia elata* Bl. f. *glauca* S. Chow in Yun-nan (B), *Gastrodia elata* Bl. f. *elata* in Hu-nan (C), powder of *Gastrodia elata* Bl. (D), and slices of *Gastrodia elata* Bl. (E)

(二) 細胞株

人類 oligodendroglioma cell line (Hs683) No.60519 (代數：29)：購自食品工業研究所生物資源保存及研究中心 (Bioresource Collection and Research Center) 新竹,

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臺灣

(三) 藥品試劑

1. gastrodin (GAS) (purity: 99%): 美國羅格斯大學食品科學系何其儻老師提供
2. acetic acid: Sigma, St. Louis, Missouri, U.S.A.
3. acetonitrile (ACN): Biotic Chemical Co., Ltd., Taipei, Taiwan
4. 40% acrylamide/Bis (29:1) solution: Bioman, Taipei, Taiwan
5. ammonium persulfate (APS): Bioman, Taipei, Taiwan
6. antibiotics-antimycotics (AA) solution (100X, 100 units penicillin, 100μg streptomycin and 0.25 μg amphotericin B per mL in saline): Caisson Laboratories, Utah, U.S.A.
7. ascorbic acid: Sigma, St. Louis, Missouri, U.S.A.
8. bolvine serum albumin: Bio-Rad, Hercules, California, U.S.A.
9. bradford protein assay: Bio-Rad, Hercules, California, U.S.A.
10. bromophenol blue (BPB): Bio-Rad, Hercules, California, U.S.A.
11. chaps: Bio-Rad, Hercules, California, U.S.A.
12. 3,4-dihydroxyphenethylamine (DA): Sigma, St. Louis, Missouri, U.S.A.
13. dimethyl sulfoxide (DMSO): Sigma, St. Louis, Missouri, U.S.A.
14. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT): Sigma, St. Louis, Missouri, U.S.A.
15. dithiothreitol (DTT): Bioman, Taipei, Taiwan
16. dulbecco's modified eagle's medium (DMEM, high glucose, with L-glutamine, pyridoxine hydrochloride, 110 mg/l sodium pyruvate): Sigma, St. Louis, Missouri, U.S.A.
17. enhanced chemiluminescent HRP substrate (ECL): Merck Millipore, Darmstadt, Germany
18. ethylenediamine tetraacetic acid (EDTA): Riedel-de Haen, Seelze, Germany

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19. fetal bovine serum (FBS): Biological Industries, Israel
20. glycine: Bioman, Taipei, Taiwan
21. homovanillic acid (HVN): Sigma, St. Louis, Missouri, U.S.A.
22. hydrophobic immobilon-P poiy vinylidene fluoride (PVDF) transfer membrane 0.22 and 0.45 μm (0.22 and 0.45 micron filter) pore size: Merck Millipore, Darmstadt, Germany
23. hydrogen chloride: J.T. Backer, Pennsylvania, U.S.A.
24. hydroxy chloride: Sigma, St. Louis, Missouri, U.S.A.
25. 4- hydroxy-3-methoxybenzaldehyde (VAN): Sigma, St. Louis, Missouri, U.S.A.
26. 4-hydroxybenzyl alcohol (HBA): Sigma, St. Louis, Missouri, U.S.A.
27. 4-hydroxybenzyl aldehyde (HB): Sigma, St. Louis, Missouri, U.S.A.
28. 5-hydroxyindoleacetic acid (5-HIAA): Sigma, St. Louis, Missouri, U.S.A.
29. 5-hydroxytryptamine (5-HT): Sigma, St. Louis, Missouri, U.S.A.
30. iodoacetamide (IAA): Sigma, St. Louis, Missouri, U.S.A.
31. isoproterenol: Sigma, St. Louis, Missouri, U.S.A.
32. lower buffer 4X: Bioman, Taipei, Taiwan
33. methanol: Sigma, St. Louis, Missouri, U.S.A.
34. N, N, N', N'-tetramethylethylenediamine (TEMED): Bio-Rad, Hercules, California, U.S.A.
35. non-essential amino acids (NEAA) for MEM (100X): Caisson Laboratories, Utah, U.S.A.
36. 1-octanesulfonic acid, sodium salt (SOS): J.T.Backer Inc., Phyllipsburg, New Jersey, U.S.A.
37. pargyline: Sigma, St. Louis, Missouri, U.S.A.
38. phenylmethanesulfonylfluoride (PMSF): Sigma, St. Louis, Missouri, U.S.A.
39. prestained protein molecular weight marker: Bioman, Taipei, Taiwan

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40. PRO-PREP^{TM} protein extraction solution: Boca Scientific, Boca Raton, Florida, U.S.A.
41. protein molecular weight marker: Fermentas, Glen Burnie, Maryland, U.S.A.
42. RIPA buffer: Sigma, St. Louis, Missouri, U.S.A.
43. sodium dihydrogen phosphate (NaH_2PO_4): Merck KGaA, Darmstadt, Germany
44. sodium dodecyl sulfate: Bio-Rad, Hercules, California, U.S.A.
45. sodium phosphate dibasic: (Na_2HPO_4): Merck KGaA, Darmstadt, Germany
46. sypro® Ruby gel stain: Invitrogen, Carlsbad, California , U.S.A.
47. thiourea: Sigma, St. Louis, Missouri, U.S.A.
48. triethylamine (TEA): Tedia, Fairfield, Ohio, U.S.A.
49. tris (hydroxymethyl) aminomethane (Tris base): Bioman, Taipei, Taiwan
50. trypan blue: Biological Industries, Israel
51. trypsin: Caisson Laboratories, Utah, U.S.A.
52. Tween®20: Sigma, St. Louis, Missouri, U.S.A.
53. upper buffer 4X: Bioman, Taipei, Taiwan
54. urea: Bioman, Taipei, Taiwan
55. β-mercaptoethanol: Bio-Rad, Hercules, California, U.S.A.

(四) 血清分析套組

1. 血清皮質固醇分析套組：Enzo Life Science Inc., New York, U.S.A.。
2. 血清大腦衍生神經滋養因子 (brain-derived neurotrophic factor, BDNF) 分析套組：Merck Millipore, Darmstadt, Germany

(五) 抗體

1. dihydropyrimidinase-related protein 2 (DPYSL2, also called CRMP2) (GTX48742): Cell Signaling Technology, Inc. Danvers, Missouri, U.S.A.
2. glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (GTX100118): GeneTex, Inc. Irvine, California, U.S.A.

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3. 5-HT1A receptor (ab85615): Abcam, Cambridge, Massachusetts, U.S.A.
4. profilin1 (PFN1) (C56B8): Cell Signaling Technology, Inc. Danvers, Massachusetts, U.S.A.
5. ras homologous member A (RhoA) (GTX101066): Cell Signaling Technology, Inc. Danvers, Massachusetts, U.S.A.
6. secondary antibodies: Cell Signaling Technology, Inc. Danvers, Massachusetts, U.S.A.
7. Slit1 (GTX48742): GeneTex, Inc. Irvine, California, U.S.A.
8. Slit2 (GTX37763): GeneTex, Inc. Irvine, California, U.S.A.

(六) 實驗設備

1. 軌道式搖床: Fisher Scientific, U.K.
2. 一維電泳設備: Hoefer, U.S.A.
3. 轉漬設備: Hoefer, U.S.A.
4. 恆溫離心機 (centrifuge): Hermle Z 300 k, Wehingen, Germany
5. 恆溫離心機 (centrifuge): CL2, Thermo Electron, Waltham, Massachusetts, U.S.A.
6. 高效液相層析儀: Jasco HPLC system, Tokyo, Japan
7. 高效液相層析儀: L-7100 pump, Hitachi Co. Ltd., Tokyo, Japan
8. 電化學檢出器 (electrochemical detector, ECD): Antec, LLC, Boston, Massachusetts, U.S.A.
9. 紫外光-可見光檢測器 (UV-VIS detector): UV-2075 Plus, Tokyo, Japan
10. 分析管柱 (analysis column) 型號: phenomenex Luna C18 (250 mm × 4.6 mm, 5 μm), Torrance, U.S.A.
11. 分析管柱 (analysis column) 型號: SPOLAR C18 column, 4.6 × 250 mm, 5 μm, Shiseido Co., Ltd, Tokyo, Japan
12. 溶媒脫氣裝置 (degasser): Jasco LC-NetII/ADC, Tokyo, Japan

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13. 溶媒輸出系統 (solvent delivery system): PU-2089 Plus, Tokyo, Japan
14. 保護管柱 (guard column): Alltima C18 column, 7.5 × 4.6 mm, 5 μm, Grace Co, Ltd, U.S.A.
15. 自動注射器: L-2200 autosamper, Hitachi Co. Ltd., Tokyo, Japan
16. 震盪器 (shaker): 9D3XL, Hotech, Taipei, Taiwan
17. 酸鹼測定計: pH meter D21, Horiba, Kyoto, Japan
18. 液態氮桶 (cryogenic refrigerator): LNC-35R, PanChum Scientific, Taipei, Taiwan
19. 超低溫冷凍櫃 (ultralow temperature freezer): NU-6382, NuAire, Plymouth, Minnesota, U.S.A.
20. 加熱攪拌器: stirrer1000, Jenway, Essex, U.K.
21. 分析天秤: analytical balance AB104-S, MonoBloc, Greifensee, Switzerland
22. 冷光影像分析系統 (UVP biospectrum): AC system EC3 300, Corning, New York, U.S.A
23. 冷凝器 (cooler): EYELA Cool Ace CA-1110, Tokyo Rikakikai, Tokyo, Japan
24. 超音波振盪機 (ultrasonic liquid processor): S-4000, Misonix, Newtown, Connecticut, U.S.A.
25. 電動吸管: Pipet-aid100, Drummond Scientific, Broomall, Pennsylvania, U.S.A.
26. 滅菌釜 (autoclave): TM-325, Tomin Medical Equipment, Taipei, Taiwan
27. 無菌操作台 (laminar flow): BSC-4, Chih Chin, Taipei, Taiwan
28. 細胞培養箱 (CO_2 incubator): Forma 310, Thermo Scientific, Mariette, Ohio, U.S.A.
29. 去離子水製造機: Elix, Merck, Germeny
30. 倒立式相位差顯微鏡 (inverted phase-contrast microscope): CK40, Olympus, Tokyo, Japan
31. 過濾小飛碟 (0.45 μm nylon filters): Agela, Beijing, China

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32. 光度計 (light meter): LI-250A, LI-COR Biosciences Inc., Lincoln, Nebraska, U.S.A.

二、實驗方法

(一) 動物實驗

1. 天麻水萃物 (WGE)、天麻苷 (GAS) 和天麻苷元 (HBA) 之動物實驗劑量篩選

根據 Chen 等人 (2008) 在 WGE 之抗憂鬱研究顯示有效劑量為 0.5 和 1 g/kg bw。其中，WGE 劑量在 0.5 g/kg 有最佳的抗憂鬱效果。因此，在動物預實驗所選定的 WGE 劑量為 0.5 g/kg，而 GAS 和 HBA 含量分別在 WGE 中佔 3.03% 和 0.17%，以此比例回推 GAS 和 HBA 所需攝取的對應劑量，但在 FST 的不泳動實驗結果顯示 GAS 和 HBA 在此劑量下無法降低大鼠不泳動之情形。綜合 Lim 等人 (2007) 和 Jia 等人 (2014) 分別針對 GAS 進行腹腔注射及口服以藥物動物學進行研究之成果，GAS 之生物可利用率 (bioavailability) 為 14.2%，由此推究其原因可能是 GAS 的生物可利用率較低而無法顯著提供生物活性。因此，目前在 GAS 和 HBA 研究方面，動物實驗所使用的口服劑量範圍分別在 60 到 400 mg/kg bw (An et al., 2003; Cai et al., 2008; Zhang et al., 2014; Xie et al., 2007) 和 25 到 100 mg/kg bw (Yu et al., 2010; Lim et al., 2007)。在神經科學研究方面，Zhang 等人 (2014) 以不可預期之壓力模式 (chronic unpredictable stress, CUS) 下發現餵食大鼠 100 和 200 mg/kg bw 可促進神經新生。綜上所述，WGE 參考 Chen 等人 (2008) 之最佳劑量 0.5 g/kg bw，GAS 參考 Zhang 等人 (2014) 之有效劑量 100 mg/kg bw，而 HBA 參考目前神經研究所使用的動物最大劑量 100 mg/kg bw (Lim et al., 2007)，進一步以 FST 評估 GAS 和 HBA 是否具有抗憂鬱之效果。

2. 動物飼養

由樂斯科生物科技股份有限公司購入四週齡 SD 雄性大鼠，秤重後依體重

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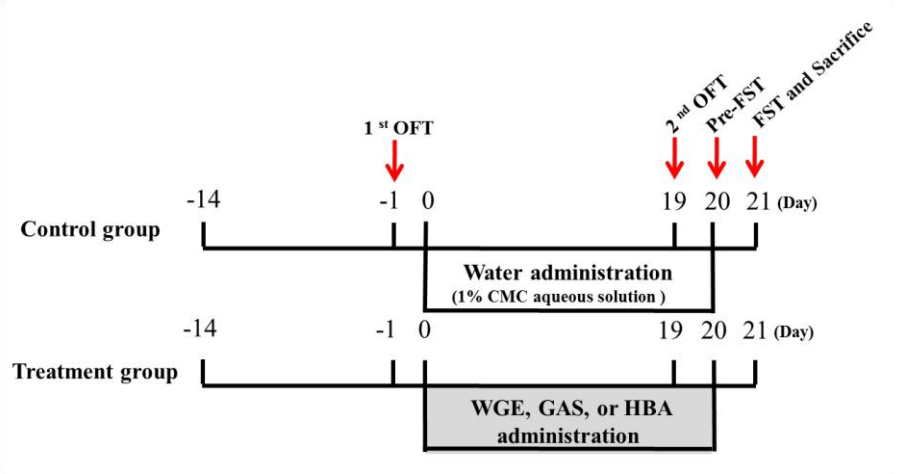
採 S 型排序方式分為四組，分別為控制組 (control, CTL)、天麻水萃物組 (WGE)、天麻苷組 (GAS) 與 天麻苷元組 (HBA)，並以一隻一籠方式飼養於 12 小時日夜循環與溫濕度管控 (23 ± 2°C、60 ± 10% RH) 的動物房，圖八為 FST 誘導類憂鬱動物模式中樣品餵食與動物行為試驗之時間。

預飼養兩週後開始管餵樣品 21 天，CTL 組餵食每公斤體重 10 ml 的 1% 羧甲基纖維素 (carboxymethyl cellulose, CMC)(10 ml/kg bw)、WGE 組餵食每公斤體重 0.5 克的天麻水萃物 (0.5 g/kg bw)、GAS 組餵食每公斤體重 100 毫克的 GAS (100 mg/kg bw)、HBA 組餵食每公斤體重 100 毫克的 HBA (100 mg/kg bw)。本實驗餵食 WGE 之劑量參照本實驗室先前研究成果所訂 (陳，2008)。



圖八、強迫游泳試驗誘導類憂鬱動物模式中樣品餵食與動物行為試驗之時間

Figure 8. Schematic diagrams represent design for studying anti-depressive-like activity of WGE by using FST animal model. OFT: Open field test; FST: forced swimming test; CMC: carboxymethyl cellulose; WGE: Water extract of *Gastrodia elata* Bl.; GAS: gastrodin; HBA: 4-hydroxybenzyl alcohol.

3. 開放空間試驗 (open field test, OFT)

在預養期最後一天與第一次 FST 前一天給予一次 OFT 試驗，試驗方法參考 McCarthy 等人之分析方法與實驗裝置並稍加修飾 (McCarthy et al., 1995; Blizard et al., 1975)。實驗裝置為一個長 76 公分、寬 57 公分、高 35 公分的黑

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色壓克力空箱，其中央光照強度為 1.6 ± 0.1 μmole s^{-1} m^{-2}，並於裝置上方 2 公尺處放置一攝影機方便記錄及觀察。實驗開始前先將大鼠自動物房移到行為室於黑暗中適應一小時，然後將大鼠放入黑色壓克力箱的中央並開始錄影 5 分鐘，結束後將大鼠移回鼠籠，用 70% 乙醇將箱子擦拭乾淨，後續以 TopScanLite™ 版本 2.0 軟體分析大鼠自主活動情形。

4. 強迫游泳試驗 (forced swimming test, FST)

(1) FST 的行為觀察

參照 Porsolt 等人於 1977 年所提出的方法，將大鼠放入直徑 15 公分和高 30 公分的玻璃圓桶內 (水深 20 公分高)，水溫維持約 25°C，使大鼠無法碰觸圓桶底部而強迫泳動。FST 分兩天進行，第一天進行 15 分鐘預試驗 (pre-test) 誘導大鼠產生類憂鬱的行為，24 小時後進行 5 分鐘試驗 (Porsolt et al., 1977)，第二天試驗全程錄影，後續以 ForcedSwimScan™ 軟體分析試驗時大鼠泳動情形。

(2) 動物犧牲與檢體採集

第二天 FST 後隨即進行二氧化碳深度迷昏後斷頭，去除大鼠頭蓋骨取出全腦，並清除腦膜與外部微血管後放置於腦模內，腦部依 Glowinski 氏法 (Glowinski and Iversen, 1966) 於前後腦比例約 2：3 的位置切下，分別分離額葉皮質 (cortex)、紋狀體 (striatum)、海馬迴 (hippocampus) 與杏仁核 (amygdala) 等四部分 (圖九)，放入抗凍小管置於 -80°C 冰箱保存；另一方面，大鼠斷頭後馬上收集血液並將其靜置在室溫下 1 小時待凝集，於 4°C 下以 2750 ×*g* 離心 20 分鐘，取其上清液分裝於 2 ml enppendorf，置於 -80°C 冰箱保存。

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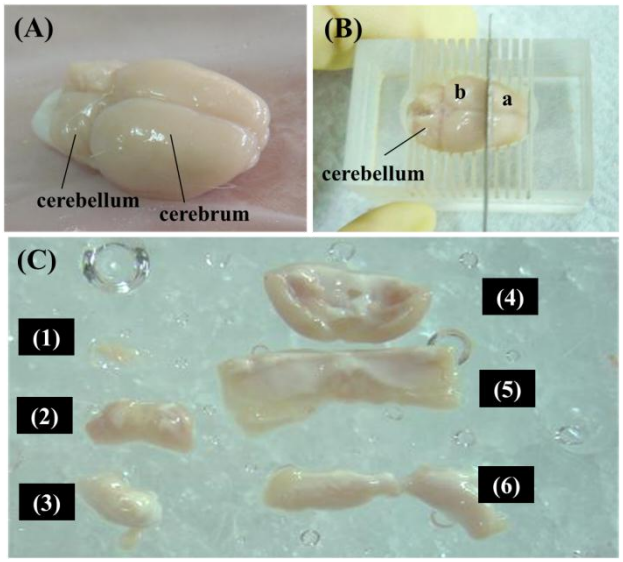

圖九、腦組織及分割後各部位 (A) 全腦、(B) 大腦下刀位置和 (C) 腦組織各部位：(1) 杏仁核、(2) 紋狀體、(3) 脊髓、(4) 皮質 (a)、(5) 皮質(b) 和 (6) 海馬迴

Figure 9. Photographs of the section of the brain (A) whole brain; (B) the cutting side of cerebrum; (C) section parts: (1) amygdala, (2) striatum, (3) spinal cord, (4) cortex (a), (5) cortex (b), and (6) hippocampus.

5. 行為分析軟體

OFT 所使用的軟體為 TopScanLite™ 版本 2.0，將大鼠在空箱中活動的影片置入，設定空箱長、寬度與底部劃分成 (A) 中央區域 (center) 與 (B) 外圍區域 (periphery)，外圍區域再分成四個與中央區域相同面積的區塊，記錄其活動穿越格線的次數 (bouts)、移動時間 (duration)、移動距離 (distance) 及移動速度 (velocity) 來分析其活動情形。

FST所使用的軟體為 ForcedSwimScan™ 版本 2.0，將大鼠泳動影片置入，設定水缸頂部、底部、邊框與水面高度。軟體會自動偵測大鼠四肢泳動情形，並將大鼠於水中的活動分成：掙扎 (struggling 或 climbing)、游泳 (swimming) 與不泳動 (immobility) 等活動行為。其中，潛水 (diving) 歸類於泳動行為；非自願性下沉 (pass diving) 歸類於不泳動行為。經分析後即可顯示出類憂鬱症的情況，當不泳動的時間越長表示大鼠越憂鬱。

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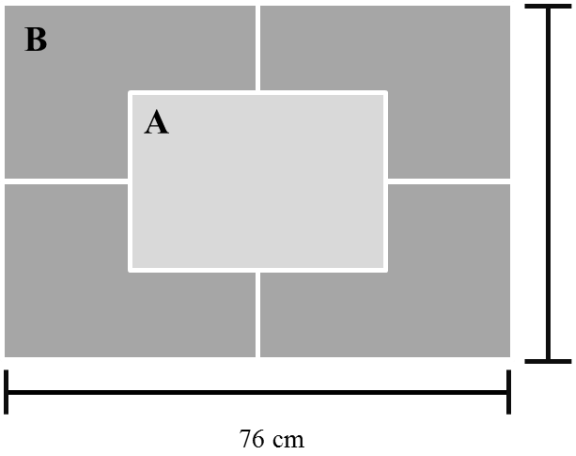

6. 腦部單胺類物質之檢測

(1) 腦組織之萃取

萃取方式參考 Cheng 等學者 (1993) 的方法，調整後進行。首先配製含 10^{-7} M ascorbic acid、1.5 mg/100 ml pargyline、50 pg/μl isoproterenol 之 0.1 N HCl 均質液並放在 4°C 冰箱中備用。取大鼠腦組織加入 1 ml 均質液後在冰浴下以組織均質機均質，接著在 4°C 下以 9000 ×*g* 離心 20 分鐘，取出上清液並保存於 -80°C，分析前再以 0.45 μm nylon 膜過濾。

(2) 單胺類物質之檢測

上清液過濾後以 HPLC-ECD 分析單胺類物質與其代謝產物之含量 (陳，2008)。

(i) 電化學檢出器 (Antec, LCC, Boston, Massachusetts, U.S.A.) 偵測條件如下：

Range: 10 nA

Filter: 1 Hz

AppE cell: 0.511 V

(ii) 層析設備及分析條件如下：

高效液相層析儀：L-7100 pump, Hitachi Co. Ltd., Tokyo, Japan

自動注射器：L-2200 autosamper, Hitachi Co. Ltd., Tokyo, Japan

分析軟體：PeakABC (Great Tide Instrument Co., Ltd., Taipei, Taiwan)

分析管柱：SPOLAR C18 column, 4.6 × 250 mm, 5 μm (Shiseido Co., Ltd, Tokyo,

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Japan)

保護管柱：Alltima C18 column, 7.5 × 4.6 mm, 5 μm (Grace Co, Ltd, U.S.A.)

移動相：每一公升含 20.5 g NaH_2PO_4、185 mg EDTA (ethylenediamine tetraacetic acid)、130 mg 1-octanesulfonic acid, sodium salt (SOS) 149.1 mg KCl 及 200 ml methanol，以磷酸調至 pH 3.11

流速：0.5 ml/min

烘箱溫度: 35°C

分析時間：35 分鐘

7. 以西方墨點法 (Western blotting) 探討天麻水萃物及其主要活性成分對大鼠腦部 Slit-Robo 路徑相關調控蛋白之影響

(1) 蛋白質萃取與定量

使用蛋白質萃取液 (lysis buffer) 前每 10 ml 加入一顆 PMSF 和 30 mg DTT，使其含有 1 mM PMSF 與 65 mM DTT。將耐凍小管中的腦組織放入研缽並與液態氮共同磨成粉末，加入 1 ml lysis buffer 研磨均勻，使腦組織粉末完全溶於 lysis buffer 中。萃取液在 4°C 下以超音波細胞破碎儀破碎 2 分鐘 (50 W；每 30 秒停機 10 秒)，放入 4°C 冰箱靜置 1 小時後再以離心機離心 (4°C、17500 ×g、2 小時)，取上清液過程避免取到漂浮在液面的油脂，分管保存於 -80 °C 冰箱。蛋白質萃取液後續以 Bradford protein assay 定量，先將定量呈色試劑加入超純水稀釋 5 倍後，再以 1 號濾紙 (Whatman) 過濾後備用，標準品為牛血清蛋白 (bovine serum albumin, BSA)，並配製成 0、50、100、200、250 及 400 μg/ml 用以製作標準曲線，在 96 孔盤上樣品與標準品加入 10 μl，並各加入 10 μl 去離子水及 200 μl 5 倍稀釋之 Braford reagent，同時做三重複，以 ELISA reader 讀取 (波長 595 nm)。將樣品吸光值代入標準曲線中計算蛋白質濃度。

(2) 蛋白質電泳分析 (SDS-PAGE electrophoresis)

利用十二烷基硫酸鈉聚丙烯醯胺凝膠電泳的系統進行蛋白質電泳分析

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(sodium dodecylsulfate polyacrylamide gel electrophoresis, SDS-PAGE)。配製之膠體分上下兩層，上層為含 5% acrylamide 的 stacking gel，下層 running gel 的 acrylamide 比例隨蛋白質分子量大小而做調整 (Slit1 和 Slit2：10% acrylamide、CRMP2、Rho A 和 5-HT1A：12% acrylamide、PFN1：15% acrylamide) 配製完成後膠體裝置在電泳槽內，加入適量的電泳緩衝液 (running buffer，含 25 mM Tris-base pH 8.3、0.2 M glycine 和 0.15% SDS)。取定量濃度的蛋白質加入適量的6 倍濃度的 SDS loading buffer (1% SDS、5% β-mercaptoethanol、20% glycerol、0.1% bromophenol blue 和 50 mM Tris-HCl (pH 6.8))，於 95^{o}C 乾浴器下加熱 10 分鐘後冷卻至室溫，將各蛋白質及標準分子量標幟蛋白質 (protein marker, GeneMark) 依序注入上層膠體樣品槽中，通以 80 V 的電壓，待 SDS loading buffer 由 stacking gel 進入 running gel 後，將電壓調整為 100 V，直到 SDS loading buffer 跑到膠體底部後停止。

(3) 轉漬 (transfer) 與轉漬膜阻斷反應 (blocking)

預先剪裁與蛋白質電泳膠相同大小的 PVDF 膜 (Slit1、Slit2、CRMP2 和 5-HT1A 使用 0.45 μm PVDF 膜；RhoA 和 PFN1 使用 0.22 μm PVDF 膜) 和兩片相同大小的濾紙，以乾淨的容器將 PVDF 膜以 100% 甲醇浸泡 5 分鐘，再浸泡於超純水中將甲醇除去，最後浸泡在預冷的轉漬液中完成活化。

蛋白質電泳完成後取出膠片並去除 stacking gel，在其右上角去一截角做記號後以以 TBST (含 25 mM Tris-HCl, pH 7.5、150 mM sodium chloride 和 0.05% Tween® 20) 搖洗 3 次 (每次 5 分鐘)。打開三明治夾黑色夾朝下，轉印的堆疊順序為海棉片、濾紙、膠片、 PVDF 膜、濾紙和海棉片，堆疊時注意避免氣泡的殘留，扣上夾板後放入 transfer tank，黑色面及白色面分別朝向負極及正極，以 450 mA 轉漬 18 小時。轉漬完成以 TBST 搖洗 PVDF 膜 3 次 (每次 5 分鐘)，接著以含 5%脫脂奶粉的 TBST 在室溫下搖洗 2 小時候移除，再以 TBST 搖洗 PVDF 膜 3 次 (每次 5 分鐘)。

(4) 抗體結合反應

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依目標蛋白質的分子量裁切 PVDF 膜後加入一級抗體 (primary antibody)，在 4°C 冰箱中搖洗至少 6 小時後將一級抗體吸除 (一級抗體重複使用不超過 3 次)。之後將一級抗體結合後的 PVDF 膜以 TBST 搖洗 5 分鐘 3 次以去除多餘一級抗體，再加入二級抗體 (secondary antibody) 搖洗 1 小時後將二級抗體吸除 (二級抗體最多重複使用 2 次)。將已結合二級抗體的 PVDF 膜以 TBST 搖洗 5 分鐘 3 次後即可進行顯色與照膠。

(5) 顯色

本研究所使用的顯色方法為 enhanced chemiluminescence (ECL)，利用氧化劑激發二級抗體上所鍵結的發光物質，並以 UVP 電泳影像照相系統 (UVP AutoChemi™ system) 照相，並存為 TIF 檔，最後以 Image J (NIH) 分析定量。

(6) 抗體脫附 (stripping)

將 Stripping buffer (glycine: 0.9 g、NaCl: 11.688 g、SDS: 10 g、去離子水補至 1 L，使用前以 1：1000 的比例加入 β-mercaptoethanol 加入膜上並搖洗半小時，將一級抗體與蛋白質之間的鍵結打斷。脫附反應的 PVDF 膜再以 TBST 搖洗 5 分鐘 3 次後即可重新進行阻斷與抗體結合反應。

8. 血清皮質固醇檢測

血清經解凍至室溫後稀釋十倍，以市售套組 Corticosterone ELISA kit 進行血清皮質固醇濃度分析，於 405 nm 波長下讀取吸光值，並利用標準曲線計算血清皮質固醇濃度。

9. 血清大腦衍生神經滋養因子 (brain-derived neurotrophic factor, BDNF) 檢測

血清經解凍後稀釋四倍，以市售套組 ChemikineTM BDNF sandwich ELISA kit 進行 BDNF 濃度分析，於 450 nm 波長下讀取吸光值，並利用標準曲線計算 BDNF 濃度。

10. 肝、腎、脾組織切片之檢測

第二天 FST 後隨即進行二氧化碳深度迷昏並斷頭取出全腦，另一方面將大鼠腹部剖開取出肝臟、腎臟及脾臟並個別秤重，將最大葉肝臟、腎臟及脾臟完全

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浸置於裝有 10% 的中性福馬林 (formaline) 之檢體瓶中，視情況搖晃檢體瓶防止臟器貼附於瓶底而沒浸到福馬林造成腐敗。臟器經過一週固定後委託國立中興大學獸醫病理學研究所廖俊旺教授以石蠟包埋與切片製作，進行蘇木紫－伊紅染色 (hematoxylin and eosin stain, HE stain) 病理染色，並以 Shackelford 等人 (2002) 之病理評估標準協助判讀組織之病理變化，如表三所示。

表三、病理評估標準

Table 3. Pathological nomenclatures.

Observation fate:
Gross finding:
No abnormalities (NA)
Left (L); Right (R)
Bilateral (B)
Grade 1 (+1): Minimal (0-10%)
Grade 2 (+2): Mild (11-20%)
Grade 3 (+3): Moderate (21- 40%)
Grade 4 (+4): Marked (41-100%)
Histopathological nomenclatures:
No significant lesions (NSL)
Distribution: Focal, Multifocal, Local Extensive, and Diffuse
Degree[a]: Minimal, Slight, Moderate, Moderate/Severe, and Severe/High
Duration: Acute, Subacute, and Chronic
Exudate: Serous, Fibrinous, and Purulent
Modification: Degeneration and Necrosis

[a]Degree of lesions was graded from zero to five depending on severity: 0 = Not present; 1 = minimal (< 1%); 2 = slight (1-25%); 3 = moderate (26-50%); 4 = moderate/severe (51-75%); 5 = severe/high (76-100%).

(二) 細胞實驗

1. Hs683 細胞培養條件

將 Hs683 培養於含 10 ml complete DMEM (DMEM：88%、胎牛血清：10%、抗生素：1%、非必須胺基酸：1%) 之 10-cm 培養皿中，置於含 5% CO_2、37^oC 之細胞培養箱中，每三天更換一次培養液。

2. 細胞解凍

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將含細胞液之冷凍小管從液態氮桶取出，迅速置於 37°C 水浴槽使其解凍。完全解凍之後將其吸取至含有 9 ml complete DMEM 的 10-cm 培養皿中，搖勻後於細胞培養箱中培養，於隔日更換一次培養液。

3. 細胞繼代

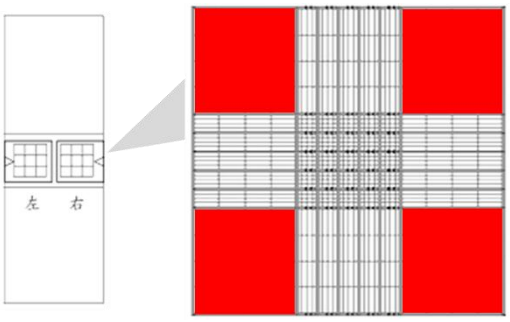
當 Hs683 細胞生長達培養皿面積之八分滿時吸除培養液，以 5 ml PBS 清洗兩次，加入 1 ml trypsin 使其均勻散佈於培養皿中，並將培養皿置於 37°C 培養箱中 5 分鐘，隨後加入 5 ml 培養液終止 trypsin 之作用並將細胞均勻打散。將培養液放置離心機中以 377 ×*g* 離心 5 分鐘後移除培養液，加入 3 ml 新培養液後震盪使細胞懸浮，取 1 ml 細胞液加入含 9 ml 新培養液之培養皿中混勻，置於細胞培養箱繼續培養。

4. 細胞冷凍

將 Hs683 以繼代的方式收集細胞，以 377 ×*g* 離心 5 分鐘使細胞沉澱後去除上清液，加入預冷之培養液 (含 5% DMSO) 使細胞均勻懸浮，將其移至冷凍小管中並放置於抗凍盒中，於 -80°C 冰箱儲存 24 小時後，再將冷凍小管放入液態氮中長期保存。

5. 細胞計數

將細胞液與 trypan blue stain (0.4%) 等體積稀釋，均勻混合後將其注入血球計數盤中，以倒立式相差顯微鏡觀察並計數細胞，取九宮格中最邊界之四格作為細胞計數的區域，計算公式為：四大格計數之細胞數/4 × 2 × 10^{-4} (每一方格的體積為 0.1 cm × 0.1 cm × 0.01 cm = 10^{-4} cm^3) 計算而得之數值即為每毫升細胞液中所含之細胞數目。



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6. 天麻水萃物及其主要活性成分對 Hs683 細胞存活率之影響

(1) 原理

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) 為可溶於水之黃色化合物，在活細胞中可以經由粒線體上之琥珀酸脫氫酶 (succinate dehydrogenase) 催化，接收進入呼吸鏈的電子而還原成不溶於水的結晶 (MTT formazen)，以 DMSO 溶解後呈現藍紫色。由於在死細胞中無此反應，因此 MTT formazen 的生成量與活細胞數目成正比。

(2) MTT 配製方法

避光狀態下秤取 0.25 g 的 MTT 粉末，裝入包上鋁箔紙的 50 ml 離心管，以 50 ml 已滅菌 PBS 的溶解，在無菌操作台內利用 0.22 μm 過濾膜過濾，儲存於 4^{o}C 冰箱，使用前以培養液稀釋十倍即可。

(3) 種細胞及樣品處理

將 Hs683 以 10^5 cell/mL 種於 96 孔盤 (200 μl/well) 待 5 小時細胞貼附後，每個 well 以 200 μl PBS 清洗一次，分別加入 200 μl/well 含有天麻苷 (GAS) (0、50、100、200、400、800 和 1000 μM) 和天麻苷元 (HBA) (0、50、100、200、400、800 和 1000 μM) 之培養液分別在培養箱培養 24 和 48 小時。

(4) 呈色、比色及結果判讀

在培養 24 和 48 小時後吸除培養液，加入 300 μl/well 含 0.5 mg/mL MTT 的培養液置於培養箱培養 3 小時，接著移除培養液並加入 300 μl/well DMSO 避光靜置 10 分鐘，以分光光度計讀取波長 570 nm 之吸光值。以 DMSO 吸光值作為 blank 校正，未添加待測樣品的組別作為控制組，細胞存活率 (%) 是與控制組作比較，計算公式如下：

細胞存活率 (%) = (處理組吸光值/控制組吸光值)× 100%

7. 以西方墨點法 (Western blotting) 探討天麻水萃物及其主要活性成分對 Hs683 細胞之 Slit-Robo 路徑中 Slit1 蛋白表現之影響

(1) 種細胞及樣品處理

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將 Hs683 以 10^5 cell/ml 種於含有 9 ml complete DMEM 的 10-cm 培養皿中待 5 小時細胞貼附後，分別以 5 ml PBS 清洗兩次，加入 10 ml 天麻水萃物 (WGE) (0.5 mg/ml)、GAS (50、100 和 200 μM) 和 HBA (50、100 和 200 μM) 之培養液，分別在培養箱培養 24 小時。

(2) 蛋白質萃取、定量與標準化

Hs683 與 WGE (0.5 mg/ml)、GAS (50、100 和 200 μM) 和 HBA (50、100 和 200 μM) 分別培養 24 小時後吸除培養液，以 5 ml PBS 清洗 2 次後加入 1 ml trypsin，置於細胞培養箱培養 5 分鐘後，再加入 5 ml DMEM 打散細胞後移至 15 ml 離心管中，以 377 ×*g* 離心 5 分鐘去清除上清液，接著加入 1 ml PBS 將細胞移至 1.5 ml enpendorf 以 8250 ×*g* 離心 30 秒移除上清液。最後加入 100 μl protein extraction buffer (含 1 錠 PhosStop/10 ml) 與細胞震盪 1 分鐘混合均勻，置於 -20°C 冰箱反應一小時，在 4°C 下以 11250 ×*g* 離心 5 分鐘，收集上清液至 1.5 ml enpendorf 並保存於 -80 °C 冰箱備用。定量方面以牛血清蛋白 (bovine serum albumin, BSA) 作為標準品，配製 0、50、100、200、250 及 400 μg/ml 以製作標準曲線，將樣品蛋白質稀釋 30 倍，分別取 10 μl 依序加入於 96 孔盤中，並製作三重複。之後加入 10 μl 去離子水和 200 μl 5 倍稀釋之 Braford reagent 進行反應，以 ELISA reader 讀取 (波長 595 nm)。將樣品吸光值代入標準曲線中計算蛋白質濃度。依據定量結果，以去離子水、樣品緩衝液將每一組蛋白質萃取液調整濃度為 1.67 μg/μl (25 μg/15 μl) (包含 6X SDS loading buffer (1% SDS；5% β-mercaptoethanol；20% glycerol；0.1% bromophenol blue；50 mM Tris-HCl pH 6.8))，套上防爆夾以乾浴鍋於 95°C 加熱 10 分鐘，置於 -20°C 冰箱保存，盡量在兩週內分析完畢，以防蛋白濃度失準。

(3) 蛋白質電泳分析 (SDS-PAGE electrophoresis)

利用十二烷基硫酸鈉聚丙烯醯胺凝膠電泳的系統進行蛋白質電泳分析。配製之膠體分上下兩層，上層則為含 5% acrylamide 的 stacking gel，下層為含 10% acrylamide 的 running gel。實驗操作流程皆與動物實驗一致。

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(4) 轉漬 (transfer)、抗體結合反應及顯色

實驗流程皆與動物實驗相同。

(三) 化學分析實驗

1. 建立天麻水萃物主要生物性成分最適萃取及分析條件

(1) 天麻水萃物之製備

首先將紅天麻切片 (slices) 依據本實驗室先前所建立的萃取方法進行萃取 (陳，2008)，萃取方式依序如下，slices 與去離子水以 1：7 (w/v) 的比例於沸水下迴流萃取 1 小時後，移出萃取液後再以天麻片與去離子水以 1：5 (w/v) 的比例於沸水下迴流萃取 50 分鐘，將兩次萃取液混合後冷卻至室溫，經 0.45 μm nylon 膜過濾後進行冷凍乾燥，置於乾燥箱中備用。後續秤取凍乾之天麻水萃粉末配製成 5000 ppm 的溶液，分析前在以 0.45 μm nylon 膜過濾。

(2) 天麻苷濃度檢測

過濾後之溶液以 HPLC-UV 分析天麻水萃物中天麻苷的含量。

(i) 紫外光-可見光檢測器 (UV-2075 Plus, Tokyo, Japan) 偵測條件如下：

偵測波長：220 nm

(ii) 層析設備及分析條件如下：

高效液相層析儀：Jasco HPLC system, Tokyo, Japan

溶媒脫氣裝置：Jasco LC-NetII/ADC, Tokyo, Japan

溶媒輸出系統：PU-2089 Plus, Tokyo, Japan

分析軟體：Chrompass (version 1.7.403.1, Jasco)

分析管柱：phenomenex Luna C18 (250 mm × 4.6 mm, 5 μm), Torrance, U.S.A.

移動相：參考 Liu 等人 (2002) 之分析條件並加以修飾，以移動相分別為 0.2% 磷酸水溶液 (A 液) 及 100% 乙腈 (ACN) (B 液) 進行梯度沖提 (gradient elution) 分析。

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Time (min)	A (water, 0.2%H_3PO_4)	B (ACN)
0	95	5
7	95	5
15	80	20
20	75	25
25	5	95
28	5	95
30	95	5
35	95	5

流速：1 ml/min

烘箱溫度：35^{o}C

分析時間：35 分鐘

2. 分析方法確效 (method validation)

依據國際醫藥法規協和會 (International Conference on Harmonization, ICH) 之指引 ICH Q2A 及 Q2B (International Conference on Harmonisation, 1995; 1997) 與衛生福利部食品化學檢驗方法之確效規範 (衛生福利部，2012)，進行成分析分法確效。此外，臺灣藥品優良製造確效作業基準中第八十四條有詳細解釋其定義：各種分析方法之系統適當性、準確度、精密度、最低檢測濃度、最低定量濃度、專一性、線性及再現性等，均視各該分析方法之本質、目的與需要分別建立、並視各該分析方法分別確效之；執行時並記錄成檔 (衛生福利部，1999)。因此，本次實驗確效分析項目如下：

(1) 專一性 (specificity)

目的在證明當檢體溶液中存在雜質、分解物、賦形劑等成分時，本分析具有明確的評估標的分析物之能力。作法為將天麻苷 (GAS)、天麻苷元 (HBA)、對羥基苯甲醛 (HB) 和香草醛 (VAN) 等標準品母液混合後注入 HPLC，記錄各標準品滯留時間，並計算檢品溶液與天麻苷標準品母液中天麻苷層析峰的滯留時間

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差值比率。

(2) 系統適用性 (system suitability)

系統適用性係建立於儀器配備、電子配件、分析操作及待分析之檢品等共同組成一完整的系統。本項試驗評估分離率 (resolution, R) 與樣品重複六次注入分析時之相對標準差 (relative standard derivation, RSD)。其他成分對天麻苷層析峰之分離率之合格標準為 R>1.5，相對應標準差之合格標準為 RSD≦2%。

(3) 線性 (linearity)

將天麻苷標準品以去離子水連續稀釋五個不同濃度，其範圍包含天麻粉水萃物的 3000 μg/ml 之 20%、60%、100%、140% 和 180% (濃度分別為 10、30、50、70 和 90 μg/ml)；在此範圍內標準品濃度與層析峰面積之間應具有良好線性關係，合格標準為判定係數 (coefficinet of determination) $R^2 \geqq 0.995$。

(4) 準確度 (accuracy)

分析方法的準確度是用來表現所檢測出來的值與一公認的之真值或一公認之對照值間的接近程度。作法為添加檢品貯備溶液與標準品貯備溶液，使各標的分析物總量達一般分析濃度之 60%、100% 和 140%，所得結果代回標準品之線性回歸方程式，求得回推濃度，再計算其回收率，以確認此種分析方法之準確度，回收之合格標準應介於 95.0%~105.0%。

(5) 精密度 (precision)

顯現自同一均質樣品多重取樣，於規定條件下所得到的一系列檢測值間之接近程度。實驗精密度之評估包括重複性 (repeatability)、中間精密度 (intermediate precision) 及再現性 (reproducibility)，依必要性選擇執行。其定義如下:

(i) 重複性

重複性係以同一實驗室於同一批次執行該檢驗方法，所得之結果予以評估。

(ii) 中間精密度

中間精密度代表實驗室內精密度，係以同一實驗室執行該檢驗方法，於不同分析日期、分析人員、分析設備等，所得之結果予以評估。

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(iii) 再現性

再現性代表實驗室間精密度，係以不同實驗室執行該檢驗方法，所得之結果予以評估。本實驗精密度之評估方法選用重複性和中間精密度，RSD 合格標準分別應≦10% 和 14% (衛生福利部，2012)。

(6) 範圍 (range)

在天麻苷一般分析濃度之 20%~180% 範圍間，檢測結果的線性、準確度、精密度均應符合既定標準。

(7) 最低檢測濃度 (limit of detection) 及最低定量濃度 (limit of quantification)

配製一般分析濃度 5、7 及 9 μg/ml 之天麻苷標準溶液，注入 HPLC 各分析三次，以回應值的標準差 (σ) 與斜率 (S) 為基礎代入公式計算：LOD=3.3σ/S；LOQ=10σ/S。

(8) 耐變性 (robustness)

耐變性為該分析方法故意給予微小之變動，其分析能力是否能在些微變動下不受任何影響，用來評估該方法於例行工作時之可靠性。耐變性之評估，可將下列之層析條件作小幅度的改變：

波長：±1 nm

流速：±0.03 ml/min

3. 天麻加工形態對熱水萃取天麻中活性成分含量之影響

將紅天麻粉末 (powder) 和切片 (slices) 依據陳 (2008) 的熱水萃取方法進行萃取。將天麻片與去離子水以 1:7 (w/v) 的比例於沸水下迴流萃取 1 小時後，移出萃取液後再加入 5 倍體積的去離子水於沸水下再萃取 50 分鐘，將兩次萃取之上清液混合後冷卻至室溫，經 0.45 μm nylon 膜過濾後進行冷凍乾燥，置於乾燥箱中備用。天麻水萃凍乾粉末以去離子水回溶並配製成 5000 ppm 的溶液，以 HPLC 進行天麻苷及天麻苷元含量分析。

4. 萃取溶劑對天麻中活性成分含量之影響

熱水萃取法如前項所述，而乙醇萃取方法是依據臺灣中藥典第二版之公告並

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加以修飾 (衛生福利部，2013)，將天麻片與 70% 乙醇以 1：25 (w/v) 的比例於沸水下迴流萃取 1 小時，上清液冷卻置室溫後經 0.45 μm nylon 膜過濾後進行冷凍乾燥，置於乾燥箱中備用。熱水萃取與乙醇萃取後之凍乾粉末分別以去離子水和含 3% 乙腈的去離子水回溶，並配製成 5000 ppm 的溶液進行天麻苷及天麻苷元含量分析。

5. 天麻變種與產地對熱水萃取天麻中活性成分含量之影響

GRY、GRH 和 GBY 為未經切片的乾燥天麻塊莖，使用前先參照羅 (2010) 的方法將其蒸軟後進行切片，經冷凍乾燥後置於乾燥箱中備用。天麻苷及天麻苷元含量分析方式如前項熱水萃取法所述。

(四) 統計分析

上述實驗所得到之實驗數據，將採用 SAS 9.2 電腦統計套裝軟體中之變異數分析 (analysis of variance，簡稱 ANOVA)。在動物行為分析方面配合 Duncan's test 進行統計；單胺類分析、Slit-Robo 路徑之西方墨點法分析、細胞存活率方面配合 Dunnett's multiple comparison test 進行統計；不同溶劑萃取天麻對天麻苷含量變化之影響方面，以 Student's t test 進行統計；天麻產地與變種對天麻苷含量變化，以 Duncan's test 進行統計，以客觀分析實驗數據間是否達到顯著性差異。

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伍、實驗結果

一、天麻水萃物及其主要活性成分對類憂鬱動物行為之影響

(一) 動物飼養

大鼠在飼養期間每週秤取飼料、飲水與體重兩次，了解大鼠在飼養期間的生活情形。在飲水、攝食及體重方面，由圖十的結果顯示各組間均無顯著差異。

(二) 動物行為

1.開放空間試驗 (OFT)

Pre-FST 前一天進行 OFT 試驗，探討餵食大鼠天麻水萃物 (WGE) 及其活性成分是否會影響其行為。錄影檔以軟體 TopScanLite™ 進行分析，在影片中的空箱底部以軟體劃分成中央區域 (center) 與外圍區域 (periphery)，外圍區域再分成四個與中央區域相同面積的區塊，記錄其穿越格線的次數 (bout)、移動距離 (distance) 及移動速度 (velocity) 瞭解活動情形。由表四結果顯示，各組動物行為表現皆無統計上的差異，證實餵食大鼠 WGE 及其活性成分對其行為表現無任何影響。

2.強迫游泳試驗 (FST)

當大鼠被放入含水玻璃圓桶內時，必須確認老鼠是否全身都有沒入水中，泳動行為的錄影檔案以 ForcedSwimScan™ 軟體進行分析。將動物活動行為分為攀爬 (climbing)、掙扎 (struggling)、潛水 (diving) 與游泳 (swimming)；與不活動行為分為不泳動 (immobility) 與非自願性下沉 (pass diving)，Porsolt 等人指出在 FST 的類憂鬱模式中其動物不活動行為是類憂鬱行為的指標 (Porsolt et al., 1977)。因此，藉由動物不活動時間分析來統計大鼠類憂鬱症狀之嚴重程度。由圖十一結果顯示，給予 WGE 及其活性成分天麻苷 (GAS) 和天麻苷元 (HBA) 後，確實能降低 FST 中不泳動行為。此外，WGE、GAS 和 HBA 組之大鼠泳動時間也有顯著提高 (圖十二)，但皆不影響大鼠在水中掙扎的時間 (圖十三)。

二、天麻水萃物及其要活性成分對類憂鬱動物腦部單胺類代謝轉換率之影響

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表五為給予大鼠 21 天的天麻水萃物 (WGE) 與其活性成分天麻苷 (GAS) 和天麻苷元 (HBA) 後其大腦四個腦區 (紋狀體、額葉皮質、海馬迴和杏仁核) 的 5-HT 及其代謝產物 5-羥基引朵醋酸 (5-hydroxyindoleacetic acid, 5-HIAA) 的濃度變化，在額葉皮質和紋狀體的結果顯示，分別只有 WGE 組和 GAS 組的 5-HT 濃度有顯著提高，但是在海馬迴的結果顯示各處理組的 5-HT 濃度皆顯著較 CTL 組高。在 5-HIAA 的分析結果顯示，GAS 組的 5-HIAA 濃度在紋狀體明顯提高，在海馬迴中各組 5-HIAA 的濃度也顯著比 CTL 組高。為了有效比較各組在 5-HT 於腦中的代謝轉換率，以 5-HIAA/5-HT 比值呈現於圖十四。結果顯示，WGE、GAS 和 HBA 組在海馬迴的代謝轉換率顯著低於 CTL 組。進一步以西方墨點法分析驗證海馬迴的 5-HIAA/5-HT 代謝率是否與 5-HT1A receptor 調控有關，結果如圖十五所示，經餵食 WGE、GAS 和 HBA 後可顯著提升海馬迴中 5-HT1A receptor 之蛋白表現結果。由此結果推論 WGE、GAS 和 HBA 可促進 5-HT1A receptor 分泌 5-HT 來降低腦中代謝轉換率。

表六為大腦四個腦區的 DA 及其代謝產物二羥苯乙酸 (3,4-Dihydroxyphenylacetic acid, DOPAC) 和高香草酸 (homovanillic acid, HVA) 的含量變化，在 DA 分析結果顯示 WGE 可顯著提高額葉皮質、紋狀體和杏仁核的 DA 於腦中濃度，GAS 和 HBA 組分別顯著提高額葉皮質和杏仁核之 DA 濃度。在代謝產物 DOPAC 和 HVA 分析結果顯示，各處理組可顯著提高海馬迴中 HVA 和紋狀體中 DOPAC 的濃度。此外，GAS 和 HBA 組分別顯著降低額葉皮質中 DOPAC 和提高杏仁核的 HVA 濃度。為了有效比較各組在 DA 於腦中的代謝轉換率，以 (DOPAC+HVA)/DA 比值呈現於圖十六。在各腦區的比較分析結果顯示，WGE、GAS 和 HBA 在額葉皮質、海馬迴和杏仁核之代謝轉換率顯著低於 CTL 組。在海馬迴的結果顯示，WGE 降低 DA 代謝率的幅度最大；在額葉皮質的結果顯示，WGE 和 GAS 降低 DA 代謝率的幅度較 HBA 大。

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三、天麻水萃物及其主要活性成分對類憂鬱大鼠腦部 Slit-Robo 路徑相關調控蛋白之影響

根據先前本實驗室研究成果，天麻水萃物可能是透過 Slit-Robo 路徑影響大腦額葉皮質而達到抗憂鬱的效果 (林，2012)。因此將大鼠額葉皮質與海馬迴以西方墨點法驗證此路徑所對應的蛋白質 Slit1、Slit2、CRMP2、RhoA 及 PFN1。額葉皮質分析結果顯示，各組對 Slit1、Slit2、CRMP2、RhoA 及 PFN1 之表現量無統計上之差異 (圖十七 A)。但由圖十七 B 海馬迴的分析結果顯示，天麻水萃物 (WGE)、天麻苷 (GAS) 和天麻苷元 (HBA) 可以顯著降低負控制調節因子 Slit1 和 RhoA 之表現量，並增強正調控因子 CRMP2 和 PFN1 之蛋白表現量。由上述結果顯示，WGE、GAS 和 HBA 可調控 Slit-Robo 路徑相關蛋白在腦部的表現。

四、天麻水萃物及其主要活性成分對 Hs683 細胞之 Slit-Robo 路徑中 Slit1 蛋白表現之影響

(一) 天麻水萃物及其主要活性成分對 Hs683 存活率之影響

為了深入瞭解天麻水萃物 (WGE)、天麻苷 (GAS) 和天麻苷元 (HBA) 是否直接調控 Slit-Robo 路徑相關蛋白在腦部的表現，本研究以不影響 Hs683 細胞存活的情況下分別與 WGE、GAS 和 HBA 三種樣品進行共同培養。根據培養 24 及 48 小時後的結果如圖十八顯示，GAS 和 HBA 劑量範圍分別在 50 到 400 μM 和 50 到 800 μM 均不會影響細胞存活率。由於 0.5 mg/ml WGE 含有 3% GAS，因此 GAS 選用等同於 WGE 所含之 GAS 濃度 50 μM 作為一倍劑量，並以 100 和 200 μM GAS 分別作為兩倍和四倍劑量進行後續實驗，HBA 之劑量則參照 GAS 50、100 和 200 μM 來制定。

(二) 天麻水萃物及其主要活性成分對 Hs683 之 Slit1 蛋白表現之影響

圖十九為 WGE 與不同濃度之 GAS 和 HBA 與 Hs683 共培養 24 小時後以西方墨點法分析之結果，顯示 0.5 mg/ml 之 WGE 與 50、100、200 μM GAS

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和 100、200 μM HBA 皆顯著降低 Slit1 之蛋白表現但無劑量效應。

五、天麻水萃物及其活性成分對血清中皮質固醇之影響

當動物暴露在壓力的情況下，會經由下視丘釋放出腎上腺皮質釋放激素刺激腦下垂體合成促腎上腺皮質釋放激素，進一步促使腎上腺皮質合成皮質固醇並釋放至血液中來應付外界壓力。從圖二十之血液分析顯示，各處理組無法對 FST 之壓力誘發所釋放的皮質固醇濃度有任何調控的作用。

六、天麻水萃物及其活性成分對血清中血清大腦衍生神經滋養因子之影響

BDNF 是大腦內調控神經細胞的發育、存活及突觸成形之重要因子 (Huang and Reichardt, 2001)。由圖二十一結果顯示各處理組皆無統計上的差異。

七、肝、腎、脾組織影響之病理判讀

動物組織由中興大學獸醫病理學研究所張淑菁小姐負責組織切片及病理判讀 (RCAM 編號：141124、ADDC 編號:143102、病理編號:CO14-910 (16 HE))，切片染色結果呈現於圖二十二，判讀結果顯示連續餵食大鼠 WGE、GAS 和 HBA 至 21 天後對大鼠肝臟、腎臟及脾臟之組織均沒有發現顯著病理變化。

八、天麻的加工方式與萃取方法對天麻苷含量測定之影響

(一) 天麻苷分析方法確效

1. 專一性 (specificity)：

各標準品之滯留時間如圖二十三所示，檢品溶液與天麻苷 (GAS) 標準品溶液中 GAS 層析峰之滯留時間差值比率絕對值≦5%。

2. 系統適用性 (system suitability)

系統適用性之指標參數數據如表七所示：相對準差 (relative standard derivation, RSD) 為 8.60%、理論板數 (number of theoretical plate, N) 為 2225、

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曳尾因數 (tailing factor, T) 為 1.12、分離率 (resolution, R) 為 1.4、選擇係數 (selectivity factor, α) 為 1.15 及容積因子/滯留時間 (capacity factor, K') 為 3.41。

3. 線性 (linearity)

GAS 標準品溶液濃度於 10~100 μg/ml 之間，GAS 層析峰面積比 (y) 與溶液濃度 (x) 之線性方程式為 $y = 0.5407x + 0.54363$，判定係數 $R^2=0.9999$，呈現良好線性關係，如圖二十四所示。

4. 準確度 (accuracy)

GAS 濃度 60% (30 μg/ml)、100% (50 μg/ml) 及 140% (70 μg/ml) 溶液之 GAS 回收率分別為 97.98%、100.81% 及 101.87% (表八) 符合藥品優良製造確效作業基準回收率 80.0%~115.0% (衛生福利部，1999)。

5. 精密度 (precision)

單日三種 GAS 濃度 (30、50 及 70 μg/ml) 以 HPLC 各分析三次所得到的重複性 RSD 數值分別為 2.77、2.34 和 3.38% (表八)，雖然數值超過藥品優良製造確效作業基準≦2% (衛生福利部，1999)，卻符合衛生福利部食品藥物管理局食品化學檢驗方法之確效規範≦14% (衛生福利部，2011)。中間精密度 RSD 數值為 3.23%。

6. 耐變性 (robustness)

分別於不同移動相流速設定 (0.97、1.00 和 1.03 ml/min) 及不同波長設定 (219、220 和 221 nm)，其各系統指標性參數如表九所示，顯示確效條件在輕微變動的情況下，並不影響 N、T、R 和 α 的數值變化。

7. 範圍 (range)

GAS 在 10~100 μg/ml 濃度範圍間，檢測結果的線性及準確度已達藥品優良製造確效作業基準 (衛生福利部，1999)，而精確度僅符合衛生福利部食品藥物管理署食品化學檢驗方法之確效規範既定標準 (衛生福利部，2011)。

8. 最低檢測濃度 (limit of detection) 及最低定量濃度 (limit of quantification)

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利用 HPLC 分析此三種 GAS 標準品溶液濃度 (5、7 及 9 μg/ml) 所得到的 GAS 層析峰面積求出線性方程式為 y = 0.7x-0.1667，判定係數 R^2=0.9932 (圖二十五)，接著把這三種 GAS 標準品之層析峰面積代入此方程式，分別得到 3.27 ± 0.06、4.87 ± 0.15 和 6.07 ± 0.06 μg/ml 等 GAS 標準品溶液的實際濃度，平均標準差為 0.13，斜率為 0.7。最後將平均標準差與斜率代入公式 LOD=3.3σ/S 與 LOQ=10σ/S 得到 GAS 確效條件 LOD 為 0.60 μg/ml 和 LOQ 為 1.81 μg/ml 之理論值。

(二) 天麻加工方式對天麻苷含量之比較

請當地農民將乾燥之紅天麻分別磨成粉末 (powder) 及切成片狀 (slices) 兩種形式，利用熱水萃取後其分析結果以圖二十六呈現，顯示兩種形式對 GAS 的萃取其含量無顯著差異。

(三) 天麻萃取方法對其主要活性成分含量之比較

利用實驗室建立之水萃方法與中藥典的乙醇萃法進行紅天麻片之萃取，由圖二十七結果顯示，水萃法相較乙醇萃法可以萃取到較高量的 GAS，而 HBA 在分析方面結果剛好相反。

(四) 天麻之產地與變種對天麻苷成分含量之比較

天麻在不同產地對 GAS 含量影響之結果以圖二十八呈現，GRH 和 GRY 皆為紅天麻，經分析後顯示 GAS 的含量無統計差異；比較不同變種之 GAS 含量變化發現，GBY 之 GAS 含量顯著高於 GRY 和 GRH。

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表四、開放空間試驗中天麻水萃物、天麻苷天麻苷元對大鼠穿越次數、移動時間和移動距離之影響

Table 4. The effects of WGE, GAS, and HBA on the bouts, distance, and velocity of rats in locomotor test in OFT.

Group	n	Dose (mg/kg bw)	Bouts (count)	Distance (mm)	Velocity (mm/s)
CTL	9		32 ± 11	17758 ± 4583	358 ± 76
WGE	10	500	33 ± 10	15782 ± 6354	324 ± 127
GAS	10	100	33 ± 11	16597 ± 4861	349 ± 90
HBA	8	100	32 ± 10	15841 ± 4667	331 ± 120

Data are expressed as the means ± SD (n = 8-10). Rats were gavaged with the following samples: WGE (500 mg/kg bw), GAS (100 mg/kg bw), and HBA (100 mg/kg bw). Data were not significantly different ($p > 0.05$) from each other according to the result of ANOVA. CTL: control; WGE: Water extract of *Gastrodia elata* Bl.; GAS: gastrodin; HBA: 4-hydroxybenzyl alcohol.

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表五、大腦不同腦區之血清素及其代謝產物含量

Table 5. The amounts of serotonin and its metabolite in the different brain areas.

Group	5-HT	5-HIAA
	ng/g brain tissue	
Frontal cortex		
CTL	710 ± 101	1173 ± 180
WGE	884 ± 138*	1390 ± 214
GAS	588 ± 180	1216 ± 248
HBA	608 ± 57	1201 ± 158
Hippocampus		
CTL	424 ± 248	5279 ± 3305
WGE	1364 ± 276*	10310 ± 3075*
GAS	4042 ± 1211***	12370 ± 4753**
HBA	3103 ± 678***	9623 ± 3333*
Striatum		
CTL	982 ± 289	1228 ± 268
WGE	2149 ± 354***	1948 ± 828*
GAS	2118 ± 458***	3973 ± 1051***
HBA	759 ± 300	785 ± 264
Amygdala		
CTL	2585 ± 1865	7881 ± 4451
WGE	4288 ± 2897	9346 ± 4286
GAS	2671 ± 1377	8458 ± 3466
HBA	2749 ± 922	8329 ± 2592

Data are expressed as means ± SD (n=8-10). All samples were administered once daily for 21 days by oral gavage. WGE (500 mg/kg bw), GAS (100 mg/kg bw), and HBA (100 mg/kg bw). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (according to the result of ANOVA coupled with Dunnett's multiple comparison test) compared with CTL group. CTL: control; WGE: Water extract of *Gastrodia elata* Bl.; GAS: gastrodin; HBA: 4-hydroxybenzyl alcohol.

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表六、大腦不同腦區之多巴胺及其代謝產物含量

Table 6. The amounts of dopamine and its metabolite in the different brain areas.

Group	DA	DOPAC	HVA
	ng/g brain tissue		
Frontal cortex			
CTL	515 ± 93	300 ± 68	108 ± 38
WGE	751 ± 143***	232.± 69	122 ± 63
GAS	723 ± 121**	184 ± 46**	139 ± 55
HBA	626 ± 63	313 ± 54	73 ± 24
Hippocampus			
CTL	3911 ± 1942	2369 ± 2679	2328 ± 585
WGE	4603 ± 3406	787 ± 468	352 ± 134***
GAS	4613 ± 2341	3044 ± 4528	505 ± 113***
HBA	1782 ± 1055	2150 ± 1413	505 ± 115***
Striatum			
CTL	22532 ± 9183	8232 ± 5279	441 ± 291
WGE	53388 ± 35364**	16622 ± 5784**	450 ± 122
GAS	42831 ± 10794	15422 ± 4266*	378 ± 115
HBA	33698 ± 9997	18827 ± 6634***	375 ± 137
Amygdala			
CTL	2501 ± 2160	3375 ± 3627	362 ± 83
WGE	8503 ± 3318***	1846 ± 940	480 ± 331
GAS	7084 ± 1537**	2092 ± 1885	485 ± 248
HBA	7989 ± 1930***	1015 ± 571	636 ± 139*

Data are expressed as means ± SD (n=8-10). All samples were administered once daily for 21 days by oral gavage. WGE (500 mg/kg bw), GAS (100 mg/kg bw), and HBA (100 mg/kg bw). ***p* < 0.05, ****p* < 0.01, *****p* < 0.001 (according to the result of ANOVA coupled with Dunnett's multiple comparison test) compared with CTL group. CTL: control; WGE: Water extract of *Gastrodia elata* Bl.; GAS: gastrodin; HBA: 4-hydroxybenzyl alcohol.

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表七、系統適用性

Table 7. System suitability results of the proposed method.

Parameters	Results	Required limits
RSD	8.60%	RSD<10%
N	2225	N>2000
T	1.12	T<1.5
R	1.4	R>1.0
α	1.15	α>1.05
K'	3.41	K'>3.0

Each value is the Mean of 6 determinations.

RSD: relative standard deviation; N: numbers of theoretical plate; T: tailing factor; R: resolution; α: selectivity factor; K': capacity factor.

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表八、以 HPLC 方法測定天麻中 gastrodin 在 intra- 和 inter-day 之準確度及精密度

Table 8. Intra- and inter-day accuracy and precision of HPLC assay for gastrodin from water extracts of *Gastrodia elata* Bl.

			Recovery						Repeatability		Intermediate	
Analyst	Level (μg/ml)	Injection	Standard[a]	Sample[b] (μg/ml)	Spike[c]	Recovery (%)	Average (*n*=3)	RSD (%)	Average (*n*=9)	RSD (%)	Average (*n*=27)	RSD (%)
1	30	1	30.75	27.04	29.08	101.20	102.67	1.28	100.81	2.77		
		2	29.64	27.78	29.27	103.75						
		3	30.38	27.97	29.64	103.05						
	50	1	49.27	48.72	50.20	104.89	101.88	2.53				
		2	50.39	48.53	49.27	99.26						
		3	49.46	46.87	48.53	101.50						
	70	1	69.65	67.06	67.24	96.81	97.87	2.72				
		2	69.28	68.73	68.73	99.20						
		3	69.47	68.17	67.98	97.60						
2	30	1	29.45	29.27	29.08	98.11	96.84	1.45	97.98	2.34	100.22	3.23
		2	29.64	29.08	28.71	95.62						
		3	28.71	30.38	29.08	96.77						
	50	1	48.90	48.53	47.79	96.21	99.62	1.46				
		2	49.27	48.16	49.27	102.26						
		3	47.61	48.90	48.35	100.39						
	70	1	68.54	68.91	67.24	95.68	97.47	1.15				
		2	68.17	68.54	67.43	97.28						
		3	67.43	68.17	67.61	99.45						
3	30	1	31.81	31.81	31.98	101.08	99.10	3.97	101.87	3.38		
		2	31.63	33.01	31.46	94.56						
		3	31.29	32.49	32.15	101.65						
	50	1	52.10	49.69	52.44	105.94	104.64	1.90				
		2	51.93	49.69	52.27	105.63						
		3	51.24	50.38	51.41	102.35						
	70	1	74.11	72.56	74.80	103.94	101.87	2.18				
		2	73.94	71.19	72.39	99.53						
		3	73.08	73.94	74.28	102.12						

[a]Standard: determination of gastrodin content by injecting each standard solution.

[b]Sample: determination of gastrodin content by injecting each sample preparation.

[c]Spike: determination of gastrodin content by injecting each spiked solution.

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表九、耐變性

Table 9. Results of robustness study of HPLC assay for gastrodin from water extracts of *Gastrodia elata* Bl.

	Wavelength (nm)			**Flow rate (ml/min)**		
	219	**220**	**221**	**0.97**	**1.00**	**1.03**
N	2218	2270	2322	2241	2270	2289
T	1.14	1.13	1.13	1.10	1.13	1.18
R	1.40	1.44	1.39	1.39	1.44	1.40
α	1.21	1.22	1.23	1.22	1.22	1.21

N: number of theoretical plate; T: tailing factor; R: resolution; α: selectivity factor.

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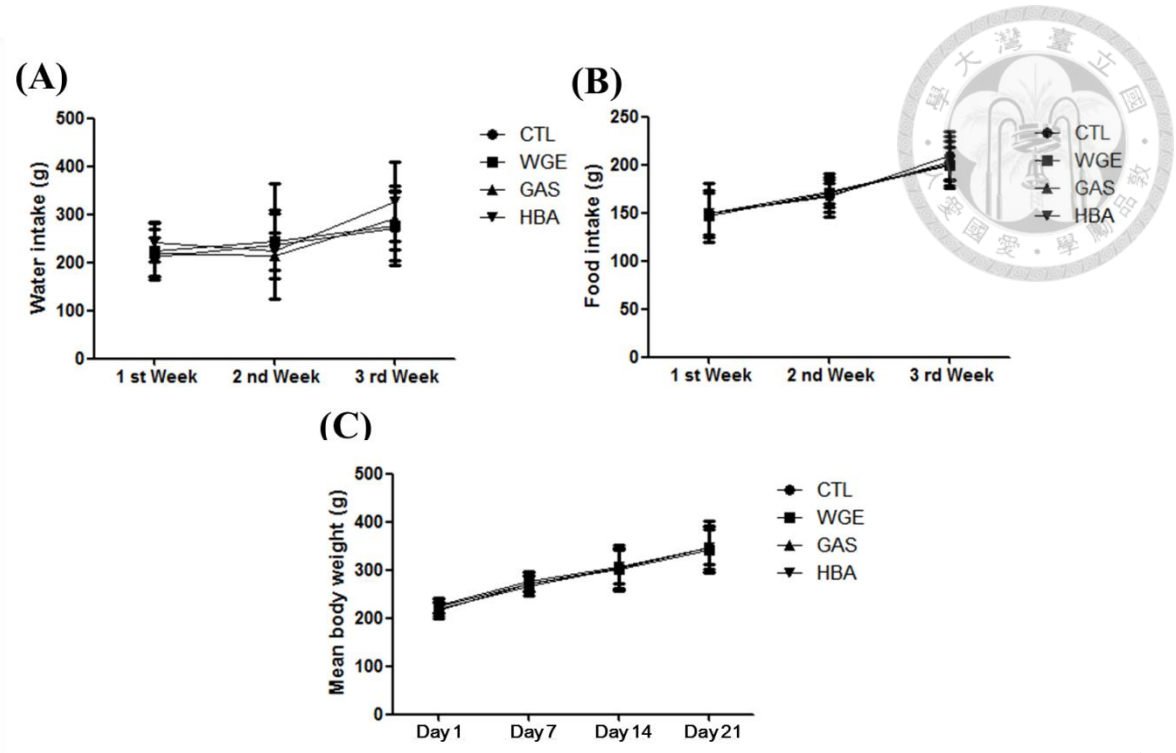

圖十、飼養期間飲水攝取量 (A)、攝食量 (B) 和 體重 (C) 之變化

Figure 10. Effects of WGE, GAS, and HBA on water intake (A), food intake (B), and body weight (C) in SD rats. Each value represents means ± SD (n=8-10). WGE, GAS, and HBA were administered once daily for 21 days by oral gavage. WGE (500 mg/kg bw), GAS (100 mg/kg bw), and HBA (100 mg/kg bw). Data were not significantly different ($p > 0.05$) from each other according to the result of ANOVA. CTL: control; WGE: Water extract of *Gastrodia elata* Bl.; GAS: gastrodin; HBA: 4-hydroxybenzyl alcohol.

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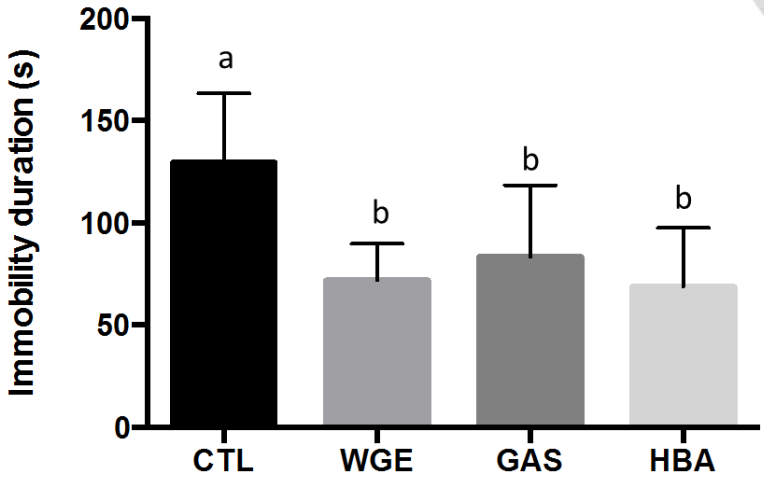

圖十一、天麻水萃物、天麻苷和天麻苷元對大鼠在強迫游泳試驗不泳動時間之影響

Figure 11. The effects of WGE, GAS, and HBA on the immobility time of rats in FST. Data are expressed as means ± SD (n=8-10). All samples were administered once daily for 21 days by oral gavage. WGE (500 mg/kg bw), GAS (100 mg/kg bw), and HBA (100 mg/kg bw). [ab] data not sharing the same letter are significantly different ($p < 0.05$) from each other according to the result of ANOVA coupled with Duncan's multiple range test. CTL: control; WGE: Water extract of *Gastrodia elata* Bl.; GAS: gastrodin; HBA: 4-hydroxybenzyl alcohol.

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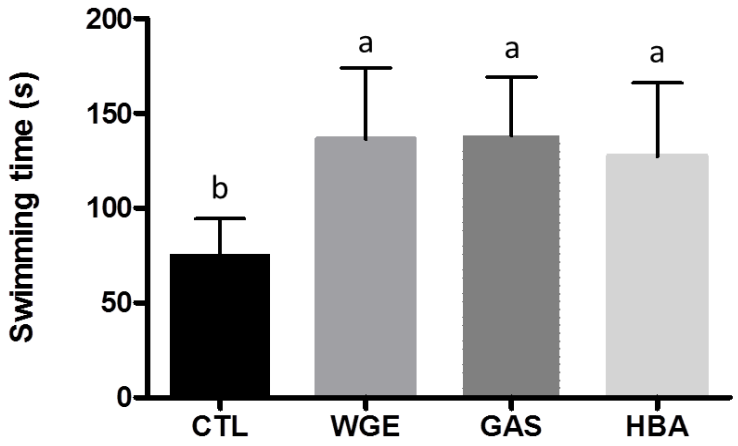

圖十二、天麻水萃物、天麻苷和天麻苷元對大鼠在強迫游泳試驗泳動時間之影響

Figure 12. The effects of WGE, GAS, and HBA on the swimming time of rats in FST. Data are expressed as means ± SD (n=8-10). All samples were administered once daily for 21 days by oral gavage. WGE (500 mg/kg bw), GAS (100 mg/kg bw), and HBA (100 mg/kg bw). [ab] data not sharing the same letter are significantly different ($p < 0.05$) from each other according to the result of ANOVA coupled with Duncan's multiple range test. CTL: control; WGE: Water extract of *Gastrodia elata* Bl.; GAS: gastrodin; HBA: 4-hydroxybenzyl alcohol.

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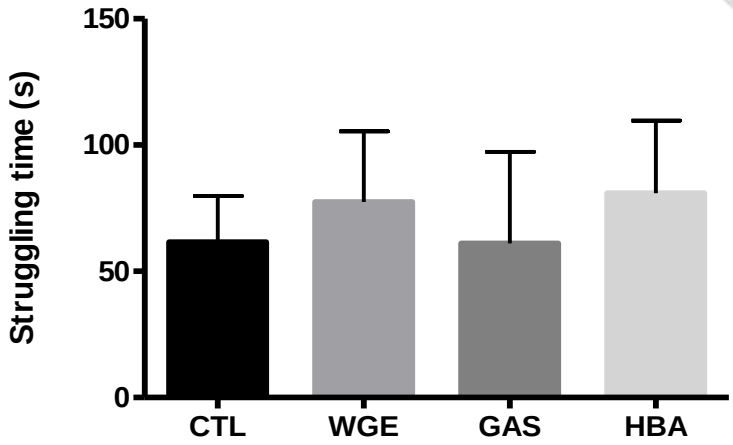

圖十三、天麻水萃物、天麻苷和天麻苷元對大鼠在強迫游泳試驗掙扎時間之影響

Figure 13. The effects of WGE, GAS, and HBA on the struggling time of rats in FST. Data are expressed as means ± SD (n=8-10). All samples were administered once daily for 21 days by oral gavage. WGE (500 mg/kg bw), GAS (100 mg/kg bw), and HBA (100 mg/kg bw). Data were not significantly different ($p > 0.05$) from each other according to the result of ANOVA. CTL: control; WGE: Water extract of *Gastrodia elata* Bl.; GAS: gastrodin; HBA: 4-hydroxybenzyl alcohol.

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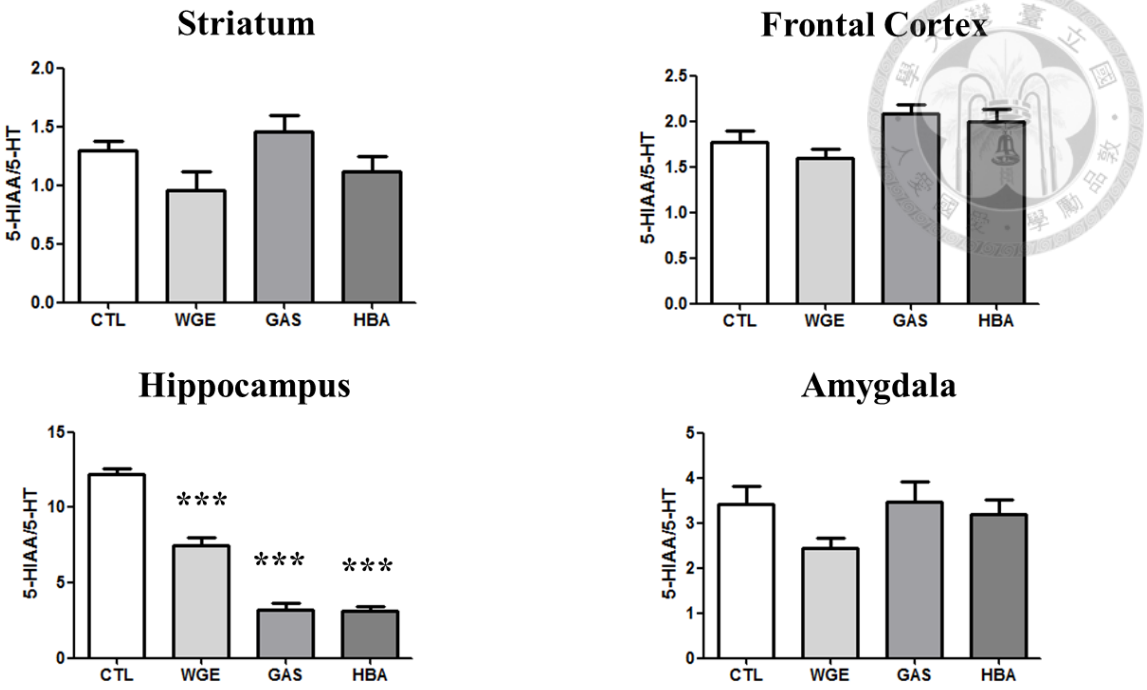

圖十四、天麻水萃物、天麻苷和天麻苷元對不同腦區之血清素代謝轉換率之影響

Figure 14. The effects of WGE, GAS, and HBA on the serotonin turnover (Ratio of 5-HIAA to 5-HT). Data are expressed as means ± SD (n=8-10). All samples were administered once daily for 21 days by oral gavage. WGE (500 mg/kg bw), GAS (100 mg/kg bw), and HBA (100 mg/kg bw).***$p < 0.001$ (according to the result of ANOVA coupled with Dunnett's multiple comparison test) compared with CTL group. CTL: control; WGE: Water extract of *Gastrodia elata* Bl.; GAS: gastrodin; HBA: 4-hydroxybenzyl alcohol.

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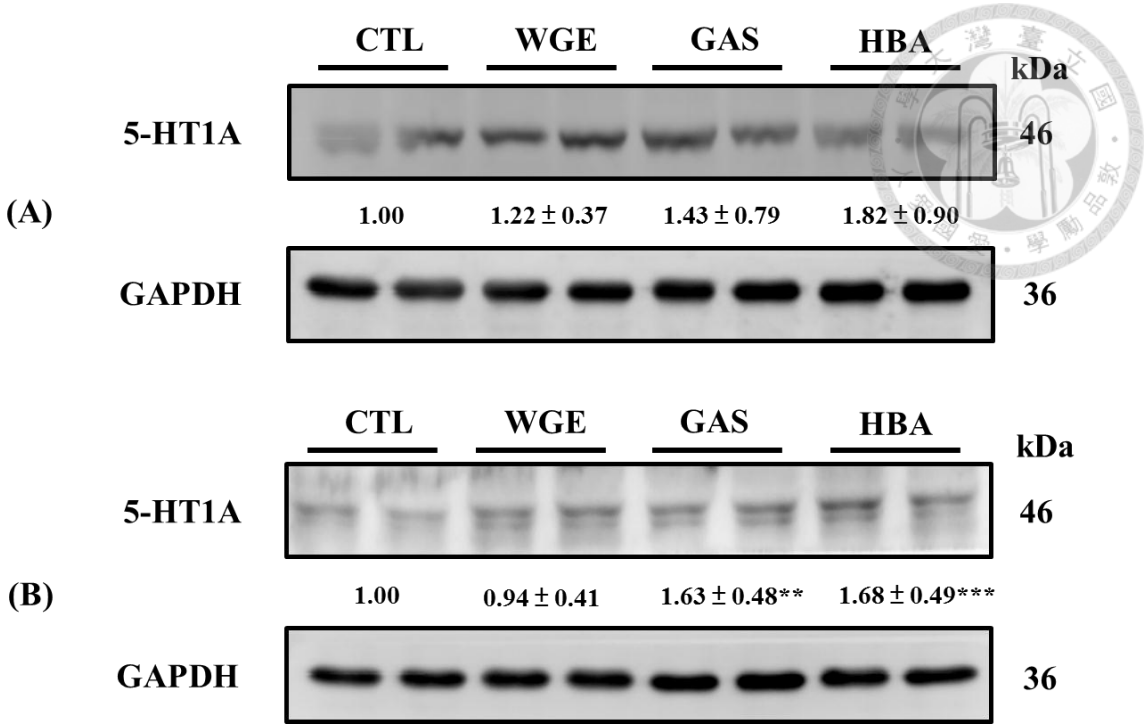

圖十五、以西方墨點法分析額葉皮質 (A) 和海馬迴 (B) 中 5-HT1A 受器的蛋白表現

Figure 15. Western blotting analysis of 5-HT1A receptor in frontal cortex (A) and hippocampus (B). (n=8-10). The value on the bottom of each group represents the ratio of expressed levels, which were normalized with the expression of GAPDH and relative to the CTL group.**$p < 0.01$, ***$p < 0.001$ (according to the result of ANOVA coupled with Dunnett's multiple comparison test) compared with CTL group. CTL: control; WGE: Water extract of *Gastrodia elata* Bl.; GAS: gastrodin; HBA: 4-hydroxybenzyl alcohol.

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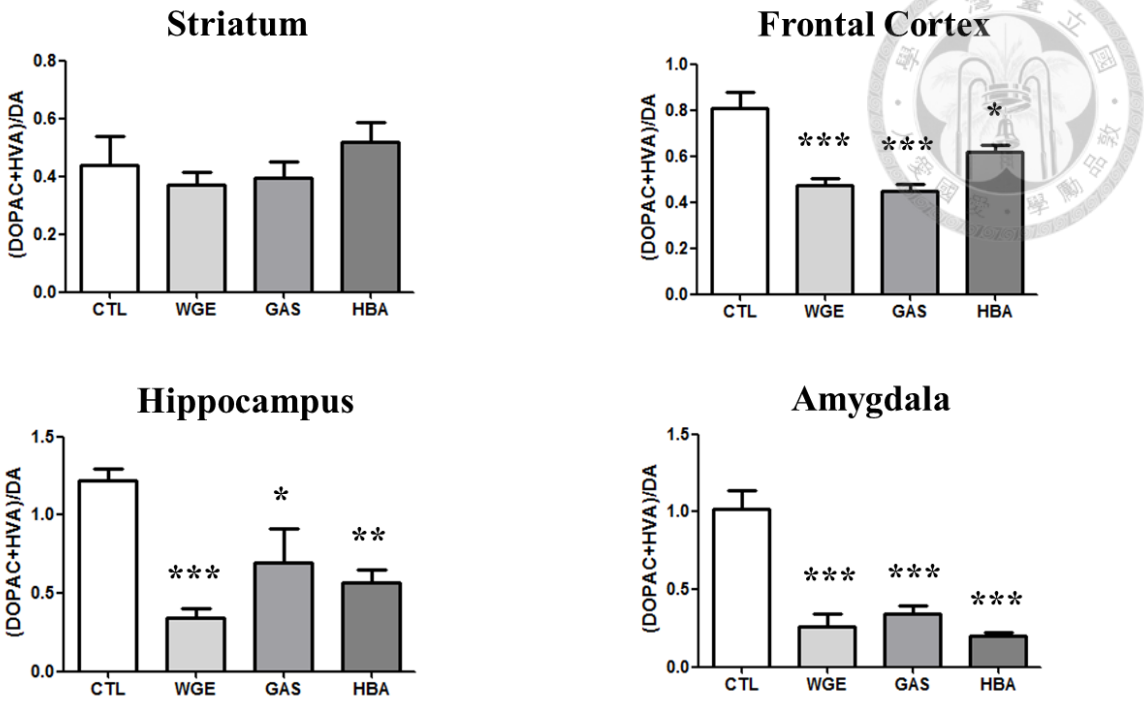

圖十六、天麻水萃物、天麻苷天和麻苷元對不同腦區之多巴胺代謝轉換率之影響

Figure 16. The effects of WGE, GAS, and HBA on the dopamine turnover (Ratio of (DOPAC + HVA)/DA). Data are expressed as means ± SD (n=8-10). All samples were administered once daily for 21 days by oral gavage. WGE (500 mg/kg bw), GAS (100 mg/kg bw), and HBA (100 mg/kg bw). ***p* < 0.05, ****p* < 0.01, *****p* < 0.001 (according to the result of ANOVA coupled with Dunnett's multiple comparison test) compared with CTL group. CTL: control; WGE: Water extract of *Gastrodia elata* Bl.; GAS: gastrodin; HBA: 4-hydroxybenzyl alcohol.

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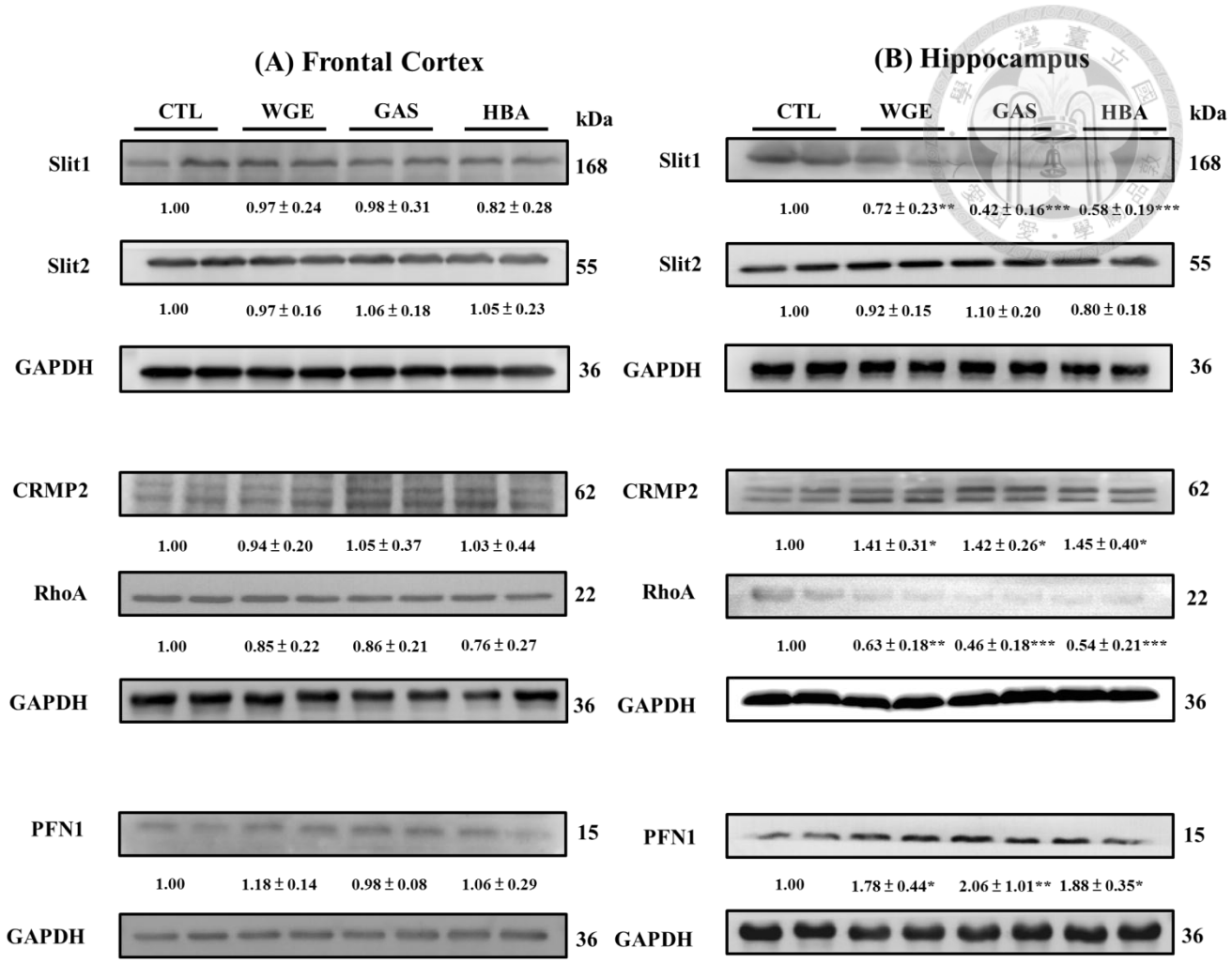

圖十七、以西方墨點法驗分析額葉皮質 (A) 與海馬迴 (B) 中 Slit-Robo 路徑的相關調控蛋白表現

Figure 17. Western blotting analysis of the regulators in the Slit-Robo pathway in frontal cortex (A) and hippocampus (B). (n=8-10). The value on the bottom of each group represents the ratio of expressed levels, which were normalized with the expression of GAPDH and relative to the CTL group. ***p* < 0.05, ****p* < 0.01, *****p* < 0.001 (according to the result of ANOVA coupled with Dunnett's multiple comparison test) compared with CTL group. CTL: control; WGE: Water extract of *Gastrodia elata* Bl.; GAS: gastrodin; HBA: 4-hydroxybenzyl alcohol.

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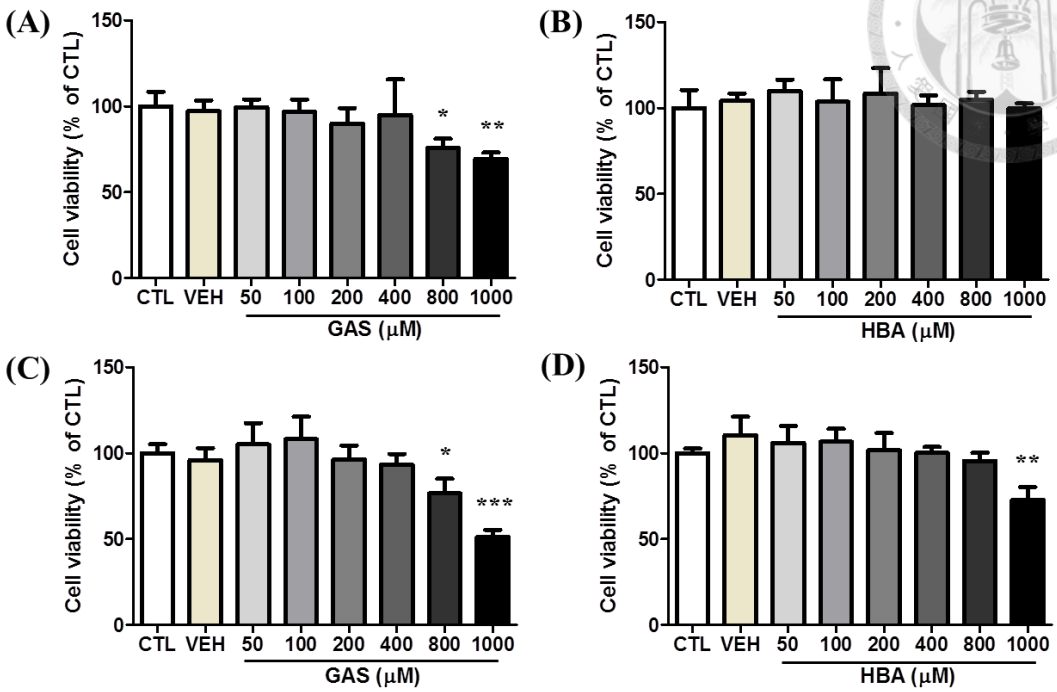

圖十八、不同濃度之天麻苷和天麻苷元處理 24 小時 (A) (B) 與 48 小時 (C) (D) 對 Hs683 細胞存活率

Figure 18. Effects of different concentration of GAS (A) (B) and HBA (C) (D) on the cell viability in Hs683 cell for 24 and 48 hr. Each value represents means ± SD (n=3). ***p* < 0.05, ****p* < 0.01, *****p* < 0.001 (according to the result of ANOVA coupled with Dunnett's multiple comparison test) compared with CTL group. CTL: control; VEH: vehicle; HBA: 4-hydroxybenzyl alcohol.

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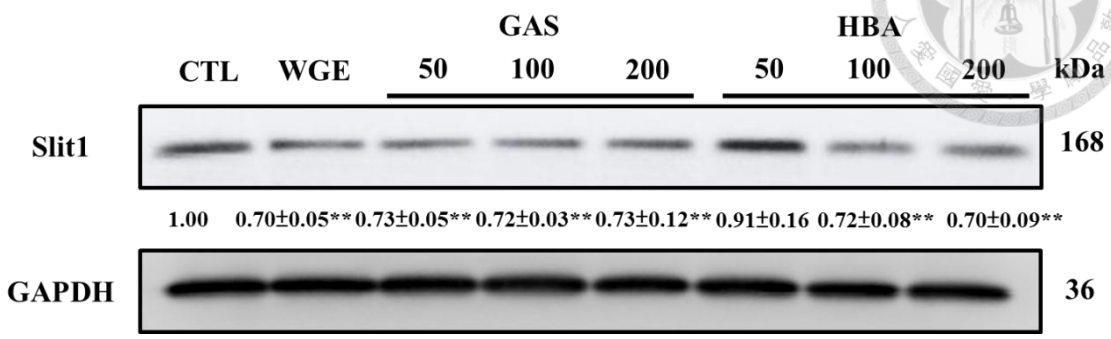

圖十九、天麻水萃物、天麻苷和天麻苷元與 Hs683 細胞共培養 24 小時後對 Slit1 蛋白表現之影響

Figure 19. Effects of WGE, GAS, and HBA on the expression of Slit1 in Hs683 for 24 hr. (n=3). The value on the bottom of each group represents the ratio of expressed levels, which were normalized with the expression of GAPDH and relative to the CTL group. ***p* < 0.01 (according to the result of ANOVA coupled with Dunnett's multiple comparison test) compared with CTL group. CTL: control; WGE: Water extract of *Gastrodia elata* Bl.; GAS: gastrodin (50, 100, and 200 μM); HBA: 4-hydroxybenzyl alcohol (50, 100, and 200 μM).

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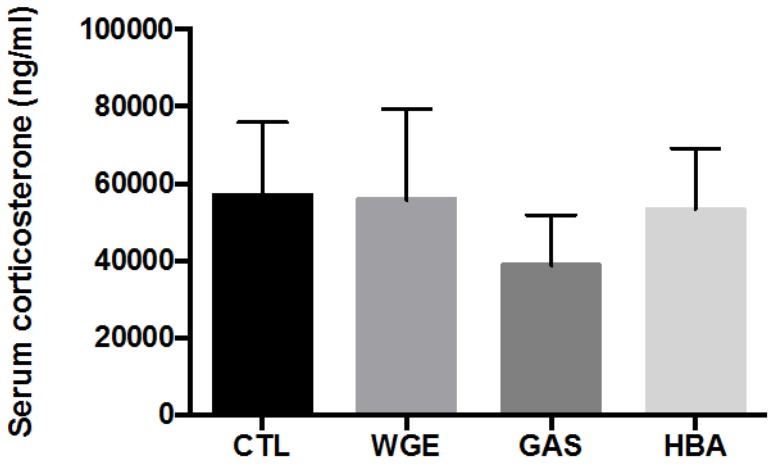

圖二十、大鼠血清皮質固醇濃度

Figure 20. Corticosterone concentration in rats serum. Data are expressed as means ± SD (n=8). Data were not significantly different ($p > 0.05$) from each other according to the result of ANOVA. CTL: control; WGE: Water extract of *Gastrodia elata* Bl.; GAS: gastrodin; HBA: 4-hydroxybenzyl alcohol.

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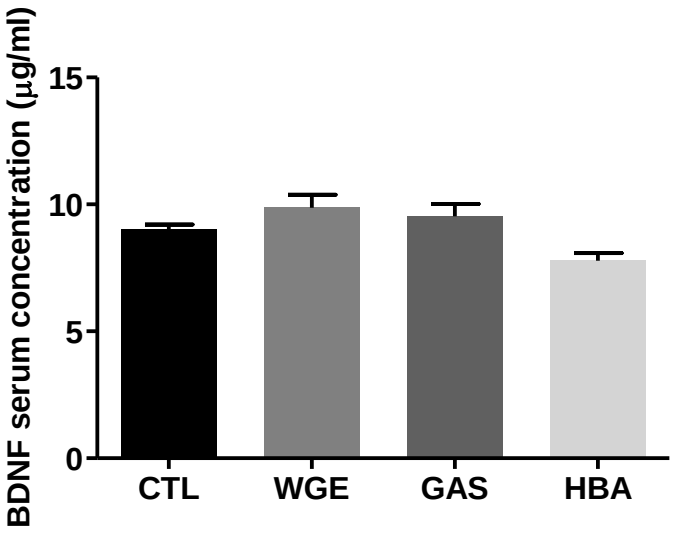

圖二十一、大鼠血清大腦衍生神經滋養因子濃度

Figure 21. BDNF concentration in rats serum. Data are expressed as means ± SD (n=8-10). Data were not significantly different ($p > 0.05$) from each other according to the result of ANOVA. CTL: control; WGE: Water extract of *Gastrodia elata* Bl.; GAS: gastrodin; HBA: 4-hydroxybenzyl alcohol.

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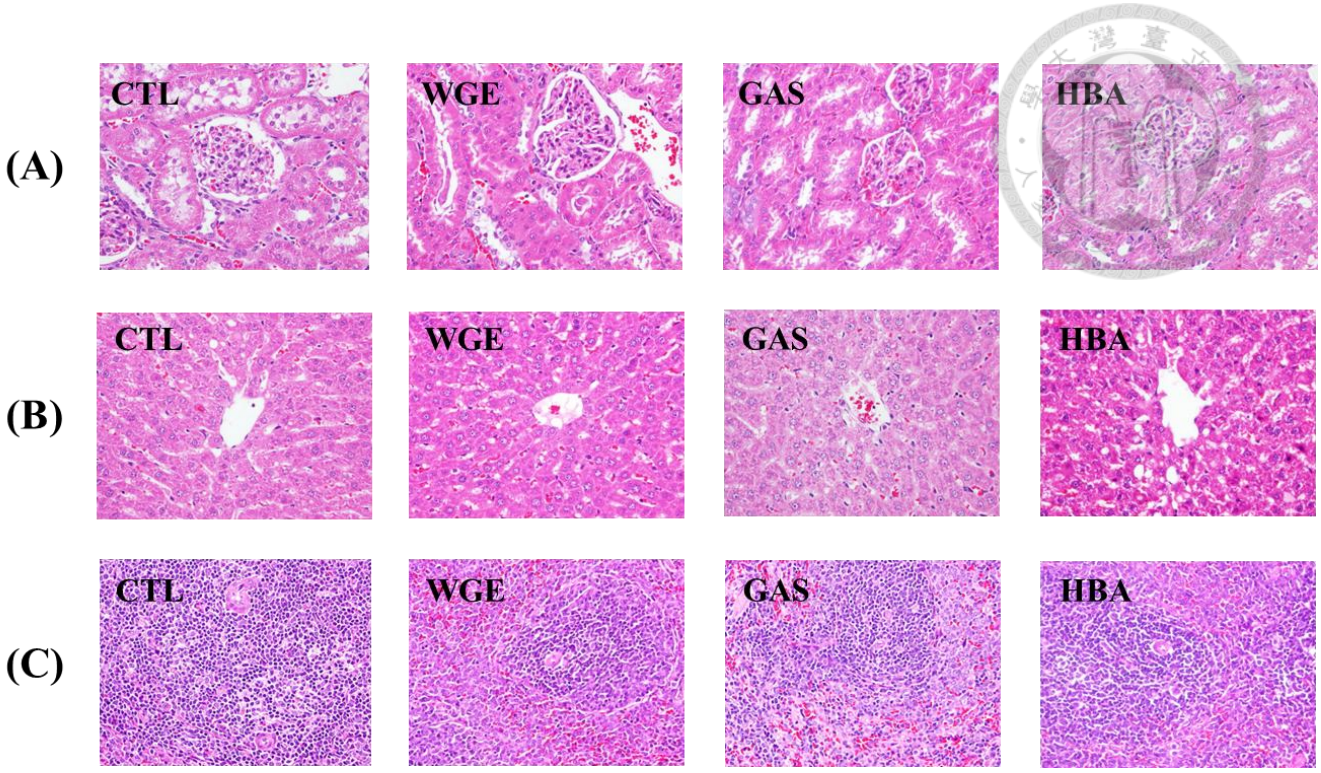

圖二十二、在強迫游泳試驗下天麻水萃物、天麻苷和天麻苷元對大鼠腎臟 (A)、肝臟 (B) 及脾臟 (C) 之組織病理切片結果

Figure 22. Representative histopathological sections of kidney (A), liver (B), and spleen (C) of rats in the FST (Original magnification, 400x). All samples were administered once daily for 21 days by oral gavage WGE (500 mg/kg bw), GAS (100 mg/kg bw), and HBA (100 mg/kg bw). No significant lesion but diffuse glycogen infiltration in liver might be related to unfasten overnight before sacrificed. CTL: control; WGE: Water extract of *Gastrodia elata* Bl.; GAS: gastrodin; HBA: 4-hydroxybenzyl alcohol.

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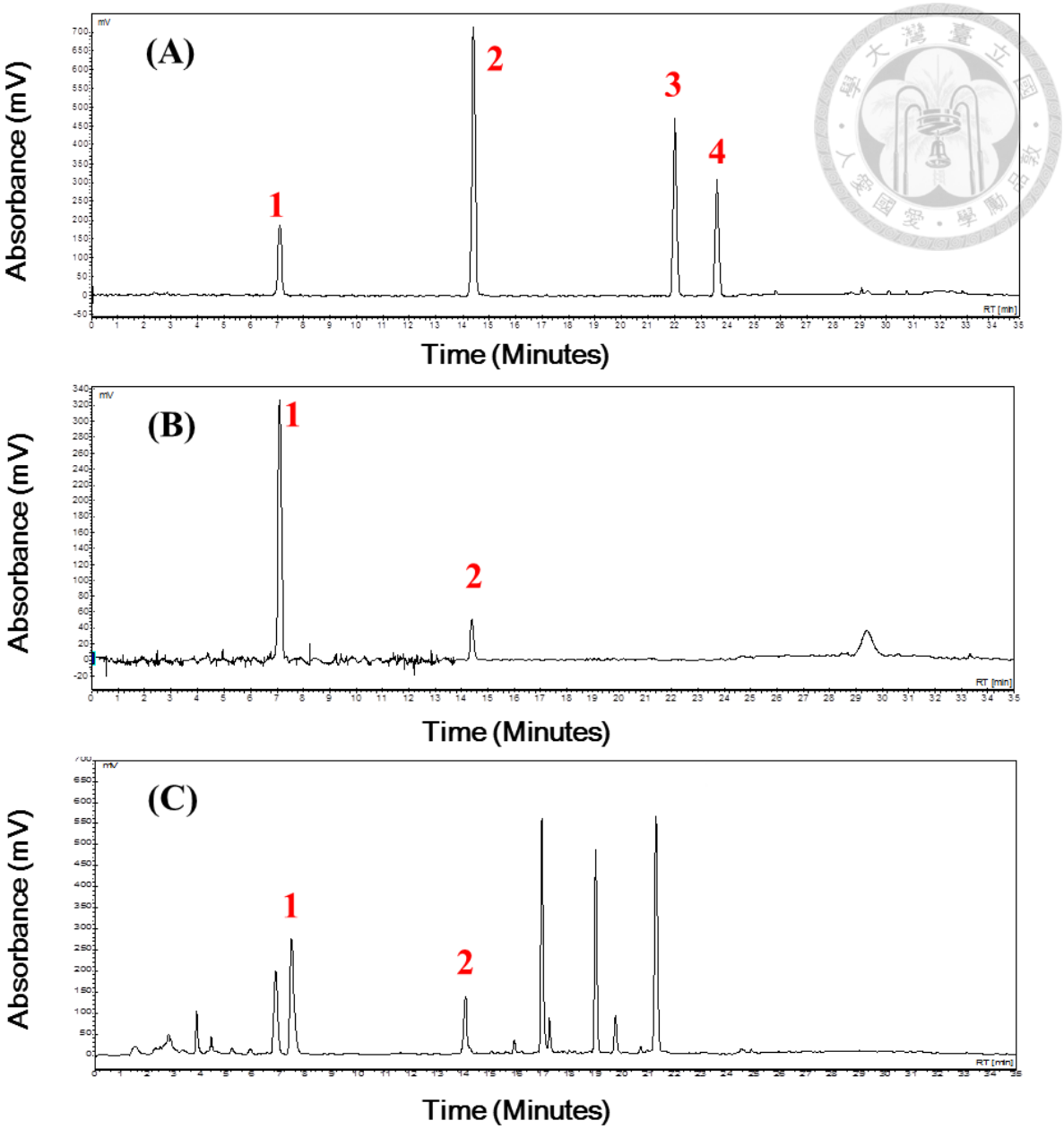

圖二十三、標準品天麻苷、天麻苷元、對羥基苯甲醛和香草醛 (A)、天麻水萃物 (柯達公司提供) (B) 和天麻水萃物 (自行萃取) (C) 之 HPLC 圖譜

Figure 23. HPLC chromatogram (A) of GAS (1), HBA (2), HB (3), and VAN (4) (50 mg/l) standard; HPLC chromatogram (B) of WGE (2500 mg/l) from Pharmaceutical Company; HPLC chromatogram (C) of WGE (3000 mg/l). GAS: gastrodin, HBA: 4-hydroxybenzyl alcohol, HB: 4-hydroxybenzyl aldehyde, VAN: 4-hydroxy-3-methoxybenzaldehyde, WGE: Water extract of *Gastrodia elata* Bl.

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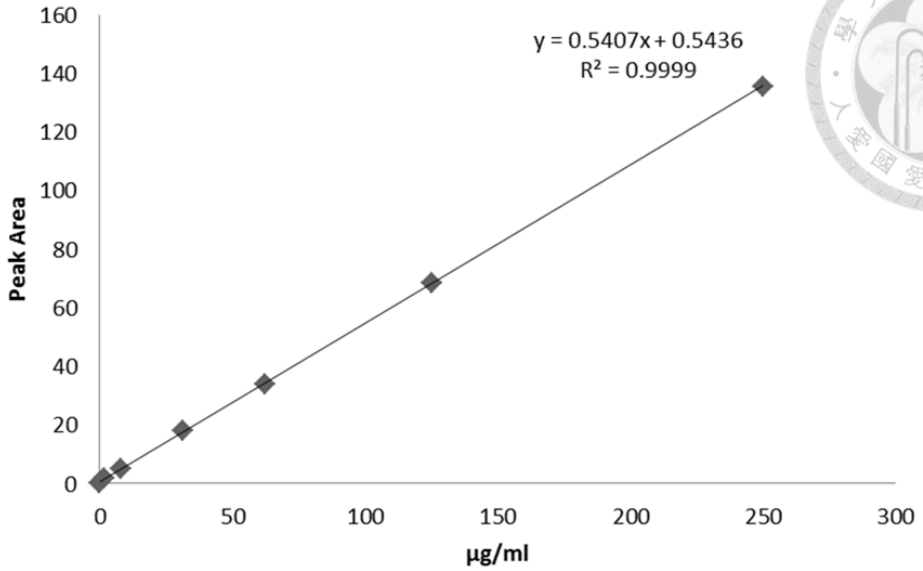

圖二十四、標準品天麻苷之回歸曲線

Figure 24. Calibration curve obtained with gastrodin standard solutions using the proposed HPLC method.

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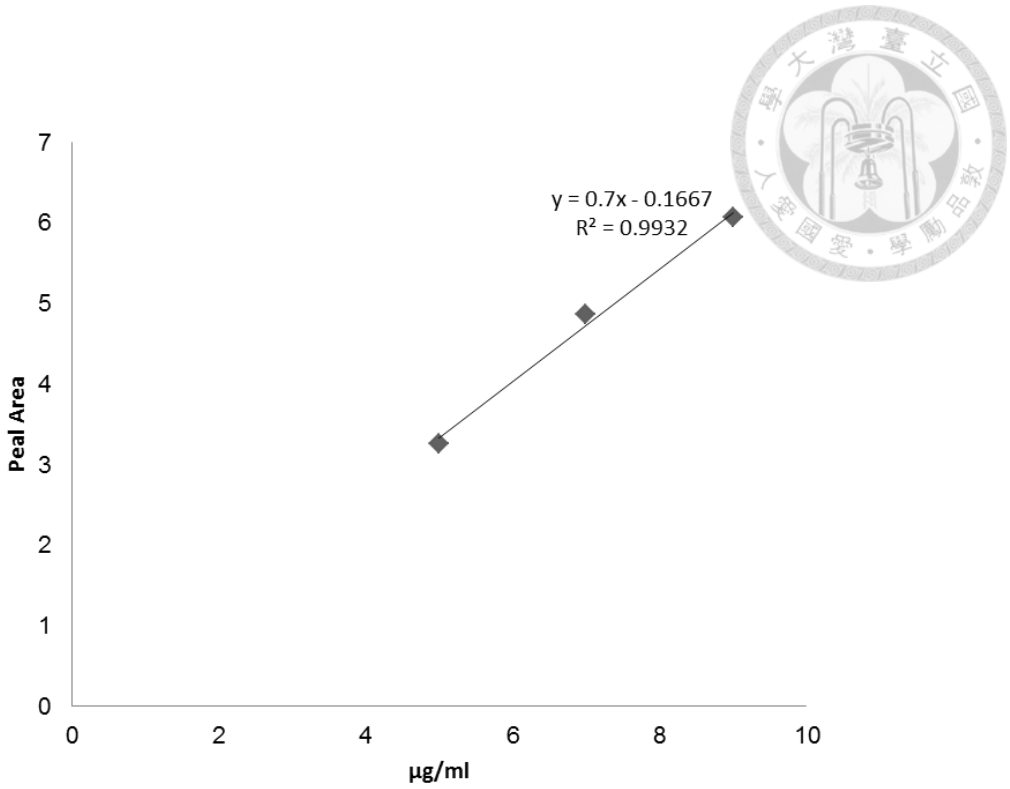

圖二十五、標準品天麻苷最低檢測濃度與定量濃度之回歸曲線

Figure 25. Calibration curve obtained with gastrodin standard solutions for limit of detection and quantification using the proposed HPLC method.

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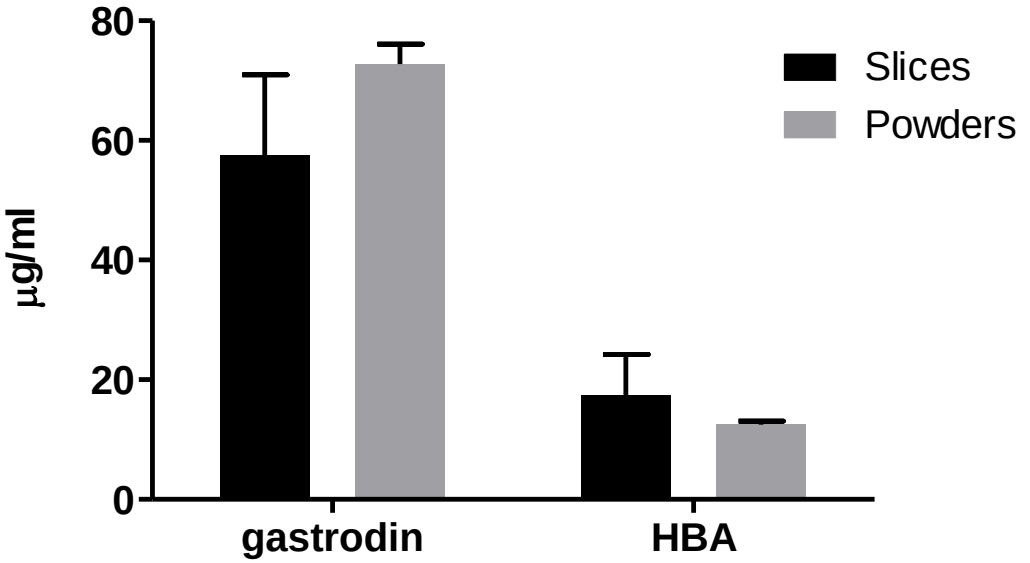

圖二十六、水萃天麻片與天麻粉末中天麻苷和天麻苷元之含量變化

Figure 26. The analysis of gastrodin and HBA quantities from slices and powder of *G. eleta* Bl. under water extraction by HPLC. Each value represents mean ± SD (n=3). Compared with water and ethanolic extraction, the data were not significantly defferent ($p > 0.05$) according to the result of ANOVA. HBA: 4-hydroxybenzyl alcohol.

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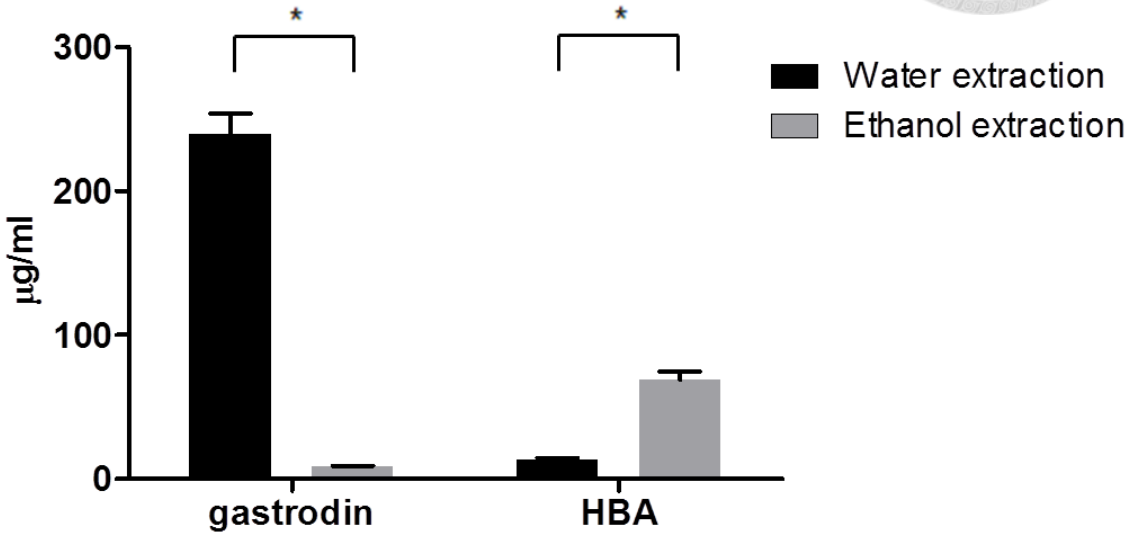

圖二十七、萃取溶劑對天麻中天麻苷和天麻苷元之含量變化

Figure 27. The analysis of gastrodin and HBA quantities under water and ethanolic extraction by HPLC. Each value represents mean ± SD (n=3). ***p* < 0.05 (according to the result of Student's t test) compared with water and ethanolic extraction. HBA: 4-hydroxybenzyl alcohol.

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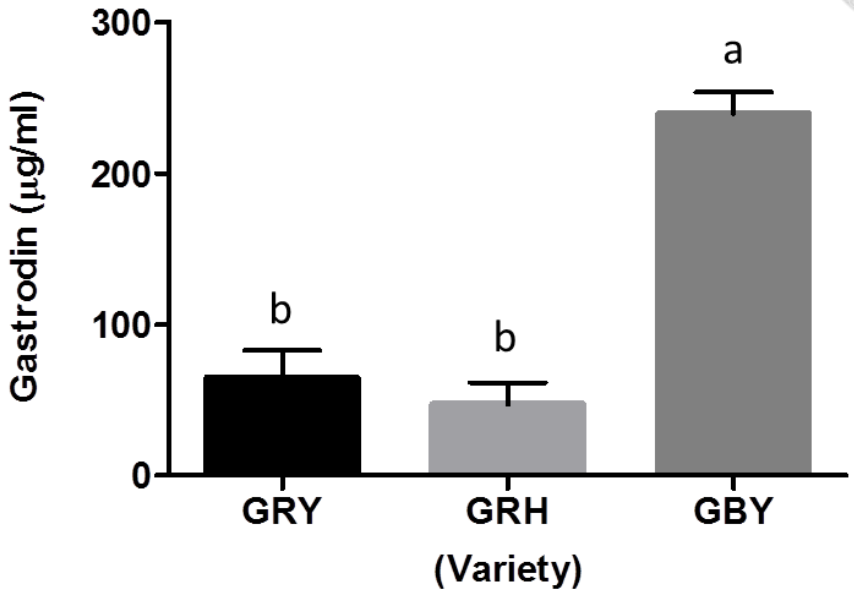

圖二十八、不同變種及產地對天麻中天麻苷含量之變化

Figure 28. The analysis of gastrodin quantity from different variety sources under water extraction by HPLC. Each value represents mean ± SD (n=3). [ab] data not sharing the same letter are significantly different ($p < 0.0001$) from each other according to the result of ANOVA coupled with Duncan's multiple range test. GRY: *Gastrodia elata* Bl. f. *elata*. from Yun-nan, GRH: *Gastrodia elata* Bl. f. *elata*. from Hu-nan, GBY: *Gastrodia elata* Bl. f. *glauca* S. Chow from Yun-nan.

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陸、討論

一、動物飼養與類憂鬱行為誘發結果之探討

(一) 大鼠飼料、水攝取量與體重之變化

在 21 天之飼養過程中每天給予大鼠口服天麻水萃物 (WGE)、天麻苷 (GAS) 與 天麻苷元 (HBA)，在各組飲食、飲水及體重進行交叉比對結果顯示皆與 CTL 組無統計差異，由此結果可以證實 服用 WGE、GAS 和 HBA 並不會影響動物的飲食、飲水及體重。值得注意的是餵食大鼠 WGE 和 GAS 過程中發現老鼠情緒比 CTL 和 HBA 組沉穩。過去文獻提到 GABA 主要是抑制神經興奮，具有抗焦慮和鎮靜之效果 (Purdy et al., 1991)，An 等人 (2003) 在癲癇研究發現 GAS 可抑制 GABA-transaminase、succinic semiadehyde dehydrogenase 和 succinic semialdehyde reductase 等 GABA 降解酵素之活性，藉由維持腦中 GABA 濃度來緩和癲癇症狀。因此，推論 WGE 和 GAS 可能影響腦部 GABA 濃度產生鎮靜之現象。

(二) 開放空間試驗

學者 Hall 在 1934 年描述當動物放置在一個新環境後，因為情緒緊張所導致運動減少及自主神經系統活躍，使動物排便和排尿次數提高。他認為這就是焦慮的指標 (Hall, 1934)。然而，Bindra 和 Thompson 認為焦慮與排尿和排便之間有一定程度的關係，但與恐懼沒有直接的關係 (Bindra and Thompson, 1953)。

依據 Walsh 和 Cummins 在 1976 年 發表評估行為及焦慮程度模式中，將動物穿越廣場中心的次數與時間作為焦慮程度的指標，穿越中心次數越多其焦慮的程度越低 (Walsh and Cummins, 1976)。為了在 OFT 獲得更一致性的分析，Brown 等人 (1999) 將行為分析項目分述如下，包括：

1. Line Crossing: 在此空間的地板用線劃分成數個網格後，分析老鼠用四肢穿越網格與網格之間的次數。

2. Center square entries: 老鼠行走過中央的次數。

3. Center square duration: 老鼠在中央的的時間。

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4. Rearing: 老鼠以後腳站立的次數。

5. Stretch attend postures: 老鼠頭部和肩部向前延伸及縮回的頻率。

6. Grooming: 老鼠在原地理毛的時間。

7. Freezing:老鼠完全靜止不動的時間。

8. Urination:老鼠排尿的次數。

9. Defecation: 老鼠排便的次數。

在神經方面與治療藥物的研究，OFT 主要運用在正式實驗前的藥物對動物行為影響之初步評估，而本次實驗是依據 McCarthy 等人與 Blizard 等人之分析方法稍加修飾 (McCarthy et al., 1995; Blizard et al., 1975)，至於分析時間的長短是根據 Cholerisa 等人的 OFT 分析報告，研究顯示當分析時間越長，其在評估動物行為的誤差會越大，因為動物會慢慢適應一個不熟悉的空間，而焦慮情況會在分析初期發生，其中分析五分鐘為主要誤差的分水嶺，因此本實驗 OFT 分析時間為五分鐘 (Cholerisa et al., 2001)。以 OFT 探討天麻水萃物與其活性成分對動物行為之影響方面，其中央穿越次數、移動時間、移動距離與移動速度皆與 CTL 組無統計上的差異，雖然餵食 WGE 和 GAS 過程中之大鼠情緒較 CTL 和 HBA 組沉靜，但此結果證實本實驗樣品皆不會在 FST 之前影響動物情緒而造成自主行為的改變。Guo 等人 (2013) 在機械性痛覺過敏 (mechanical hyperalgesia) 之研究中也評估過注射 GAS 對小鼠自主行為的影響，其結果與本實驗在 GAS 的結果一致。

(三) 強迫游泳試驗

在抗憂鬱藥物研究方面，由於對病因相關知識的不足，目前研究人員在精神病理學針對神經化學和行為表現對動物進行藥物開發 (Katz, 1981)。目前已知的抗憂鬱用藥分為理論型及經驗型，前者依據一些憂鬱症假說而開發出來的用藥，後者是醫生依據過去患者使用後的治療經驗來治療下一位病患。由於患有憂鬱症的病人可能還夾帶著其他精神疾病，研究學者很難在這複雜的生理反應中找到引發憂鬱症的真正機制。因此，Porsolt 等人 (1977) 建立了動物的 FST 模式，其

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優點在於誘發老鼠產生類憂鬱情況的一致性。

Borsini 和 Meli (1988) 將 Porsolt 等人 (1977) 利用 FST 模式研究各種憂鬱藥對類憂鬱動物的泳動行為之影響結果整理成表十，報告顯示 citalopram、trazodone、salbutamol 和 zimelidine 之服用對動物不泳動時間沒有任何影響，有些憂鬱用藥甚至會造成不泳動時間的增加，Borsini 和 Meli (1988) 認為可以藉由藥物治療的作用機制及此表統計的結果來避免 FST 假陽性的結果。

本研究對 FST 中動物的行為結果分為不泳動行為 (immobility)、泳動行為 (swimming)、及掙扎行為 (struggling)。不泳動行為是 FST 主要測量的指標，當動物有類憂鬱情況時會出現四肢不動的漂浮情況，其不泳動時間與類憂鬱情況呈正相關。由於 noradrenergic 用藥可增加突觸間正腎上腺素 (NE) 含量，藉由提高動物在 FST 過程中的掙扎時間來降低動物不泳動之絕望行為 (Porsolt et al., 1979; Cryan et al., 2005)。因此，將掙扎情況也列入本次 FST 的分析，結果顯示大鼠服用 WGE、GAS 和 HBA 不顯著影響動物掙扎時間，推測此三種樣品無調控正腎上腺素系統之效果。在不泳動時間方面，WGE、GAS 和 HBA 能顯著降低大鼠在 FST 中不泳動的時間，而 WGE 降低大鼠在 FST 不泳動行為主要是透過降低血清素與多巴胺等神經傳導物質在腦中的代謝率達到抗憂鬱來緩解 (Chen et al., 2008)。本研究也在動物犧牲後，進一步探討 GAS 和 HBA 是否影響腦部神經傳導物質代謝來降低不泳動之情況。

二、腦部單胺類神經傳導物質代謝速率之變化

過去文獻指出腦部神經傳導物質與情緒有緊密的關聯性 (Van Praag, 1982; Hughes et al., 2004)。因此，單胺類假說為憂鬱症研究的主流，主要將重點放在血清素系統 (serotonergic system) 和多巴胺系統 (dopaminergic system) 於腦中的代謝情況。實驗室先前研究已證實天麻水萃物 (WGE) 可藉由降低血清素 (5-HT) 和多巴胺 (DA) 在腦部的代謝而達到抗憂鬱之功效 (Chen et al., 2008)，本次研究進一步探討 WGE 之抗憂鬱功效是否來自其活性成分天麻苷 (GAS) 和天麻苷

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表十、抗憂鬱藥物對雄性大鼠在強迫游泳試驗中抗憂鬱行為之影響

Table 10. Effect of drugs reported to exert antidepressant activity in man on FST in rats.

Drug	Dose	% of immobility time (control = 100) after			
	(mg/kg)	1	or	2–3	Administrations
Amineptine	10			80	Borsini et al. 1981
	20			40–65	Borsini et al. 1981
	40			10	Borsini et al. 1981
Amitriptyline	1	99	Gorka and Wojtasik 1980		
	3	92	Gorka and Wojtasik 1980		
	3.2			81	Satoh et al. 1984
	3.75			75	Porsolt et al. 1977
	5	83–94	Kawashima et al. 1986; Zabrowska-Lupina 1980	137	Cooper et al. 1980
	7.5	74	Araki et al. 1984	77–78	Ogawa et al. 1984; Porsolt et al. 1977
	10	83–97	Gorka and Wojtasik 1980; Kawashima et al. 1986; Kitada et al. 1981; Zabrowska-Lupina 1980	72–74	Cooper et al. 1980; Satoh et al. 1984
	15	70	Araki et al. 1984	27–68	Cooper et al. 1980; Kitada et al. 1981; Miyauchi et al. 1981; Ogawa et al. 1984; Porsolt et al. 1977
	30	70–80	Araki et al. 1984; Kitada et al. 1981		
	32			43	Satoh et al. 1984
Buproprion	5			85	Cooper et al. 1980
	10			51	Cooper et al. 1980
	15			21	Cooper et al. 1980
Carbamazepine	40			50	May et al. 1985
Citalopram	16			88	Porsolt et al. 1979
	32			109	Porsolt et al. 1979
	64			103	Porsolt et al. 1979
Clomipramine	3.2			95	Satoh et al. 1981
	5			78–79	Porsolt et al. 1979; Porsolt 1981
	10	100	Kawashima et al. 1986	89–90	Porsolt et al. 1978a, 1979; Satoh et al. 1981
	20			92–94	Porsolt et al. 1979; Porsolt 1981
	30	100	Araki et al. 1985		
	32			74	Satoh et al. 1981
Clorgyline	2.5			96	Porsolt 1981
	5			99	Porsolt 1981
	10			75	Porsolt 1981
Danitracen	0.5	99	Gorka and Wojtasik 1980		
	1.5	84	Gorka and Wojtasik 1980		
	5	62	Gorka and Wojtasik 1980		
	10	62	Herman et al. 1981		
Deprenyl	2.5			77	Porsolt 1981
	5			59	Porsolt 1981
	10			10	Porsolt 1981
Desipramine	3.2			91	Satoh et al. 1984
	5			94	Porsolt 1981
	7.5	89	Araki et al. 1981		
	10	67–100	Araki et al. 1981; Kawashima et al. 1986; Kitada et al. 1981; Kostowski 1985	66–90	Borsini et al. 1981; Drago et al. 1985 Porsolt et al. 1977, 1979 Pulvirenti and Samanin 1986; Satoh et al. 1984
	15	82	Araki et al. 1981		
	20	67–95	Borsini et al. 1981; Kitada et al. 1986; Kostowski et al. 1984a, b; Plaznick et al. 1985b	30–55	Borsini et al. 1981; Drago et al. 1985; Kitada et al. 1981, 1983, 1986; Miyauchi et al. 1981, 1984; Porsolt et al. 1977; Vaccheri et al. 1984
	30	57–79	Araki et al. 1984, 1985; Kitada et al. 1981		
	32				
	40			27–41	Borsini et al. 1981; Porsolt et al. 1979; Satoh et al. 1984

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Table 10 (continued)

Drug	Dose	% of immobility time (control = 100) after			
	(mg/kg)	1	or	2–3	Administrations
Dexamisole	5	92	Przegalinski et al. 1980		
	10	82	Przegalinski et al. 1980		
	20	68	Przegalinski et al. 1980		
	40	22	Przegalinski et al. 1980		
Doxepine	10	77	Gorka and Wojtasik 1980		
	30	63	Gorka and Wojtasik 1980		
	60	61	Gorka and Wojtasik 1980		
Fluoxetine	3	110	Gorka et al. 1979b		
	10	113	Gorka et al. 1979b		
	20			96	Porsolt et al. 1979
	30	94	Gorka et al. 1979b		
	40			63	Porsolt et al. 1979
	80			48	Porsolt et al. 1979
Fluradoline	40			62	Borsini, unpublished results
Fluvoxamine	10	95	Maj et al. 1982		
	20	94	Maj et al. 1982		
	40	98	Maj et al. 1982		
Imipramine	1	93	Gorka and Wojtasik 1980; Gorka et al. 1979b		
	3	90	Gorka and Wojtasik 1980; Gorka et al. 1979b		
	3.2			84	Satoh et al. 1984
	5	73	Zebrowska-Lupina 1980	98	Cooper et al. 1984
	7.5	63–100	Araki et al. 1985; Porsolt et al. 1978a	72–96	Porsolt et al. 1978a, b
	10	73–94	Gorka and Janus 1985; Gorka and Wojtasik 1980; Gorka et al. 1979b; Kawashima et al. 1986; Zebrowska-Lupina 1980	48–83	Cooper et al. 1980; Drago et al. 1984 Satoh et al. 1984
	15	63–86	Porsolt 1981; Porsolt et al. 1978a	14–79	Cooper et al. 1980; Duncan et al. 1985 Porsolt et al. 1978a, b, 1979b
	20			45–58	Drago et al. 1985; Duncan et al. 1985
	30	61–100	Araki et al. 1984, 1985; Duncan et al. 1985; Porsolt 1981; Porsolt et al. 1978a	31–60	Duncan et al. 1985; Koyuncuoglu et al. 1982; Porsolt 1981; Porsolt et al. 1978a, 1979b; Martorana and Nitz 1979
	32			44	Satoh et al. 1984
Iprindole	3	98	Gorka and Wojtasik 1980		
	7.5			110	Porsolt et al. 1977
	10	104	Gorka and Wojtasik 1980		
	15			98	Porsolt et al. 1977
	30	80	Gorka and Wojtasik 1980	80–94	Duncan et al. 1985; Porsolt et al. 1977
	40			64–73	Kitada et al. 1981; Porsolt et al. 1977
	60	69	Gorka and Wojtasik 1980	107	Porsolt et al. 1977
Iproniazide	15			88	Porsolt et al. 1978a
	20			87	Drago et al. 1985
	30			79	Porsolt et al. 1979
	60			53–58	Drago et al. 1985; Porsolt et al. 1979
	120			96	Porsolt et al. 1979
Maprotiline	8			130	Porsolt et al. 1979
	16			88	Porsolt et al. 1979
	32			58	Porsolt et al. 1979
Mianserine	3	96	Gorka and Wojtasik 1980		
	3.75			98	Porsolt et al. 1977
	7.5			90	Porsolt et al. 1977
	10	87–102	Gorka and Wojtasik 1980; Kitada et al. 1981		
	15			66	Porsolt et al. 1977
	30	78–97	Gorka and Wojtasik 1980; Kitada et al. 1981	70–83	Kitada et al. 1981; Porsolt et al. 1977
	60	98	Gorka and Wojtasik 1980	121	Porsolt et al. 1977

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Table 10 (continued)

Drug	Dose	% of immobility time (control = 100) after			
	(mg/kg)	1	or	2–3	Administrations
Nialamide	40			103	Porsolt et al. 1978b
	80			57	Porsolt et al. 1978b
	100			37	Porsolt et al. 1978b
Nisoxetine	7.5			101	Porsolt et al. 1979
	15			101	Porsolt et al. 1979
	30			67	Porsolt et al. 1979
Nomifensine	2.5			72–82	Borsini et al. 1981; Porsolt et al. 1978a, b
	5			10–89	Borsini et al. 1981; Gorka et al. 1985 Porsolt et al. 1977, 1979
	10	62	Herman et al. 1981	4–17	Borsini et al. 1981; Duncan et al. 1985 Porsolt et al. 1978a, b
Nortriptiline	5	87	Zebrowska-Lupina 1980	87	Porsolt et al. 1978a
	10	73	Zebrowska-Lupina 1980	80	Porsolt et al. 1978a
	20			39	Porsolt et al. 1978a
Pargyline	25			66	Duncan et al. 1985
Pirlindole	10			83	Martorana and Nitz 1979
	20			71	Martorana and Nitz 1979
Salbutamol	16			117	Porsolt et al. 1979
	32			105	Porsolt et al. 1979
	64			107	Porsolt et al. 1979
Tandamine	4			99	Porsolt et al. 1979
	8			92	
	16			77	
Toloxatone	25			90	Porsolt et al. 1978a
	50			74	Porsolt et al. 1978a
	100			56	Porsolt et al. 1978a
	200			53	Porsolt et al. 1978a
Tranylcypromine	1			94	Martorana and Nitz 1984
	1.25			85	Porsolt 1981
	2			60	Martorana and Nitz 1984
	2.5			36	Porsolt 1981
	5			10	Porsolt 1981
TRH	1			91	Ogawa et al. 1984
	2			81	Ogawa et al. 1984
	10			61	Ogawa et al. 1984
Trazodone	2.5			103	Porsolt 1981
	10	0	Herman et al. 1981		
	50			101–104	Gorka et al. 1985; Porsolt 1981
	100			97	Porsolt 1981
Viloxazine	7.5			97	Porsolt et al. 1978a
	12.5			96	Porsolt et al. 1978a
	25			82	Porsolt et al. 1978a
	30			80	Porsolt et al. 1978a
	50			67	Porsolt et al. 1978a
Zimelidine	3.2			103	Satoh et al. 1984
	10			91	Satoh et al. 1984
	32			84	Satoh et al. 1984

Per cent values indicate the minimum-maximum range of effect (Borsini and Meli, 1988)

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元 (HBA)。大鼠經過連續 21 天口服 WGE、GAS 和 HBA 後以 FST 誘導類憂鬱行為，發現此三種樣品可顯著降低 5-HT 和 DA 在腦部之代謝。其中，WGE、GAS 和 HBA 對多巴胺系統的影響較血清素系統顯著，而 WGE 對多巴胺系統有較顯著影響之現象也於先前 Chen 等人 (2008) 研究成果中發現。

腦中單胺類的代謝受到單胺類受器 (monoamine receptors) (Hsieh et al., 1998; Guo et al., 2013; Wang et al., 2014)、單胺類合成酵酶 (monoamine-synthesizing enzymes) (Davis and Carlsson, 1973; Shin et al., 2011) 和單胺類氧化酶 (monoamine oxidase) (Riederer and Youdim, 1986) 的影響。5-HT1A 受器是一種促進 5-HT 分泌的單胺類受器，Wang 等人 (2014) 指出 GAS 可藉由提高 5-HT1A 受器與降低 5-HT 轉運子 (transporter) 的表現，降低 5-HT 代謝率改善 3,3'-Iminodipropionitrile 誘導神經受損造成的記憶衰退。GAS 也被做為治療機械性痛覺過敏 (mechanical hyperalgesia) 之研究素材，發現 GAS 透過影響 5-HT1A 受器來達到止痛的效果 (Guo et al., 2013)。Hsieh 等人 (1998) 在 cycloheximide 誘導動物記憶受損的研究中發現 HBA 透過調控 5-HT1A 和 5-HT2 受器可以改善動物記憶衰退之程度。綜合以上研究， GAS 和 HBA 與調控單胺類受器 5-HT1A 受器有高度的關聯性。因此，進一步以西方墨點法來分析 5-HT1A 受器在額葉皮質與海馬迴的表現，結果顯示海馬迴中 5-HT1A 受器的表現量在 WGE、GAS 和 HBA 組有顯著提升，此現象可以清楚解釋 5-HT 代謝率為何在海馬迴中有顯著影響。

酪胺酸羥化酶 (tyrosine hydroxylase) 為合成 DA 的關鍵酵素，主要將酪胺酸 (tyrosine) 轉變為 L-dopa (Iuvone et al., 1978)。在 Shin 等人 (2011) 的研究指出天麻可改善 methamphetamine 誘導 dopaminergic 毒性模式中所產生的氧化壓力，降低小鼠紋狀體的氧化傷害，顯著提高 tyrosine hydroxylase 的表現與活性。另外，Kumar 等人 (2013) 在 1-methyl-4-phenylpyridinium (MPP+)/1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) 誘導帕金森氏症之動物模式中發現，經給予 GAS 後具有恢復 tyrosine hydroxylase 蛋白表現的能

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力。根據本實驗室先前在 PC12 細胞實驗中研究發現，DA 的代謝率降低主要是受到 WGE 和 GAS 對 tyrosine hydroxylase 的調控，透過 tyrosine hydroxylase 促進 DA 合成來提高腦內濃度 (Lin et al., 2016)。綜合以上研究結果，推測本實驗在 DA 所觀察到的現象可能是透過此機制來達成。

單胺氧化酶 (monoamine oxidase, MAO) 主要在分解動物顱內的神經傳導物質，其可分為 MAOA 與 MAOB 兩種，前者與 5-HT、NE 及 DA 有高度的親和力，而後者則與 phenylethylamine 及 benzylamine 有高親和力 (Shih et al., 1999)。其中，在 corpus striatum、thalamus 和 brainstem 的 MAO 較其他腦區活躍 (Fowler et al., 1987)。MAOA 和 MAOB 此兩種酵素主要在維持突觸間單胺類濃度的平衡，有學者研究發現 MAO 活性的異常與帕金森氏症、憂鬱症與精神障礙呈現高度相關係性 (Benedetti and Dostert, 1989)。本實驗室先前研究已證實天麻水萃物會降低動物額葉皮質與海馬迴等大腦區域的單胺類神經傳導物質 (5-HT 與 DA) 代謝轉換率，改善動物經 FST 後的類憂鬱情況，並發現天麻水萃物、GAS 和 HBA 與 PC12 共培養下可間接降低 MAOA 活性的效果，而 GAS 甚至能直接抑制 MAOA 的活性 (陳，2008；Lin et al., 2016)。綜合本實驗的研究成果顯示，動物服用 GAS 和 HBA 後，可透過調控單胺類受器和 MAOA 來減緩單胺類在腦中的單胺類代謝轉換率。

三、神經可塑性 (neuroplasticity) 機制之探討

神經可塑性 (neuroplasticity) 定義為神經系統對內在 (生長發育) 或外在 (學習、疾病治療和環境) 的刺激所做出的反應，其中，包括：重組神經結構、功能和連結 (Cramer et al., 2011)。神經系統可因適應內外在因子而增強功能 (Cohen et al., 1997)，像在成人海馬迴中藉由神經新生來幫助記憶的形成；反之，神經系統功能會降低甚至產生傷害 (Nudo et al., 1996)，像中風所造成的神經受損導致語言及行動障礙。

本實驗室先前在 FST 動物模式下以基因體及蛋白質體學的技術發現，天麻

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水萃物 (WGE) 對大鼠抗憂鬱功效與神經可塑性有高度關聯性 (陳，2011；林，2012)。在基因體學部分將大鼠三個腦區 (額葉皮質、海馬迴和紋狀體) 利用微陣列分析來探討天麻抗憂鬱可能的機制，並結合即時聚合酶鏈式 (real-time PCR) 驗證。微陣列分析結果以 Kyoto Encyclopedia of Genes and Genomes (KEGG) 資料庫進行相關路徑之交叉比對，顯示在大鼠前額葉皮質和海馬迴中與 axongenesis、neurogenesis、nervous system development 及 dopamine secretion 具有高度相關性。經由即時聚合酶鏈式實驗驗證 WGE 顯著提升神經可塑性 (neuroplasticity) 相關的基因表現，如: *Map1b*、*RhoA*、*profilin-1* 及 *CRMP2* (陳，2011)。在蛋白質體學部分以二維膠體電泳分析 WGE 對大鼠三個腦區 (額葉皮質、海馬迴和紋狀體) 蛋白質表現，以軟體評估有差異的蛋白，再以質譜儀鑑定蛋白，結果顯示天麻水萃物抗憂鬱的機制與 Slit-Robo 路徑和神經細胞骨架重組具有高度關聯性，兩路徑下游皆對應到 actin 與 tubulin。其中，Slit-Robo 路徑還包括：CRMP2 和 PFN1 等蛋白 (林，2012)。在西方墨點法結果顯示，WGE 顯著影響負調控因子 Slit1、Slit2 和 RhoA 與正調控因子 CRMP2 和 PFN1，此現象與二維膠體電泳和系統生物資訊軟體的結果相互呼應 (林，2012)。

神經滋養因子 (neurotrophic factors) 是提供神經元存活和維持成熟神經元正常運作及成長的重要營養來源。在天麻苷 (GAS) 和天麻苷元 (HBA) 與神經滋養因子的相關研究中指出，GAS 可藉由恢復海馬迴中神經滋養因子 glial fibrillary acidic protein 和 brain-derived neurotrophic factor (BDNF) 來改善動物在 chronic mild stress 誘導下所產生的類憂鬱行為 (Zhang et al., 2014)。此外，HBA 證實可促進 BDNF、glial cell line-derived neurotrophic factor 和 myelin basic protein 的表現來改善 MCAO 腦部缺血所產生的氧化傷害 (Yu et al., 2010; Kam et al., 2011)。然而，在血清分析發現 WGE、GAS 和 HBA 無法有效降低皮質固醇的濃度，亦無法提高 BDNF 的濃度。推測可能是因為 FST 只給予短暫的壓力，此模式無法在短期誘導出皮質固醇分泌過量所造成的神經內分泌失調，進而無法看到皮質固醇影響 BDNF 濃度變化的現象。即使如此，臨床研究上發

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現腦部的神經滋養 (neurotrophic) 和細胞可塑性 (cellular plasticity) 與雙極憂鬱症患者具有高度關聯性 (Soeiro-de-Souza et al., 2012)，藉此更加深我們對 GAS 和 HBA 是否影響神經可塑性的好奇心。綜合先前在 WGE、GAS 和 HBA 的神經研究結果推測， WGE 具有神經可塑性的功效可能來自 GAS 和 HBA。因此，以西方墨點法優先驗證 Slit-Robo 路徑中的相關調控蛋白表現。

Slit-Robo 路徑是影響神經細胞生長、神經細胞骨架中軸突生長錐或樹突動力學的重要路徑 (Whitford et al., 2002)。有研究發現降低 Slit 相關蛋白的表現可提高神經可塑性，因此指出 Slit-Robo 路徑與神經可塑性具有高度關聯性 (Lin and Isacson, 2006; Borrell et al., 2012)。當神經細胞分化後會長出軸突結構，此時神經細胞具有一基因 commissureless (*Comm*) 調控 *Robo* 表現，若 *Comm* 不表現時，會促使 *Robo* 正常製造並運輸至生長錐上表現，接收 Slit 的訊息後使軸突發展時不會跨過中線 (midline)，即轉向與中線平行生長。反之，會與 *Robo* 結合並促使其自然分解而不會使 Robo 受器表現於生長錐，使神經細胞無法接收 Slit 的訊息，而被 Netrin-1 吸引跨過中線，跨過中線後，*Comm* 即會停止表現而使 *Robo* 正常製造後接受 Slit 的訊息，造成軸突轉向與中線平行生長 (Dickinson and Duncan, 2010)。本研究在西方墨點法結果顯示，大鼠口服 WGE、GAS 和 HBA 後可顯著降低 Slit-Robo 路徑中的負調控因子 Slit1 和 RhoA 的蛋白在海馬迴的表現，其中 Slit 蛋白由神經膠質細胞沿著中樞神經系統的中線分泌，與軸突上的 Robo 受器結合。成年哺乳類的神經新生主要在海馬迴，Kaneko 等人 (2010) 發現成年哺乳類的新生神經細胞會分泌 Slit1 來幫助其移行 (migration) 至大腦皮質，Lin 和 Isacson (2006) 在胚胎幹細胞實驗中證實 Netrin-1 可促進神經突觸的增生，Slit1 則有抑制突觸增生的效果 (圖二十九)，透過先前的研究結果可解釋在本實驗中為何 WGE、GAS 和 HBA 可顯著影響降低海馬迴中 Slit1 表現的現象。

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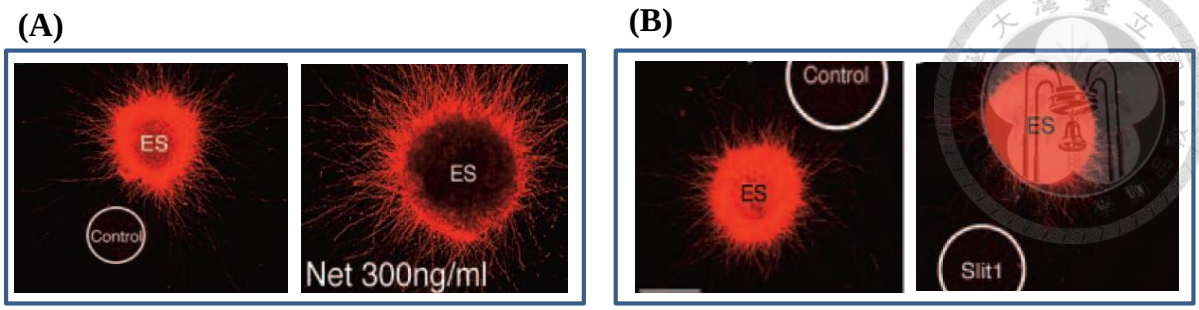

(Lin and Isacson, 2006)

圖二十九、Net (A) 和 Slit1 (B) 對胚胎幹細胞突觸發展的影響

Figure 29. The effect of neurite development of enhancesembryonic stem (ES) with Netrin-1 (Net) (A) and Slit (B) treatment.

Hs683 是人類寡樹突細胞株，Rossi 等人 (2005) 發現其具有分泌 Slit1 的能力。為了瞭解 WGE、GAS 和 HBA 是否可直接抑制神經膠細胞分泌 Slit1，後續以 Hs683 細胞進行評估。WGE 0.5 mg/ml 之劑量主要參考 Lin 等人 (2016) 在 PC12 中 MAOA 的研究，由於柯達所提供之 WGE 含有 3% GAS，表示在 0.5 mg/ml WGE 下含有 50 μM GAS，因此 GAS 細胞實驗採用 50 μM、100 μM 和 200 μM 等劑量與 Hs683 細胞株共同培養，在 HBA 劑量選擇上因目前鮮少有神經膠細胞的研究發表，所以劑量選擇參照 GAS 劑量。根據結果顯示 WGE、GAS 和 HBA 確實可直接降低 Hs683 在 Slit1 的蛋白表現。

RhoA 亦為 Slit-Robo 路徑中重要抑制神經突發展的負調控因子 (Benarroch, 2007)，當 GTP-RhoA 被 SRGAP 水解為 GDP-RhoA 時則具有活性，GDP-RhoA 會活化 Rho-associated protein kinase (ROCK) 而抑制肌動蛋白的聚合 (Schmandke et al., 2007)，此機制回應 Sin 等人 (2002) 所發現的現象——降低 RhoA 表現可提高神經可塑性。在本次研究結果顯示 WGE、GAS 和 HBA 也有能力顯著降低 RhoA 在海馬迴的表現。

此外，先前研究指出 WGE 促進 Slit-Robo 路徑中的正調控因子 CRMP2 和 PFN1 等白的表現，藉由提高神經可塑性來改善大鼠經 FST 誘導所產生的類憂鬱行為 (林，2012)。CRMP2 富含於海馬迴神經細胞中生長的軸突 (Inagaki, et

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al., 2001; Fukata et al., 2002; Yoshimura et al., 2006)，Yang 等人 (2013) 在誘導憂鬱之動物模式中發現 CRMP2 在腦部的蛋白表現顯著降低；PFN1 是 PFN 家族成員中的一員，目前研究發現其參與多種調控，包括各種細胞過程 (細胞骨架動力學，膜運輸，和核運輸)、神經元分化及突觸可塑性的調節 (Birbach, 2008)。近期在 Martins-de-Souza 等人 (2012) 的病理報告指出，MDD 患者腦部組織的 PFN1 蛋白表現顯著低於正常人。根據本研究在 CRMP2 和 PFN1 的西方墨點法分析結果顯示，除了 WGE 組可顯著提升海馬迴中此兩蛋白的表現外，GAS 和 HBA 組也得到類似結果。

本研究在動物預實驗中發現依據 WGE 中 GAS 和 HBA 含量換算而得的劑量，無法改善大鼠在 FST 不泳動之情況，但卻能在較高劑量 GAS (100 mg/kg bw) 和 HBA (100 mg/kg bw) 達到抗憂鬱效果，此現象推測與生物可利用率 (bioavailability) 有關。在 GAS 的藥物動力學研究中，Lim 等人 (2007) 和 Jia 等人 (2014) 分別以靜脈注射與口服 GAS 評估在大鼠體內的吸收及代謝情況，兩研究數據經過計算後發現 GAS 的生物可利用率為 14.2%，因此可解釋為何 GAS 在預實驗中無法改善大鼠類憂鬱情況，但 HBA 是否也因低生物可利用率造成在低劑量無改善大鼠類憂鬱情況仍需進一步確認。

四、分析確效的建立與不同變因對天麻中天麻苷含量變化之比較

天麻苷 (GAS) 為天麻主要活性成分之一 (Hsieh et al., 1997; Liu et al., 2002)，可用於鑑別天麻 (衛生福利部，2013)，經本動物實驗結果證實 GAS 具有改善老鼠類憂鬱行為的功效，假使未來以天麻開發調節憂鬱情緒之保健食品或抗憂鬱用藥之替代藥物，其採購將以 GAS 含量為導向。依據臺灣中藥典第二版對天麻鑑別標準，規定稀乙醇抽出物不得少於 14.0%，水抽出物不得少於 18.0%，所含 GAS 不得少於 0.20% (衛生福利部，2013)。其中，藥典提供 GAS 之 TLC 定量法操作易人為因素造成誤差；HPLC 之分析方法因其移動相沒有以梯度方式沖提，降低定量天麻中 GAS 的準確性。因此，本實驗利用 HPLC 進行天麻中 GAS

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的定性與定量，進而建立 HPLC 的確效方法。HPLC 分析條件採用先前實驗室建立的方法 (林，2010) 對柯達提供的 WGE 進行分析，其分析圖譜與標準品比對後確實含有高量的 GAS 與少量天麻苷元 (HBA)，但是實驗室自行購買的天麻樣品其水萃物的成分相當多，原先建立的分析方法無法提供適當的圖譜解析度。藉此參考 Liu 等人 (2002) 的分析條件並加以修飾，此分析條件在自行萃取的 WGE 與柯達提供的 WGE 都能提供良好的解析度。GAS 確效方法中之精密度重複性數值雖然超過藥品優良製造確效作業基準≦2% (衛生福利部，1999)，但天麻在臺灣已列為可供食品使用之原料，所以仍符合衛生福利部食品藥物管理署食品化學檢驗方法之確效規範≦10%。此外，中間精密度也在確效規範≦14%之範圍內 (衛生福利部食品藥物管理署，2012)。

為了找出萃取 GAS 較佳的天麻加工方式，我們將紅天麻粉末 (powder) 和切片 (slices) 兩種常見加工方式以實驗室建立的水萃方式進行萃取，並以 HPLC 比較 GAS 含量變化。一般情況下，粉末的表面積與溶劑或空氣的接觸面積遠大於其他形狀，因此推測天麻粉末應該可以萃取到較切片高量的 GAS，但實驗結果顯示兩種加工型態之 GAS 含量無顯著差異。有學者指出天麻大部分活性成分溶於水與醇類，但由於其所含的澱粉在受熱後易產生糊化現象，提高後續分離純化的困難度，因此大部分天麻研究多採醇類萃取，其醇濃度在 50~70% 為佳 (吳，2009)。本次萃取實驗也發現天麻粉經熱水萃取後產生澱粉糊化的現象，濃稠的糊化溶液相當難分離上清液；而乙醇萃部分雖然不受糊化影響，但其上清液在膜過濾的阻塞率也較天麻切片上清液高。綜合上述結果，後續實驗皆以切片的形式進行溶劑萃取。在比較不同萃取溶劑對 GAS 含量變化影響方面，GAS 在水萃法中的含量遠高於乙醇萃法，顯示 GAS 有較高的親水性。陳 (2011) 分別以去離子水、甲醇及 70% 乙醇對天麻進行熱迴流萃取，測定萃取液的抗氧化活性及神經保護效果。其結果顯示在 α,α-diphenyl-β-picryl -hydrazyl (DPPH) 自由基清除能力、還原力試驗和相對還原力、超氧歧化酶 (superoxide dismutase) 活性、ABTS 陽離子自由基清除活性及抑制硫代巴比妥酸鹽反應產物 (thiobarbituric

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acid reactive substances, TBARS) 生成之結果乙醇萃取物皆有最高活性，並具有最佳神經保護效果 (陳，2011)。雖然乙醇萃取物在抗氧化及神經保護研究皆有較高的活性，但主要活性成分為 HBA 為主，在相關研究也證實 25 和 50 mg/kg bw 的 HBA 可以預防缺血性腦中風的神經氧化傷害 (Kam et al., 2011; Yu et al., 2010)，而本次萃取實驗主要目的是找出 GAS 最適萃取溶劑，因此以水萃法進行後續天麻產地與變種的比較。為了找出中國大陸何處栽培的天麻變種有較高含量的 GAS，進一步選用大陸常見的紅天麻和烏天麻來比較，在不同天麻產地分析結果顯示，天麻的 GAS 含量並不會受到種植地區的影響；但在不同天麻變種方面，雲南當地烏天麻之 GAS 含量顯著高於紅天麻，雖然烏天麻不耐乾旱又易夭折 (周等人，1987)，但仍有人工栽種的價值。

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柒、結論

在 FST 誘導動物類憂鬱模式的研究中，連續管灌餵食四週齡 SD 雄性大鼠 21 天的天麻水萃物 (WGE) (500 mg/kg bw)、天麻苷 (GAS) (100 mg/kg bw) 和天麻苷元 (HBA) (100 mg/kg bw)，均能顯著降低大鼠在水中不泳動的時間 (immobility time)，顯示具有抗憂鬱的效果。以 HPLC 分析腦部單胺類神經傳導物質血清素 (5-HT) 和多巴胺 (DA) 的代謝變化，顯示 WGE、GAS 和 HBA 皆能顯著降低類憂鬱動物腦部過剩的單胺類代謝率，維持 5-HT 和 DA 在腦中的濃度。其中，這三種樣品對多巴胺系統的影響較大。此外，在西方墨點法分析發現 WGE、GAS 和 HBA 可降低海馬迴中 Slit-Robo 路徑負控制因子 Slit1 和 RhoA 之蛋白表現，並促進正調控因子 CRMP2 和 PFN1 之蛋白表現，經 Hs683 細胞實驗證實 WGE 和 GAS 可直接調控 Slit-Robo 路徑。在 GAS 分析確效與最適萃取方法開發方面，分析方法符合衛生福利部藥物管理署食品化學檢驗方法之確效標準，後續比較天麻加工方式、變種與產地對 GAS 含量的影響顯示，天麻之 GAS 含量變化與萃取溶劑和變種呈現正相關。其中，以烏天麻切片經熱水萃取所得到的 GAS 最符合經濟效益。

綜合以上研究成果，研究證實 GAS 和 HBA 是提供 WGE 影響腦部單胺類系統和神經細胞骨架重組改善憂鬱症狀的活性成分，而後續所建立的 GAS 分析確效、天麻萃取方式與原料來源的評估，以利往後調節憂鬱情緒保健食品開發之原料品質管理，並取代現今臨床抗憂鬱用藥，希冀憂鬱症在未來不再是文明社會的高負擔疾病。

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Original Article

Method development and validation for the high-performance liquid chromatography assay of gastrodin in water extracts from different sources of *Gastrodia elata* Blume

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ABSTRACT

Gastrodia elata Blume is commonly used as a medical herb in China for ameliorating headaches, dizziness, and convulsions. In previous studies, water extracts of *G. elata* Bl. (WGE) have demonstrated potential to act as therapeutic agents to improve depression-like symptoms in rats. As gastrodin (GAS) is a major active compound in WGE, its quantitation in WGE is important for quality control. The objective of this study was to develop an optimized and validated reversed-phase high-performance liquid chromatography method for the analysis of GAS in different sources of WGE. We evaluated the GAS content in varieties of *G. elata* Bl. including *G. elata* Bl. f. *glauca* S. Chow and *G. elata* Bl. f. *elata*. We also evaluated the GAS content of the latter variety from two different origins, Yun-nan and Hu-nan. The results indicate that the amount of GAS analyzed in WGE from *G. elata* Bl. f. *glauca* S. Chow is five times higher than that of *G. elata* Bl. f. *elata* from Yun-nan and Hu-nan. A significant difference in GAS content was observed between varieties of *G. elata* Bl., although not between locations of origin.

1. Introduction

Rhizoma Gastrodiae is the dried tuber of *Gastrodia elata* Blume (*G. elata* Bl., Orchidaceae), a prominent traditional Chinese medicinal herb. It is used to treat headaches, dizziness, convulsions, paralysis, rheumatism, and lumbago [1]. Five varieties of *G. elata* Bl. are cultivated in China, including *G. elata* f. *elata*, *G. elata* f. *glauca* S. Chow (GBY), *G. elata* f. *viridis* Makino, *G. elata* f. *flavida* S. Chow, and *G. elata* f. *alba* S. Chow [2]. Among

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these varieties, *G. elata* f. *elata* and GBY are the most widely cultivated [3,4].

Previous reports have indicated that *G. elata* Bl. can serve as an anticonvulsant [5–7], antioxidant [8–10], antiepileptic [11], memory enhancer [12–14], smooth muscle relaxant [15], protectant against amyloid β peptide-induced cell death [16], and apoptosis inhibitor [7,11,17]. Our previous studies demonstrated the antidepressant properties of water extracts (WGE) in forced-swimming tests [18,19]. Five bioactive compounds—gastrodin (GAS), vanillyl alcohol, 4-hydroxy-3-methoxybenzaldehyde (vanillin), 4-hydroxybenzyl aldehyde (HB), and 4-hydroxybenzyl alcohol (HBA)—have been isolated from *G. elata* Bl. However, the antidepressant effect of the primary bioactive compounds in *G. elata* Bl. remains unknown. GAS is regarded as the major bioactive compound in *G. elata* Bl. [20]. GAS (the structure shown in Fig. 1) has also been found to serve as a memory enhancer [13,21], anticonvulsant [22], apoptosis inhibitor [23]. Thus, the quality of *G. elata* Bl. can be evaluated by the level of GAS.

In the present study, we aimed to develop a simple, accurate, and selective method that is fully validated according to the ICH Q2A and Q2B guidelines [24,25], and which can be used to assay the active components of *G. elata* Bl. To determine the amount of GAS in different sources of WGE, we evaluated the GAS content in two varieties of *G. elata* Bl., including GBY and *G. elata* Bl. f. *elata*. We also evaluated two different origins of *G. elata* Bl. f. *elata* from Yun-nan (GRY) and from Hu-nan (GRH). Our study of the quantitation of GAS can provide useful information for selecting a source of *G. elata* Bl.

2. Experimental

2.1. Chemicals and reagents

G. elata Bl. f. *elata* (black *G. elata* Bl. from Yun-nan, GBY) and *G. elata* Bl. f *glauca* S. Chow (red *G. elata* Bl. from Yun-nan, GRY) were purchased from Chrysanthemum Village medicine market, a Chinese herb pharmacy in Kunming (Yunnan, China). *G. elata* f. *glauca* S. Chow (red *G. elata* Bl. from Hu-nan, GRH) was purchased in Hu-nan, China. These *G. elata* Bl. varieties were identified by Professor Zhi-Hong Zhou in the Chinese Medicine Cultivation Center at Yun-nan University of Traditional Chinese Medicine (Yunnan, China). GAS (p-hydroxymethylphenyl-β-D-glucopyranoside) was obtained from Professor Chi-Tang Ho in the Department of Food Science at Rutgers University (New Brunswick, NJ, USA). HBA, HB, and vanillin were purchased from Sigma (Sigma–Aldrich, St. Louis, MO, USA). All standards used were of ≥ 99.5% purity.

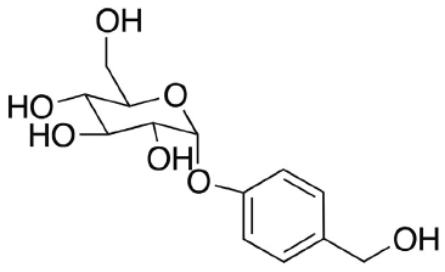

Fig. 1 – Structure of gastrodin in *Gastrodin elata* Blume.

2.2. Chromatography

2.2.1. Instrumentation and analytical conditions

The high-performance liquid chromatography (HPLC) system consisted of a PU-2089 Plus solvent delivery system (Jasco Corporation, Tokyo, Japan), an LC-NetII/ADC degasser (Jasco Corporation), and a UV-2075 Plus detector (Jasco Corporation). The data were acquired via Chrompass analytical software (version 1.7.403.1, Jasco). GAS was measured by the Jasco HPLC system with a phenomenex Luna C18 (250 mm × 4.6 mm i.d., 5 μm particle size; UV-2075 Plus detector, JASCO Corporation, Torrance, CA, USA) at 35 ± 1°C. The peaks of GAS were detected at 220 nm. The flow rate was set at 1.0 mL/min. The mobile phase was prepared freshly, filtered through a 0.22 μm polyethersulfone (PES) vacuum bottle top filter (Sigma–Aldrich) by an Aspirator AS-1 vacuum filtration apparatus (Fargo Instruments, Taipei, Taiwan) and degassed by Bransonic 5510R-DTH (Branson Ultrasonic Corporation, Danbury, CT, USA) for 30 minutes. The mobile phase consisted of a mixture of 0.02% phosphoric acid in water (solvent A) and acetonitrile (solvent B) pumped in the gradient mode as follows: 0–7 minutes, 5% B; 7–15 minutes, 5–20% B; 15–20 minutes, 20–25% B; 20–25 minutes, 25–95% B; 25–28 minutes, 95% B; 28–30 minutes, 95–5% B; and 30–35 minutes, 5% B.

The HPLC analysis method was fully validated according to the guidelines of ICH Q2A and Q2B [24,25] and the specifications of the Food Chemistry Analytical Method Validation by the Taiwan Food and Drug Administration [26]. The method was validated for linearity, precision (repeatability and intermediate precision), accuracy, specificity, detection limits, robustness (detection wavelength and the flow rate of mobile phase), and system suitability [27–30].

2.3. Preparation of *G. elata* Bl. extract

Extraction methods using ethanol and water were followed according to those in previous reports from the second edition of the Taiwan Herbal Pharmacopeia [31] and Teo et al [32] with slight modifications. For the ethanolic extraction, 1 g of slices/powder was immersed in 25 mL of 70% ethanol and refluxed in boiling water for 60 minutes. The supernatant was quickly cooled to 4°C and filtered through 0.45-μm nylon filters (Agela, Beijing, China). The ethanolic extracts (EGE) were concentrated by a rotary evaporator (Heidolph 4000; Heidolph Instruments, Schwabach, Germany) and lyophilized (SFD-25 model, Chang Juing Co., Kaohsiung, Taiwan) under high vacuum and re-dissolved with water prior to the HPLC assay. For the water extraction, 1 g of either slices or powder was immersed in 5 mL of deionized water and refluxed under boiling water for 60 minutes. Seven mL of deionized water was then added to and refluxed for another 50 minutes [33]. The WGE were collected as described above for the EGE, without the concentration step.

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2.4. Preparation of stock and working solutions

The standard stock solution was prepared by dissolving GAS in water at a concentration of 1000 μg/mL and stored at 4°C. Working standard solutions were freshly prepared by diluting the stock solution in purified water to GAS concentrations of 10 μg/mL, 30 μg/mL, 50 μg/mL, 70 μg/mL, and 90 μg/mL, respectively.

2.5. Preparation of standard and quality control samples

We prepared nine quality control (QC) samples: three WGE solutions at concentrations of 30 μg/mL, 50 μg/mL, and 70 μg/mL; three standard solutions at concentrations of 30 μg/mL, 50 μg/mL, and 70 μg/mL; and three spiked solutions at concentrations of 30 μg/mL, 50 μg/mL, and 70 μg/mL. In order to extract GAS, the concentrations of the original WGE solutions were 1800 μg/mL, 3000 μg/mL, and 4200 μg/mL, which resulted in a GAS content of 30 μg/mL, 50 μg/mL, and 70 μg/mL, respectively. All of the QC sample solutions had concentrations between 30 μg/mL and 70 μg/mL. The concentrations of the calibration standards were within the range of 10–90 μg/mL. QC samples fell within the range of the calibration curve of GAS.

3. Results and discussion

3.1. Method development and optimization

The preliminary experiments of the GAS assay were performed according to the HPLC method developed in our laboratory [34]. The HPLC chromatography was carried out by an isocratic elution of 10% acetonitrile (aqueous). A flow rate of 1 mL/min and a detection wavelength of 220 nm were used. GAS was identified according to the retention time of its peak, which was determined from a GAS standard with appropriate specificity and selectivity. However, the isocratic elution did not lead to satisfactory separation of all the peaks in a QC sample, which resulted in poor results from GAS quantification studies in our laboratory [34]. Thus, the chromatographic conditions were modified using a gradient elution method, according to Liu et al [35]. The gradient elution of a water and acetonitrile mixture resulted in lower resolution of peaks from a QC sample. Accordingly, peak resolution was improved by adding 0.2% phosphoric acid (aqueous) to the water of mobile phase. All peaks of the unknown components that were used to develop the method were well separated. GAS was identified by comparing the retention time of its peak with that of a commercial standard. HBA, an aglycone of GAS, was also identified in the HPLC chromatogram [36,37]. The results demonstrate the specificity of the developed method since none of the excipients interfered with target analytes (Fig. 2).

3.2. Validation of method

3.2.1. Linearity, limit of detection, and limit of quantitation

The calibration curve of GAS was linear within the concentration range of 10–90 μg/mL in water. The regression equation was $y = 0.5407x + 0.5436$ and the correlation coefficient (r^2) was 0.9999 (Fig. 3). Limits of detection (LoD) and quantitation (LoQ) were expressed as $LoD = 3.3\sigma/S$ and $LoQ = 10\sigma/S$ (where σ = the standard deviation of y-intercepts of regression lines and S = the slope of the calibration curve). The LoD and LoQ were calculated to be 0.5 μg/mL and LoQ was 1.52 μg/mL, respectively.

3.2.2. Accuracy and precision

The relative standard deviation (RSD) of the overall intraday precisions for the peak area ratios obtained for GAS was 8.60% ($n = 6$; Table 1). This demonstrates that the equipment used for the study functions correctly for the developed analytical method, and the method can be repeated. The data for the accuracy measurements were expressed as percent of recovery of GAS in the real samples. The samples were spiked with GAS to obtain final concentrations of 60%, 100%, and 140% to the nominal analyte concentration of 50 μg/mL. Analyte samples at each of these concentrations were injected and analyzed in triplicate by three analysts. The mean recovery of GAS in real samples was within the range of 94.56–105.94% with 3.23% of mean RSD (Table 2). However, *G. elata* Bl. is a natural product and content of GAS varies by different variety, origin, and harvest season. Because *G. elata* Bl. is a traditional functional food, the analysis method should follow the criteria for the Food Chemistry Analytical Method Validation by Taiwan Food and Drug Administration, which mean recovery and RSD range were not more than 80.00–115.00% and 14.00%, respectively. As shown in Table 2, all recoveries were within the acceptable range.

3.2.3. Robustness

To show the reliability of the HPLC analysis in the presence of varied analytical parameters, we injected solutions under the following modified chromatographic conditions: wavelength at 219 nm and 221 nm or flow rate at 0.97 mL/min and 1.03 mL/min. As shown in Table 3, the numbers of theoretical plates (N), the tailing factor (T), resolution (R), and selectivity (α) of GAS were not significantly affected by these slight changes. These results indicate the robustness of HPLC analysis despite variation of the parameters, including detection wavelength and flow rate.

3.2.4. System suitability

To confirm system suitability, the peak GAS values of 4200 μg/mL WGE were analyzed six times consecutively for RSD, N, T, R, α, and capacity factor (K′). The results of this analysis are listed in Table 1. The RSD was 8.60%, which fell within the criterion of ≤ 10.00% described in the Food Chemistry Analytical Method Validation guidelines [26]. This indicates that our results met the criteria and ours is a suitable method of analysis of the GAS content in WGE. Our analysis method is fully in compliance with the Food Chemistry Analytical Method Validation Guidelines.

3.2.5. Comparison between WGE and EGE from different sources of G. elata Bl.

In our pilot study, powder of *G. elata* Bl. was gelatinized during water extraction due to the presence of high levels of starch. The suspension from the extracts was difficult to obtain by

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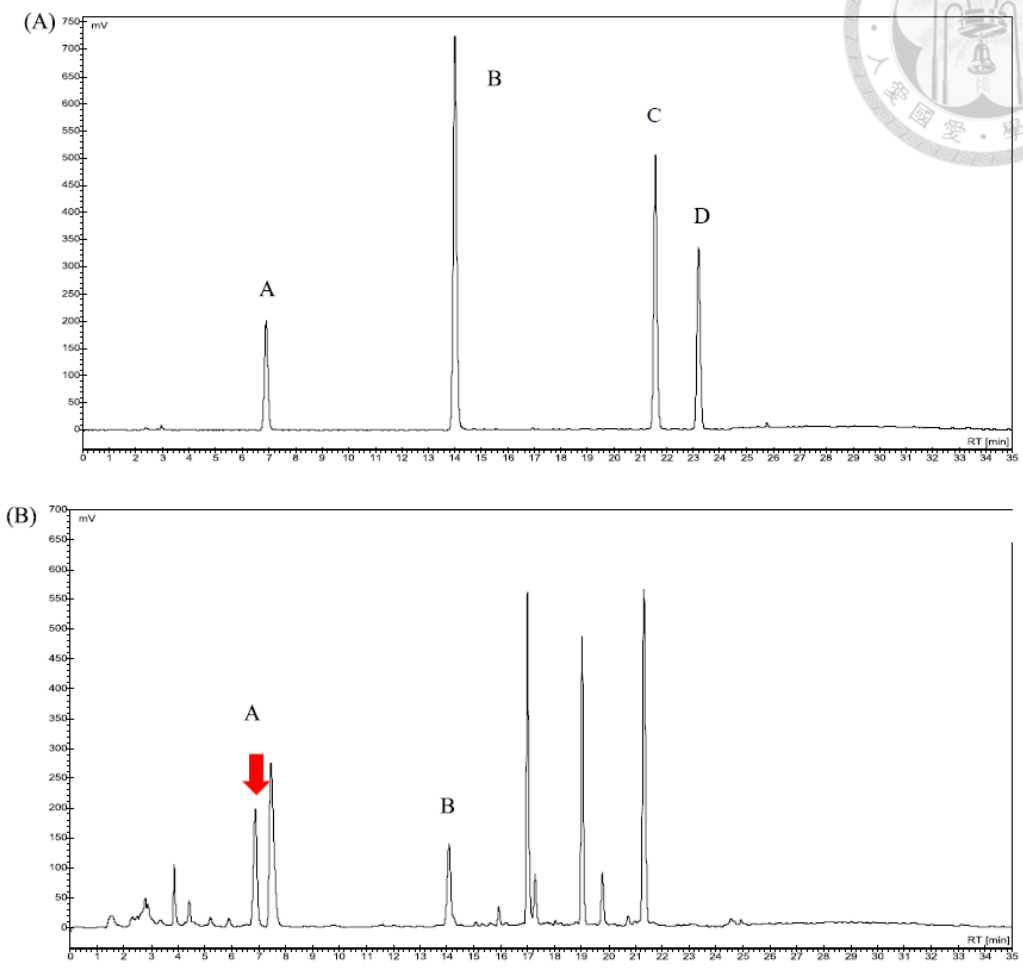

Fig. 2 – (A) Chromatogram of four standards of *Gastrodia elata* Blume by high-performance liquid chromatography; A = gastrodin, B = 4-hydroxybenzyl alcohol, C = 4-hydroxybenzyl aldehyde, D = vanillin (4- hydroxy-3-methoxybenzaldehyde). (B) Chromatogram of water extracts from *G. elata* Bl. A = gastrodin, B = 4-hydroxybenzyl aldehyde.

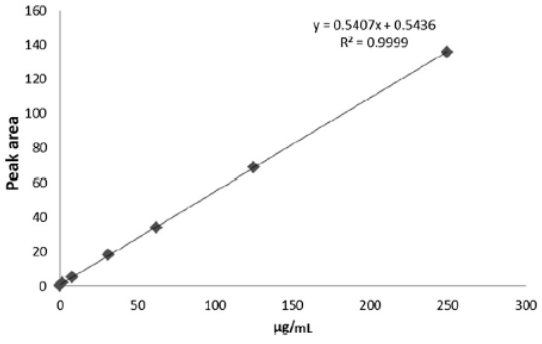

Fig. 3 – Calibration curve obtained with gastrodin standard solution using the proposed high-performance liquid chromatography method.

Table 1 – System suitability results of the proposed method.

Parameters	Results	Required limits
RSD	8.60%	RSD < 10%
N	2225	N > 2000
T	1.12	T < 1.5
R	1.4	R > 1.0
α	1.15	α > 1.05
K′	3.41	K′ > 3.0

Each value is the mean of six determinations.
α = selectivity factor; K′ = capacity factor; N = numbers of theoretical plate; R = resolution; RSD = relative standard deviation; T = tailing factor.

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Table 2 – Intra- and interday accuracy and precision of high-performance liquid chromatography assay for gastrodin from water extracts of *Gastrodia elata* Blume.

Analyst	Level (μg/mL)	Injection	Recovery			Recovery (%)	Average ($n = 3$)	RSD (%)	Repeatability		Intermediate	
			Standard[a]	Sample[b] (μg/mL)	Spike[c]				Average ($n = 9$)	RSD (%)	Average ($n = 27$)	RSD (%)
1	30	1	30.75	27.04	29.08	101.20	102.67	1.28	100.81	2.77	100.22	3.23
		2	29.64	27.78	29.27	103.75						
		3	30.38	27.97	29.64	103.05						
	50	1	49.27	48.72	50.20	104.89	101.88	2.53				
		2	50.39	48.53	49.27	99.26						
		3	49.46	46.87	48.53	101.50						
	70	1	69.65	67.06	67.24	96.81	97.87	2.72				
		2	69.28	68.73	68.73	99.20						
		3	69.47	68.17	67.98	97.60						
2	30	1	29.45	29.27	29.08	98.11	96.84	1.45	97.98	2.34		
		2	29.64	29.08	28.71	95.62						
		3	28.71	30.38	29.08	96.77						
	50	1	48.90	48.53	47.79	96.21	99.62	1.46				
		2	49.27	48.16	49.27	102.26						
		3	47.61	48.90	48.35	100.39						
	70	1	68.54	68.91	67.24	95.68	97.47	1.15				
		2	68.17	68.54	67.43	97.28						
		3	67.43	68.17	67.61	99.45						
3	30	1	31.81	31.81	31.98	101.08	99.10	3.97	101.87	3.38		
		2	31.63	33.01	31.46	94.56						
		3	31.29	32.49	32.15	101.65						
	50	1	52.10	49.69	52.44	105.94	104.64	1.90				
		2	51.93	49.69	52.27	105.63						
		3	51.24	50.38	51.41	102.35						
	70	1	74.11	72.56	74.80	103.94	101.87	2.18				
		2	73.94	71.19	72.39	99.53						
		3	73.08	73.94	74.28	102.12						

RSD = relative standard deviation; Sample = determination of gastrodin content by injecting each sample preparation; Spike = determination of gastrodin content by injecting each spiked solution; Standard = determination of gastrodin content by injecting each standard solution.

[a] Standard: determination of gastrodin content by injecting each standard solution.

[b] Sample: determination of gastrodin content by injecting each sample preparation.

[c] Spike: determination of gastrodin content by injecting each spiked solution.

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Table 3 – Results of robustness study of high-performance liquid chromatography assay for gastrodin from water extracts of *Gastrodia elata* Blume.

	Wavelength (nm)			Flow rate (mL/min)		
	219	220	221	0.97	1.00	1.03
N	2218	2270	2322	2241	2270	2289
T	1.14	1.13	1.13	1.10	1.13	1.18
R	1.40	1.44	1.39	1.39	1.44	1.40
α	1.21	1.22	1.23	1.22	1.22	1.21

α = selectivity factor; N = number of theoretical plate; R = resolution; T = tailing factor.

centrifugation and filtration through 0.45 μm nylon filters. Therefore, the powder was deemed unsuitable for GAS preparation. The HPLC chromatogram indicated that the quantities of GAS and HBA were not significantly different between slices and powder (Fig. 4). Thus, the slice from *G. elata* Bl. could be preferable over the powder for use in subsequent studies. As shown in Fig. 5, the extraction solvent affected the quantity of GAS. The amount of GAS in WGE was significantly higher than that in EGE. Since GAS is a highly water-soluble compound, its extraction efficiency was higher in water than in methanol as the extraction solvent [38,39]. Therefore, this method of water extraction can be applied to further analyses of *G. elata* Bl. According to the Chinese medicinal dictionary report, the quality of *G. elata* Bl. harvested during winter is superior to that harvested during summer [40,41]. The *G. elata* Bl. samples used in the present study were harvested during winter. To verify the quantities of GAS from different sources of *G. elata* Bl., all samples were initially steamed for 30 minutes. Once cooled, the samples were sliced, frozen, and lyophilized. The chromatography results demonstrated that the yield of GAS from GRY and GRH did not differ significantly (Fig. 6). However, we found that the amount of GAS from GBY was five times higher than the amount from GRY and GRH.

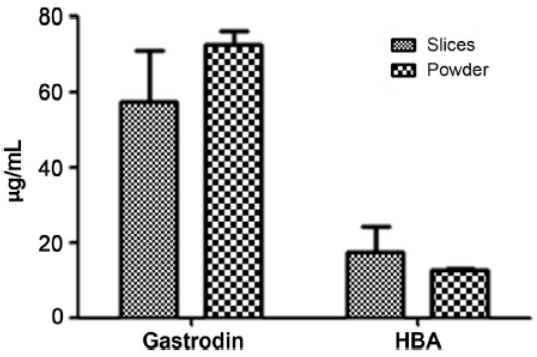

Fig. 4 – The analysis of gastrodin and 4-hydroxybenzyl alcohol (HBA) quantities from slices and powder of Gastrodia elata Blume under water extraction by high performance liquid chromatography. Each value represents mean ± standard deviation ($n = 3$). Compared with water and ethanolic extraction, the data were not significantly different ($p > 0.05$) according to ANOVA.

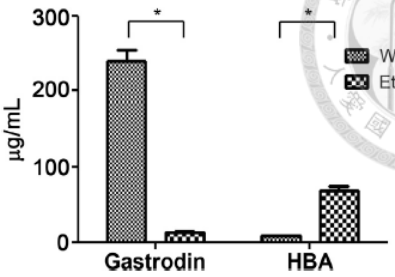

Fig. 5 – The analysis of gastrodin and 4-hydroxybenzyl alcohol (HBA) quantities under water and ethanolic extraction by high-performance liquid chromatography. Each value represents mean ± standard deviation ($n = 3$). * Indicates $p < 0.05$ (Student t test) compared with water and ethanolic extraction.

These results indicate that GBY provides a better source for GAS than GRY and GRH under water extraction.

4. Conclusion

To summarize, a specific HPLC-UV method to measure the amount of GAS in water and ethanolic extracts from different sources of *G. elata* Bl. was developed and validated. By

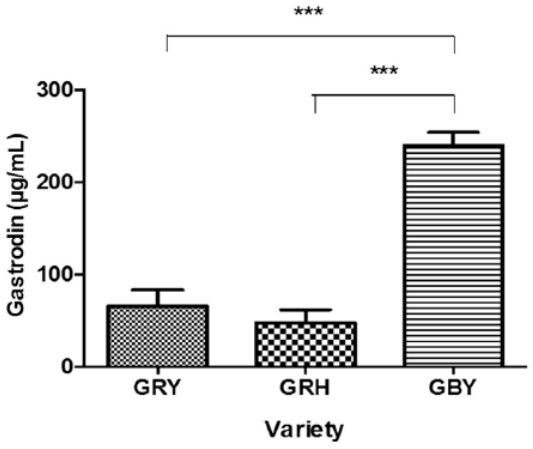

Fig. 6 – The analysis of gastrodin quantity from different variety sources under water extraction by high-performance liquid chromatography. Each value represents mean ± standard deviation ($n = 3$). The overall p value shown was obtained by analysis of variance Tukey's test for multiple comparisons and indicated a significant difference (*$p < 0.001$) between *Gastrodia elata* Blume f. *elata* from Yun-nan (GRY) and *G. elata* Bl. f. *glauca* S. Chow from Yun-nan (GBY), and also between *G. elata* Bl. f. *elata*. from Hu-nan (GRH) and GBY.**

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optimizing the chromatographic conditions, we discovered that water extraction was more efficient than ethanolic extraction. Our results indicate that GAS levels vary significantly between the different varieties of *G. elata* Bl., although not between the different origins. Therefore, this validated HPLC method can be effectively used for the quality control of WGE in the development of health products in the future. Further studies on the determinations of GAS contents from all varieties and sources of *G. elata* Bl. will be worthwhile in establishing a quality control database.

Conflicts of interest

All authors declare no conflicts of interest.

Acknowledgments

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Anti-depressant effects of *Gastrodia elata* Blume and its compounds gastrodin and 4-hydroxybenzyl alcohol, via the monoaminergic system and neuronal cytoskeletal remodeling

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ABSTRACT

Ethnopharmacology relevance: *Gastrodia elata* Blume is a highly valuable traditional Chinese medicine used in the treatment of depression. However, compounds with antidepressant effects in water extracts of *G. elata* Bl. (WGE) have not been identified. The aims of this study were to determine the major antidepressant compound in WGE and to evaluate the antidepressant effects of WGE and its active compounds which involved the monoaminergic system and neuronal cytoskeletal remodeling.

Materials and methods: Gastrodin (GAS) and 4-hydroxybenzyl alcohol (HBA) in WGE, were analyzed with high-performance liquid chromatography (HPLC)-ultraviolet detection.

The forced swimming test (FST) was used to induce depression-like symptoms in 9 weeks old male Sprague-Dawley rats. The open field test (OFT) was used to measure anxiety after WGE, GAS, and HBA treatments. The levels of monoamine such as serotonin (5-HT), dopamine (DA), and their metabolites 5-hydroxyindoleacetic acid (5-HIAA), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) were measured using HPLC-electrochemical detection. Western blotting was used to examine the 5-HT1A receptor and the neuronal cytoskeleton remodeling-related proteins, Slit, dihydropyrimidinase-related protein 2 (DPYSL2, also called CRMP2), Ras homologous member A (RhoA), and profilin 1 (PFN1) in vivo. Slit1 expression was evaluated in Hs683 cell line after treated with WGE (0.5 mg/mL), GAS (50, 100 and 100 μM), and HBA (50, 100 and 100 μM).

Results: Oral administration of WGE (500 mg/kg bw), GAS (100 mg/kg bw), and HBA (100 mg/kg bw) exhibited the anti-depressant effect by significantly reducing the immobility time in FST, monoamine metabolism including the 5-HT to 5-HIAA in the hippocampus and DA to DOPAC and HVA ratios in the frontal cortex, amygdala, and hippocampus. In the hippocampus, the expression of the neuronal cytoskeleton remodeling-related negative regulators Slit1 and RhoA were significantly down-regulated. In addition, the positive regulators CRMP2 and PFN1 were significantly up-regulated following GAS, HBA, and WGE treatments. Moreover, WGE, GAS, and HBA were directly down-regulated Slit1 expression in Hs683 cells.

Conclusion: WGE, GAS, and HBA exhibited potential anti-depressant effects in rats by decreasing monoamine metabolism and modulated cytoskeleton remodeling-related protein expression in the Slit-Robo pathway. These results suggest that WGE can be used as agent for depressive prevention.

1. Introduction

Major depression disorder (MDD) is a human psychiatric disease (Goldberg and Huxley, 1992) that affects more than 350 million people worldwide and results in a significant burden and disruption in the daily lives of patients (World Health Organization, 2012). MDD strongly correlates with suicide attempts (Chen and Dilsaver, 1996; Placidi et al., 2001), and the World Health Organization predicts that MDD will result in the highest global

Abbreviations: WGE, water extracts of Gastrodia elata Bl.; GAS, gastrodin; HBA, 4-hydroxybenzyl alcohol; HB, 4-hydroxybenzyl aldehyde; VAN, vanillin; OFT, open field test; FST, forced swimming test; MDD, major depression disorder; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; CRMP2, dihydropyrimidinase-related protein 2; RhoA, Ras homologous member A; PFN1, profilin 1; HPLC, high performance liquid chromatography; CTL, control; BDNF, brain-derived neurotrophic factor; MCAO, middle cerebral artery occlusion

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streptomycin) and grown at 37 °C under a humidified 5% CO_2 atmosphere. Adherent cells were suspended by 1 mL of trypsin–EDTA for 5 min at 37 °C.

2.3.2. Western blotting analysis

Hs683 cells were seeded at a density of 10^5 cells/mL in a 10 cm^2 dish and were incubated for 4 h. Then, medium were removed and co-incubation with WGE (0.5 mg/mL), GAS (50, 100, and 200 μM) and HBA (50, 100, and 200 μM) for another 24 h. The cells were harvested and lysed with lysis buffer containing 1 mM phenylmethanesulfonyl fluoride, 1 mM ethylenediaminetetraacetic acid, 1 μM pepstatin A, 1 μM leupeptin, 0.1 μM aprotinin, and a combination of proteinase and phosphatase inhibitors tablets (Roche, Diagnostics International AG, Rotkreuz, Switzerland). The cell lysates were stored at −20 °C for 1 h and centrifuged 12,500 rpm at 4 °C for 5 min. The supernatant was obtained, and protein was quantified with the Bradford protein assay method (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The supernatant was loaded onto gels for sodium dodecyl sulfate-polyacrylamide gel electrophoresis separation, and then electrotransferred onto polyvinylidene difluoride membranes (EMD Millipore Corporation, Billerica, MA, USA). After gel electrophoresis, Slit1 and 5-HT1A receptor were identified according to the method of Lin et al. (2014).

2.4. In vivo study

2.4.1. Animals

Male Sprague Dawley rats (4 weeks old) were purchased from BioLASCO Taiwan Co., Ltd. (Taipei, Taiwan). All animals were housed individually in a climate-controlled room, and the temperature and humidity were maintained at 23 ± 2 °C and 60 ± 10%, respectively. The animals were exposed to a 12–12 h light-dark cycle from 10 p.m. to 10 a.m. Free access to water and food was provided daily. Each animal and its water and food were weighed every morning and recorded before oral gavage. The rats were randomly separated into the following four groups (10 rats/group): control (CTL), WGE, GAS, and HBA. After 2 weeks of habituation, oral administration of the treatments was initiated and continued for 21 days with a 1% carboxymethyl cellulose aqueous solution, or 1% carboxymethyl cellulose aqueous solution containing WGE (500 mg/kg bw), GAS (100 mg/kg bw), or HBA (100 mg/kg bw). Animal behavior was examined as described below and in Fig. 1. The animal protocol was reviewed and approved by the Institutional Animal Care and Use.

Committee of National Taiwan University, Taiwan (Approval Number: NTU-101-EL-123). The protocol complied with the guidelines described in the "Animal Protection Law," amended on June 29, 2011, Hua-Zong-(1)-Yi-Tzi-10,000,136,211, Council of Agriculture, Executive Yuan, Taiwan.

2.4.2. Open field test (OFT)

The OFT was conducted in order to measure the spontaneous locomotor activity in rats (McCarthy et al., 1995). Briefly, each rat was placed individually in the center of a black plexiglass apparatus (57 cm wide × 76 cm long × 35 cm high), as a bright light was turned on over the field. The rats adapted to the apparatus in the 1st OFT. The formal test (2nd OFT) was performed on the 19th day (Fig. 1). The rats explored freely for 5 min. All test sessions were recorded with a DVD recorder. The open-field apparatus was divided into central and peripheral areas. The bouts (the number of segments crossed with all four paws), distance (cm), and velocity (cm/min) in the central and peripheral areas were further analyzed with TopScanLite™ v2.0 software (CleverSys, Inc., Reston, VA, USA).

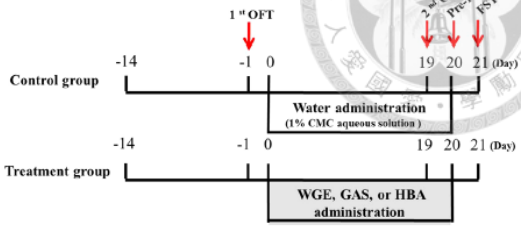

Fig. 1. Schematic diagrams represent design for studying anti-depressive-like activity of WGE by using FST animal model. OFT: open field test; FST: forced swimming test; CMC: carboxymethyl cellulose; WGE: water extract of *Gastrodia elata* Bl.; GAS: gastrodin; HBA: 4-hydroxybenzyl alcohol.

2.4.3. Forced swimming test (FST)

The FST was conducted according to the previously standardized and validated animal model protocol (Porsolt et al., 1979). As shown in Fig. 1, the FST is a 2-day procedure in which rats swim under specific conditions. On the first day (the 20th day), one rat was placed into a cylinder (35 cm tall with a 30 cm diameter that was filled with 20 ± 1 cm of water at 25 ± 1 °C) for 15 min, subsequently removed from the water and dried with towels. All rats repeated the test session for 5 min within 24 h (the 21st day) following the first session. The behavior of the rats during the FST was video recorded and the films were analyzed with ForcedSwimScan™ software (CleverSys, Inc.) in order to calculate the duration of immobility.

2.4.4. Brain sample preparation

Each rat swam for 5 min following the second session of FST and was wiped dry as soon as possible. Then, the rat was placed into an apparatus (20 cm wide × 32 cm long × 16 cm high, made of transparent plexiglass) and was deeply anesthetized with low flow rate of CO_2 (20 ± 5% volume/min). We pinched the foot of the rat to confirm the effect of anesthesia and sacrificed by decapitation later. The brains were removed, and the frontal cortex, hippocampus, striatum, and amygdala were immediately dissected according to the procedure outlined by Glowinsk and Iversen (1966). Four sections of the brain tissue were stored at −80 °C until further analysis. The frozen tissues were divided into two parts. One tissue sample was used for the monoamine metabolism analysis, and the other for western blotting.

2.4.5. Monoamine concentration analysis with the HPLC-electrochemical detector

We performed the HPLC analysis according to the method described by Hao et al. (2013). The brain tissues were homogenized in 800 μL of 0.1 N HCl ice-chilled containing 10^{-7} M ascorbic acid, 1.5 mg/100 mL pargyline, and 50 pg/μL isoproterenol for 30 s. The samples were centrifuged (10,000 rpm for 30 min), and the supernatants were filtered (0.45 μm) and subjected to HPLC in order to analyze monoamine metabolism. Each working standard solution (DA, DOPAC, HVA, 5-HT, and 5-HIAA) was prepared with the same method as that used for the homogenized solution.

HPLC was coupled with an electrochemical detector [Antec, (USA) LLC, Boston, MA, USA; 10 nA range, 1 Hz filter, and 0.511 V AppE cell] and a L−2200 autosamper (Hitachi, Ltd., Tokyo, Japan). A SPOLAR C18 column 4.6 × 250 mm i.d., 5 μm particle size; Shiseido Co., Ltd., Tokyo, Japan) and Alltima C18 guard column (7.5 × 4.6 mm i.d., 5 μm particle size; W. R. Grace & Co., Columbia, MA, USA) containing 0.17 m NaH_2PO_4, 0.63 mM ethylenediaminetetraacetic acid, 0.6 mM octane-1-sulfonic acid sodium salt,

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2 mM KCl, and 20% methanol were adjusted to a pH of 3.11 with H_3PO_4. The flow rate was 0.5 mL/min, the oven was set to 35 °C, and the injection volume was set to 20 μL. The working standard solution and samples were injected into the HPLC system, and the peaks were analyzed with PeakABC (Great Tide Instrument Co., Ltd., Taipei, Taiwan).

2.4.6. Western blotting

The frozen brain tissues were ground to powder and dissolved in lysis buffer containing 7 m urea, 2 m thiourea, 65 mM dithiothreito, 4% CHAPS, and a combination of proteinase and phosphatase inhibitors tablets (Roche, Diagnostics International AG, Rotkreuz, Switzerland). The samples were centrifuged at 18,000 rpm for 2 h at 4 °C (Lai et al., 2014). The protein quantities and gel electrophoresis were following the method as described in Section 2.3.2. After gel electrophoresis, Slit1, Slit2, RhoA, CRMP2, PFN1, and 5-HT1A were identified according to the method of Lin et al. (2014).

2.5. Statistical analyzes

The following statistical analyzes were used. In order to analyze food consumption, water consumption, bodyweight, OFT results, and immobility duration in the FSTs, one-way analyzes of variance and Duncan's multiple range tests were used to compare multiple groups. The variances of immobility duration differed significantly among the compared groups. *P* values less than 0.05 were considered significant in these analyzes ([a] and [b] denote significant difference if the same letter is not shared). In order to analyze the levels of monoaminergic neurotransmitters and western blotting results, one-way analyzes of variance and Dunnett's multiple comparison tests were used to compare the CTL group and other groups. *P* values less than 0.05 were considered significant in these analyzes (* denotes $p < 0.05$, ** denotes $p < 0.01$, and *** denotes $p < 0.001$). In all of the analyzes, the relevant symbols or horizontal lines represent the mean, and the error bars represent the standard deviation.

3. Results

3.1. Quantitative analysis of GAS and HBA in WGE

The HPLC chromatograms of four reference standards (GAS, HBA, HB, VAN) and WGE are shown in Fig. 2. The contents of GAS and HBA, the major compounds in WGE, were determined to be 30.30 mg/g and 1.43 mg/g respectively (Fig. 2B).

3.2. Effects of WGE, GAS, and HBA on body weight and daily intake of water and food

Water intake (Fig. 3A), food intake (Fig. 3B), and body weight (Fig. 3C) did not differ ($p > 0.05$) among the CTL, WGE, GAS, and HBA groups after 21 days of oral administration. These results

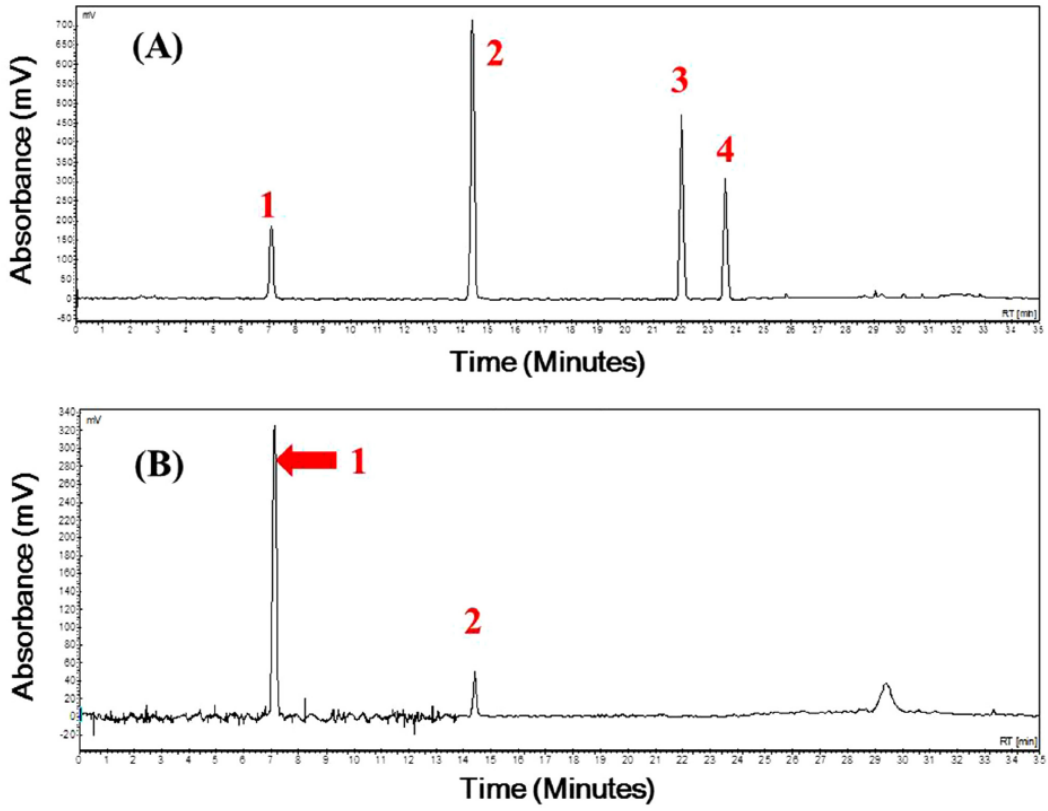

Fig. 2. HPLC chromatogram (A) of GAS (1), HBA (2), HB (3), and VAN (4) (50 mg/L) standard; HPLC chromatogram (B) of WGE (2500 mg/L) GAS: gastrodin, HBA: 4-hydroxybenzyl alcohol, HB: 4-hydroxybenzyl aldehyde, VAN: 4-hydroxy-3-methoxybenzaldehyde, WGE: Water extract of *Gastrodia elata* Bl.

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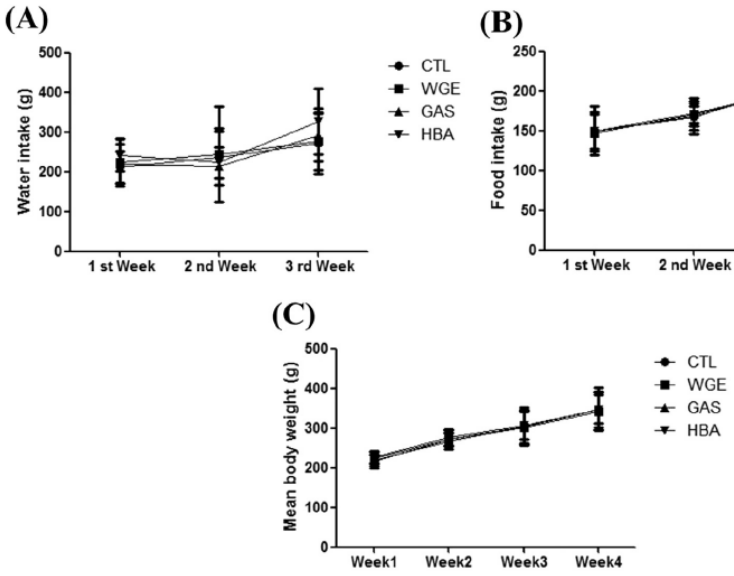

Fig. 3. Effects of WGE, GAS, and HBA on water intake (A), food intake (B), and body weight (C) in SD rats. Each value represents means ± SD (n=8–10). WGE, GAS, and HBA were administered once daily for 21 days by oral gavage. WGE (500 mg/kg bw), GAS (100 mg/kg bw), and HBA (100 mg/kg bw). Data were not significantly different ($p > 0.05$) from each other according to ANOVA. CTL: control; WGE: water extract of *Gastrodia elata* Bl.; GAS: gastrodin; HBA: 4-hydroxybenzyl alcohol.

indicated that WGE, GAS, and HBA did not affect the growth and nutrition intake of the animals (Fig. 3).

3.3. Animal behavior

3.3.1. Locomotor activity in the OFT

OFT is commonly used to assess the sedative, toxic, or stimulant effects of compounds by measuring the exploratory behavior and general activity in rodents (Gould, 2009). The anxiety behavior of rats was evaluated during 5 min of the OFT. Table 1 shows the data for the bouts (count), distance (cm), and velocity (cm/min), which were used to evaluate anxiety. These factors did not differ ($p > 0.05$) among the CTL, WGE, GAS, and HBA groups. It indicated that GAS and HBA have no effects on the spontaneous locomotor activity and animal behavior on rats.

3.3.2. Immobility time in FST

The FST is used to induce depression-like symptoms in animal models. Analysis of the immobility time during the FST can be used to evaluate the level of depression-like behavior in rats. Fig. 4 shows that the immobility times in the FST in the CTL, WGE, GAS, and HBA groups were 129.6, 71.9, 83.4, and 68.8 s, respectively, and these values differed significantly ($p < 0.05$) among different treated-groups. The oral administration of WGE, GAS, and HBA for 21 days notably decreased the immobility time.

3.4. Regional brain monoamine turnover

Fig. 5 shows the 5-HIAA/5-HT turnover rates in the rat brains, and Fig. 6 presents the turnover of DA in the rat brains. Notably, the 5-HIAA/5-HT turnover rates were significantly decreased ($p < 0.001$) in the hippocampus in the treatment groups compared with the CTL group. The metabolism speed of DA was decreased in the frontal cortex ($p < 0.001$ for WGE and GAS, and $p < 0.05$ for HBA), hippocampus ($p < 0.001$ for WGE, $p < 0.05$ for GAS, and $p < 0.001$ for HBA), and amygdala ($p < 0.001$) following the oral

Table 1
The effects of WGE, GAS, and HBA on the bouts, distance, and velocity of rats in locomotor test in OFT.

Group	Dose (mg/kg bw)	Bouts (count)	Distance (mm)	Velocity (mm/s)
CTL		32 ± 11	17,758 ± 4583	358 ± 76
WGE	500	33 ± 10	15,782 ± 6354	324 ± 127
GAS	100	33 ± 11	16,597 ± 4861	349 ± 90
HBA	100	32 ± 10	15,841 ± 4667	331 ± 120

Data are expressed as the means ± SD (n=8–10). Rats were gavaged with the following samples: WGE (500 mg/kg bw), GAS (100 mg/kg bw), and HBA (100 mg/kg bw). Data were not significantly different ($p > 0.05$) from each other according to ANOVA. CTL: control; WGE: Water extract of *Gastrodia elata* Bl.; GAS: gastrodin; HBA: 4-hydroxybenzyl alcohol.

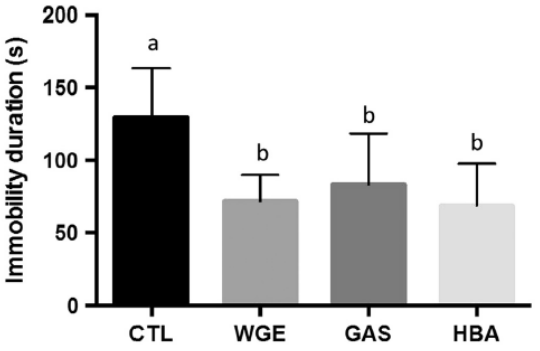

Fig. 4. The effects of WGE, GAS, and HBA on the immobility time of rats in FST. Data are expressed as means ± SD (n=8–10). All samples were administered once daily for 21 days by oral gavage. WGE (500 mg/kg bw), GAS (100 mg/kg bw), HBA (100 mg/kg bw).ab data not sharing the same letter are significantly different from each other ($p < 0.05$) by ANOVA and Duncan's multiple range test. CTL: control; WGE: water extract of *Gastrodia elata* Bl.; GAS: gastrodin; HBA: 4-hydroxybenzyl alcohol.

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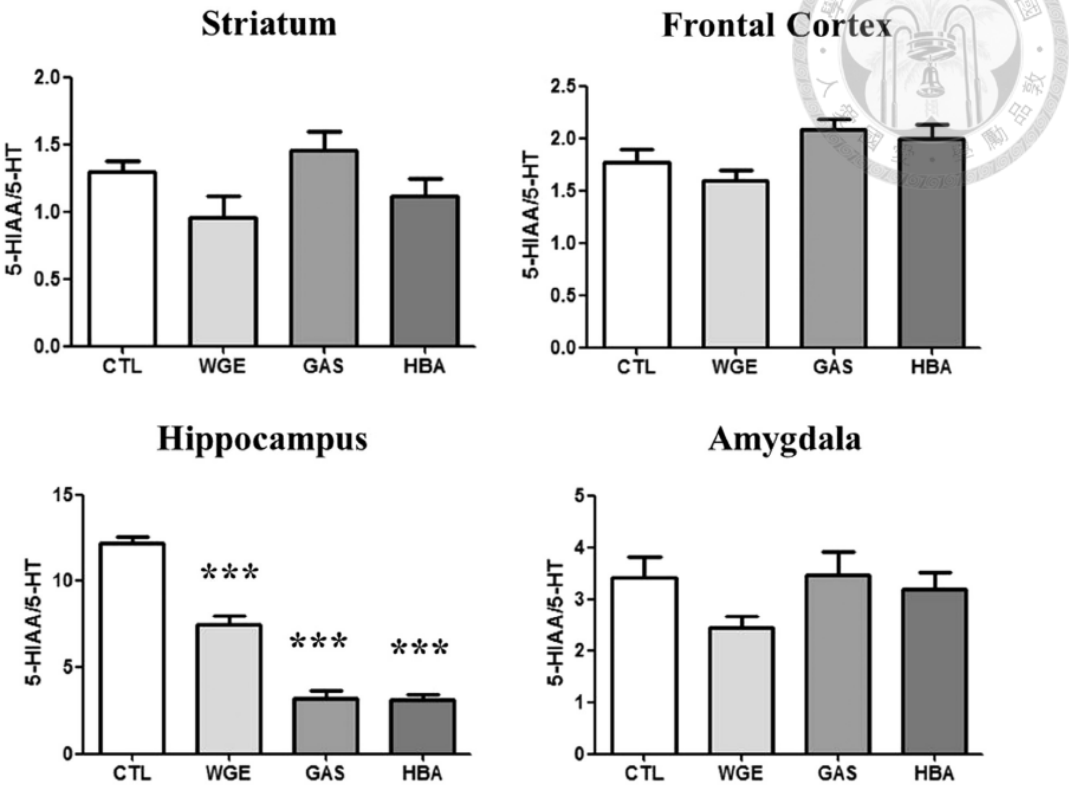

Fig. 5. The effects of WGE, GAS, and HBA on the serotonin turnover (5-HIAA/5-HT). Data are expressed as means ± SD (n=8–10). All samples were administered once daily for 21 days by oral gavage. WGE (500 mg/kg bw), GAS (100 mg/kg bw), HBA (100 mg/kg bw). ***$p < 0.001$ (according to the one-way ANOVA coupled with Dunnett's multiple comparison test) compared with CTL group. CTL: control; WGE: water extract of *Gastrodia elata* Bl.; GAS: gastrodin; HBA: 4-hydroxybenzyl alcohol.

administration of WGE, GAS, and HBA. These results indicated that WGE and its active compounds maintained monoamine levels in the rat brains following the FST.

3.5. Altered 5-HT1A receptor expression in the brain of rats

We further investigate whether 5-HT1A receptor would be modulated after all treatments. As shown in Fig. 7, the expression of 5-HT1A receptor was elevated significantly in the GAS ($p < 0.01$) and HBA ($p < 0.001$) groups compared with the CTL group in the hippocampus, but not in the frontal cortex.

3.6. Regulation of the neuronal cytoskeletal remodeling proteins related to the Slit-Robo pathway

A link between the expression of the regulators in the Slit-Robo pathway and depression are shown in the western blotting results in Fig. 8. The negative regulators (Slit1, Slit2, and RhoA) and positive regulators (CRMP2 and PFN1) were analyzed in the frontal cortex and hippocampus. Fig. 8A shows that all treatments do not affect the regulators of Slit-Robo pathway in the frontal cortex. In contrast, incremental changes in the expression of CRMP2 and PFN1 were observed in the hippocampus (Fig. 8B). Rats gavaged with WGE, GAS, and HBA exhibited a significant up-regulation in CRMP2 ($p < 0.05$) and PFN1 ($p < 0.05$, $p < 0.01$, and $p < 0.05$, respectively) expression and a significant down-regulation in Slit1 ($p < 0.01$, $p < 0.001$, and $p < 0.001$, respectively) and RhoA ($p < 0.01$, $p < 0.001$, $p < 0.001$ respectively) expression in the hippocampus.

3.7. Effects of WGE, GAS, and HBA on Slit1 expression in Hs683

SLIT family of genes have been implicated in specific cancers and have been suggested to have a restricted pattern of expression in normal cells. Rossi et al. (2005) investigated the extent and distribution of the expression of these genes in cancer cells. Hs683 is one of tumor cell types confirmed that possess Slit1 expression. Therefore, Hs683 was evaluated that Slit1 expression after all treatments. Fig. 9 shows that Slit1 was significantly down-regulated after co-cultured with WEG ($p < 0.01$), GAS (50, 100, and 200 μM; $p < 0.01$), and HBA (100 and 200 μM; $p < 0.01$).

4. Discussion

This study demonstrated that WGE and its active compounds, GAS and HBA, caused anti-depressant effects. WGE and its compounds decreased the immobility time in the FST and did not induce anxiety in the OFT (Table 1). In the monoamine analysis, WGE, GAS, and HBA decreased the turnover rate of monoamine metabolism and influenced the dopaminergic system in the hippocampus. In the western blotting results, the three treatments changed the expression of neurocytoskeletal remodeling proteins in the Slit-Robo pathway to increase neuroplasticity. Taken together, WGE, GAS, and HBA are potential antidepressant treatment candidates.

WGE ameliorated depression-like behavior in rats in the FST by decreasing the monoamine metabolism (Chen et al., 2008, 2009)

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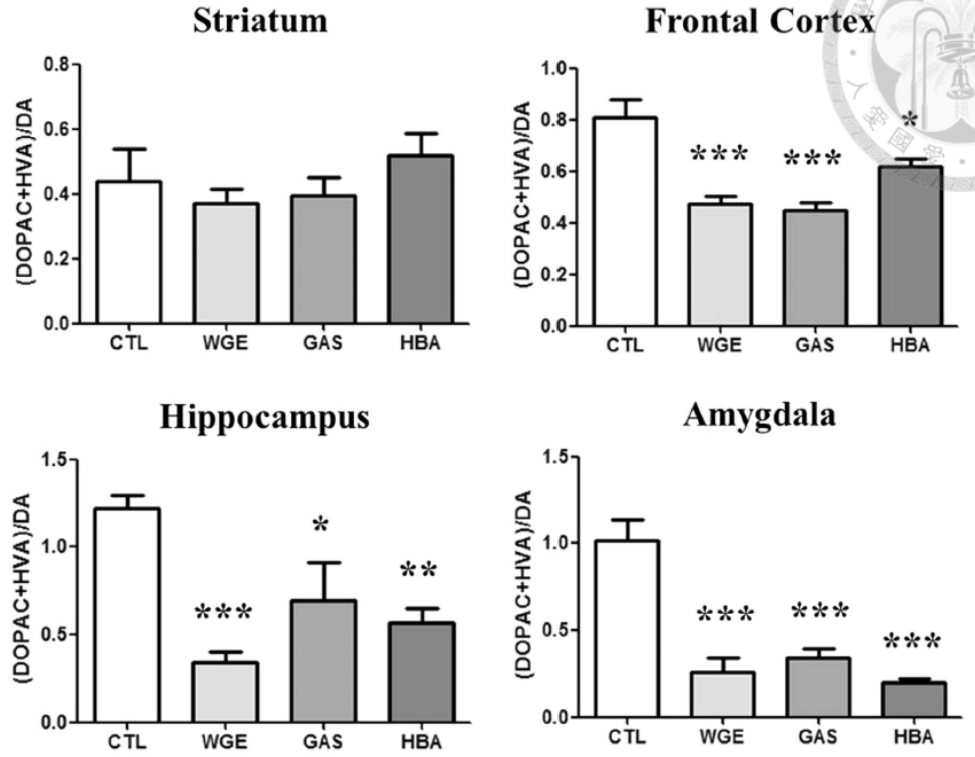

Fig. 6. The effects of WGE, GAS, and HBA on the dopamine turnover ((DOPAC+HVA)/DA). Data are expressed as means ± SD (n=8–10). All samples were administered once daily for 21 days by oral gavage. WGE (0.5 g/kg bw), GAS (100 mg/kg bw), HBA (100 mg/kg bw). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ (according to the one-way ANOVA coupled with Dunnett's multiple comparison test) compared with CTL group. CTL: control; WGE: water extract of *Gastrodia elata* Bl.; GAS: gastrodin; HBA: 4-hydroxybenzyl alcohol.

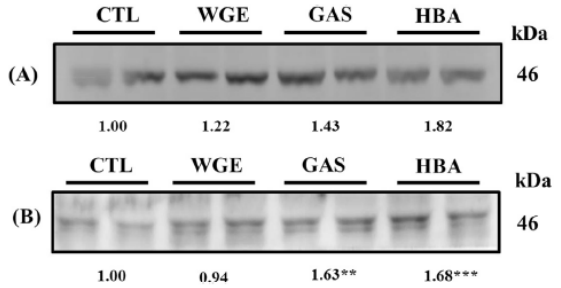

Fig. 7. Western blotting analysis of 5-HT1A receptor in frontal cortex (A) and hippocampus (B). (n=8–10). The value on the bottom of each group and the chart images represent the ratio of expressed levels, which were normalized with the expression of GAPDH and relative to the C group. $^{**}p<0.01$, $^{***}p<0.001$ (according to the one-way ANOVA coupled with Dunnett's multiple comparison test) compared with C group. CTL: control; WGE: water extract of *Gastrodia elata* Bl.; GAS: gastrodin; HBA: 4-hydroxybenzyl alcohol.

and down regulating the Slit-Robo pathway in order to increase neuroplasticity (Lin et al., 2014). Although few studies have investigated the active compounds that ameliorate depression, this study investigated the contribution of the active anti-depressant compounds in WGE. GAS and HBA separation was conducted with gradient elution in reversed-phase HPLC. Compared with the results obtained from HPLC under isocratic conditions in Lin et al. (2014), our HPLC gradient analysis method was more efficient and had a higher resolution. From our early study, GAS and HBA were existed in WGE, which be extracted by ourselves (Chen et al., 2015). According to Hsieh et al. (1997), GAS and HBA are the primary bio-active compounds in *G.elata* Bl. Both GAS and HBA facilitate memory consolidation and retrieval during passive avoidance tasks in rats (Hsieh et al., 1997). Therefore, we hypothesized that GAS and HBA are priority candidates as antidepressant agents that decrease monoamine metabolism and down-regulate the Slit-Robo pathway. In our pilot studies, we calculated the quantities of GAS and HBA, which would be administered to rats. Unfortunately, the gavaged dosage of GAS and HBA could not decrease immobility time in the FST (data not shown). To the best of our knowledge, the range of oral dosage of GAS and HBA is from 60 to 400 mg/kg bw (An et al., 2003; Cai et al., 2008; Zhang et al., 2014; Xie et al., 2007) and 25–100 mg/kg bw (Yu et al., 2010; Lim et al., 2007), respectively, in animal experiments. GAS (100 and 200 mg/kg bw) improved neurogenesis in the hippocampus of SD rats through up-regulation of BDNF expression under chronic unpredictable stress (CUS) (Zhang et al., 2014). We hypothesized that GAS would promote neuroplasticity with a reasonable dosage of 100 mg/kg bw. On the other hand, no research study has indicated the anti-depressant effects of HBA. The dosage of HBA used was 100 mg/kg bw (Lim et al., 2007).

There are three kinds of neurotransmitters, classified as amines, neuropeptides, and gases. Amines are further divided into three types, quaternary amines, monoamines, and amino acids. Since monoamines have a significant impact on the regulation of mood and emotion (Van Praag, 1982; Hughes et al., 2004), most research studies on about the monoamine hypothesis of depression have focused on the metabolism of serotonergic and dopaminergic

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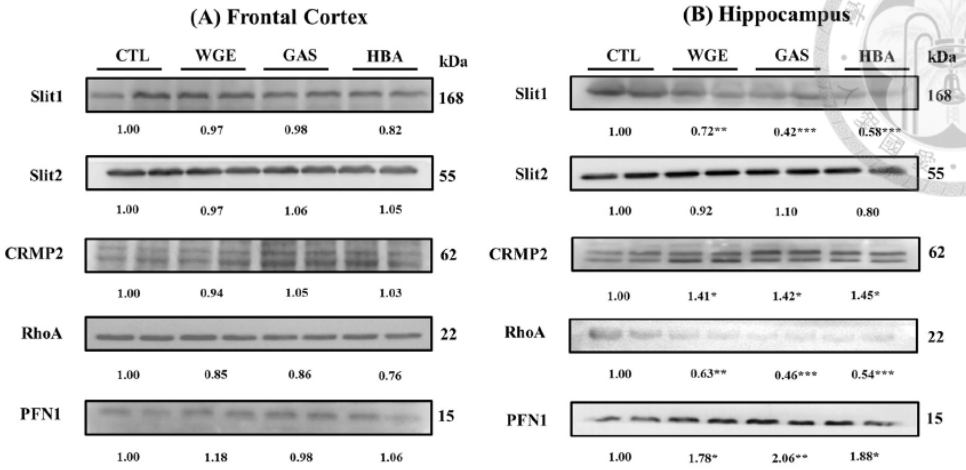

Fig. 8. Western blotting analysis of the regulators in the Slit-Robo pathway in frontal cortex (A) and hippocampus (B). (n=8–10). The value on the bottom of each group and the chart images represent the ratio of expressed levels, which were normalized with the expression of GAPDH and relative to the C group. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ (according to the one-way ANOVA coupled with Dunnett's multiple comparison test) compared with C group. CTL: control; WGE: water extract of *Gastrodia elata* Bl.; GAS: gastrodin; HBA: 4-hydroxybenzyl alcohol.

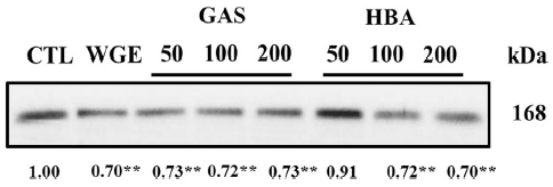

Fig. 9. Effects of WGE, GAS, and HBA on the expression of Slit1 in Hs683 for 24 h. (n=3). The value on the bottom of each group and the chart images represent the ratio of expressed levels, which were normalized with the expression of GAPDH and relative to the C group. $^{**}p<0.01$ (according to the one-way ANOVA coupled with Dunnett's multiple comparison test) compared with C group. CTL: control; WGE: water extract of *Gastrodia elata* Bl.; GAS: gastrodin (50, 100, and 200 μM); HBA: 4-hydroxybenzyl alcohol (50, 100, and 200 μM).

systems. According to our FST results, oral supplementation of WGE, GAS, and HBA for 21 days significantly reduced the immobility time (Fig. 4). As shown in Figs. 5 and 6, we found that WGE and its active compounds influence the DA turnover rate more significantly than the 5-HT turnover rate, as reported in previous studies (Chen et al., 2008). Fig. 5 shows that WGE and its active compounds, GAS and HBA, decreased the turnover rate of 5-HIAA/5-HT in the hippocampus. In addition, the turnover of DA in the frontal cortex, hippocampus, and amygdala were decreased after treatment with WGE, GAS, and HBA (Fig. 6). Monoamine metabolism is influenced by regulation of monoamine receptors (Hsieh et al., 1998; Guo et al., 2013; Wang et al., 2014), monoamine-synthesizing enzymes activity (Davis and Carlsson, 1973; Shin et al., 2011), and monoamine oxidase activity (Riederer and Youdim, 1986). 5-HT1A receptor, one kind of 5-HT receptors could promote 5-HT release. Accordingly, our result indicated that 5-HT1A receptor expressions were significantly increased in the hippocampus after oral supplementation of GAS and HBA (Fig. 7B). It may be a pathway for maintain the concentration of 5-HT in the brain by promoting 5-HT release. The phenomenon also found in some studies that GAS and HBA may increase the sensitivity of 5-HT1A receptors, thereby promoting 5-HT release (Hsieh et al., 1998; Guo et al., 2013). Tyrosine hydroxylase is a key enzyme responsible for DA synthesis. Following our other reach results (data not shown), WGE and GAS up-regulated tyrosine hydroxylase expression in rat pheochromocytoma cell line. Elevation of synthesis was the possible mechanisms to explain the lower DA turn over in treatment groups. Moreover, WGE and GAS directly inhibited the activity of monoamine oxidase A to degrading monoamine neurotransmitters in rat pheochromocytoma cell line (data not shown). Based on previous reports and our present results, we speculate that monoamine receptors, monoamine-synthesizing enzymes, and monoamine oxidases might play a role in the activity of WGE, GAS, and HBA.

Neurotrophic and cellular plasticity in the brain have a high correlation with bipolar disorder (Soeiro-de-Souza et al., 2012). GAS was demonstrated to reverse depression-like behaviors in rats exposed to chronic mild stress and restored the expression of neurotrophic factors, glial fibrillary acidic protein and brain-derived neurotrophic factor (BDNF) in the hippocampus (Zhang et al., 2014). HBA is well known to exert neuroprotective effects against transient focal cerebral ischemia in rats by up-regulation of neurotrophic factors, such as BDNF, glial cell line-derived neurotrophic factor, and myelin basic protein in a middle cerebral artery occlusion (MCAO) animal model (Yu et al., 2010; Kam et al., 2011). Based on the concept of cellular plasticity, which connect our previous proteomic results, GAS and HBA may have contributed to anti-depressant effects through the regulation of cytoskeleton rearrangement through the Slit-Robo pathway.

The Slit-Robo pathway is well known to be important in neuronal growth, and alters the cytoskeleton of neurons in order to modify the axon growth cone or dendritic dynamics (Whitford et al., 2002). Some studies have indicated that suppression of neuroplasticity is highly correlated with the Slit-Robo pathway, and the down-regulation of the Slit protein promotes neuroplasticity (Lin and Isacson, 2006; Borrell et al., 2012). The Slit proteins are secreted by glial cells along the midline of the central nervous system, and bind to Robo receptors expressed on crossing axons (Lin and Isacson, 2006; Dickinson and Duncan, 2010). From our early bioinformatics result, WGE affected the neurocytoskeletal rearrangement, which was associated with neuroplasticity. The identified proteins from the two-dimensional gel electrophoresis were associated with the actin filament, microtubule, and neurofilament. Some actin related proteins of the Slit-Robo pathway such as Slit1, CRMP2, RhoA, and PFN1 were significantly impacted following WGE treatment (Lin et al., 2014).

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Our western blotting results showed effects of not only WGE but also for GAS and HBA. The neuronal cytoskeleton remodeling-related factors, Slit1 and RhoA, were decreased in the hippocampus (Fig. 8B), but not in the frontal cortex (Fig. 8A). Kaneko et al. (2010) found that Slit1 was secreted from new neurons, which migrated in adult mammals. The evidence supported that only Slit1 and not Slit2 would be influenced. Therefore, we conducted an experiment using Hs683 cells, oligodendroglioma cell line, as an in vitro model to investigate whether WGE, GAS, and HBA could inhibit Slit1 secret. Since 0.5 mg/mL WGE contented 3% GAS, the dosage of GAS in cell culture was applied with 50, 100, and 200 μM. HBA was also evaluated with the same dosage range. As shown in Fig. 9, Slit1 was significantly down-regulated under WGE, GAS (50 μM, 100 μM, and 200 μM), and HBA (100 μM and 200 μM) treatments. According to our findings, WGE, GAS, and HBA inhibited the expression of Slit1 directly. RhoA is also involved in the Slit-Robo pathway. The down-regulation of RhoA promotes neuroplasticity (Sin et al., 2002; Lin et al., 2014). Moreover, the incremental expression of the positive factor, CRMP2, was found in the hippocampus in all the treatment groups (Fig. 8B). CRMP2 is enriched in the growing axons of hippocampal neurons (Inagaki et al., 2001; Fukata et al., 2002; Yoshimura et al., 2006). Yang et al. have indicated that CRMP2 is down-regulated in a depression-induced animal model (Yang et al., 2013). Similar to the CRMP2 results, the expression of PFN1 was increased in the hippocampus after the administration of WGE, GAS, and HBA (Fig. 8B) but not in the frontal cortex (Fig. 8A). PFN1, which is a member of the PFN family member, has been described as a regulator of multiple processes, including various cellular processes (cytoskeletal dynamics, membrane trafficking, and nuclear transport), neuronal differentiation and synaptic plasticity, and has properties proposed for a synaptic tag (Birbach, 2008). In a pathologic report, PFN1 exhibited a distinct proteome fingerprint in the brain tissues of patients with MDD (Martins-de-Souza et al., 2012). WGE increased the expression of positive regulators, such as PFN1, in a FST animal model (Lin et al., 2014).

According to our findings, the effects varied among WGE and its components. Bioavailability is a key point to explain the phenomenon. WGE has been found to reduce immobility time in the FST in our previous studies (Chen et al., 2008). However, the gavaged dosage of GAS and HBA which are equal quantities to that in WGE could not decrease immobility time in the FST (data not shown). Nonetheless, the higher dosages of GAS (100 mg/kg bw) and HBA (100 mg/kg bw) significantly reduced immobility time and modulated Slit-Robo pathway. Since the bioavailability of GAS is 14.2% (Lin et al., 2007; Jia et al., 2014), it can explain that low dosage of GAS and HBA do not reduce immobility time in the FST. Moreover, we further performed an in vitro cell showing that 0.5 mg/mL WGE, which content 50 μM GAS, reduced Slit1 expression in Hs683 cells. The similar results were found in 50 μM, 100 μM, and 200 μM GAS and 100 μM and 200 μM HBA. Based on these evidences, the low concentration of GAS or HBA could not notably affect immobility time as WGE in FST. Therefore, the antidepressant effect of WGE may be exerted by the lower amounts of active compounds including GAS and HBA.

5. Conclusion

Based on our behavioral and monoamine analyzes, WGE and its compounds, GAS and HBA, decreased the immobility time in the FST and turnover rate of monoamine metabolism. In particular, GAS and HBA influenced the dopaminergic system more than the serotonergic system. In the neuroplasticity analysis, WGE, GAS, and HBA down-regulated the Slit-Robo pathway, which modulated the cytoskeleton remodeling-related processes in the hippocampus. The decreased monoamine metabolism and altered cytoskeleton remodeling-related proteins in the Slit-Robo pathway may be possible mechanisms behind the anti-depression effects of WGE and its compounds. These findings demonstrated that GAS and HBA contributed to the antidepressant effects of WGE, and suggested that WGE is a suitable complementary treatment for depression.

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Zhang, R., Peng, Z., Wang, H., Xue, F., Chen, Y., Wang, Y., Wang, H., Tan, Q., 2014. Gastrodin ameliorates depressive-like behaviors and up-regulates the expression of BDNF in the hippocampus and hippocampal-derived astrocyte of rats. Neurochem. Res. 39, 172–179.

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EDUCATION

2011-2016	Ph.D. Department of Food Science, National Taiwan University
2009-2011	M.S. Department of Food Science, National Chiayi University
2005-2009	B.S. Department of Food Science, Fu Jen Catholic University

WORKING EXPERIENCE

2013-2014	Teaching assistant of Food and health, Food safety and regulations
2012-2013	Teaching assistant of Principle and application of Chinese medicated diet, Clinical nutrition, Food safety and regulations
2011-2012	Teaching assistant of animal cell model

LICENSES AND TRAINING PROGRAM

1. Food Technician

2. Hazard Analysis and Critical Control Points (HACCP) Training Program

MAJOR RESEARCH AREA

1. *Physiological functions of foods*

2. *Analysis of active principles in foods*

3. *Traditional Chinese dietary therapy*

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RESEARCH EXPERIENCE

2011-2013 The project: Mechanism of antidepressant effects exerted by gastrodin and HBA with forced-swimming model

In previous studies, the water extract of *Gastrodia elata* Blume has been reported to provide anti-depression effects both *in vivo* and *in vitro*. The objective of this study is to investigate anti-depression effect by the major active compounds (gastrodin and 4-hydroxybenzyl alcohol (HBA)) found in the water extract using animal model. Then, the anti-depression effects will be evaluated by forced swimming test and sucrose consumption test. The possible anti-depressant mechanisms will be evaluated by investigating the monoamine contents by HPLC. The identified mechanisms will be validated by Western Blots. Moreover, the serum will be analyzed by brain-derived neurotrophic factor (BDNF) method. Hopefully, the results will suggest anti-depression effect is exerted by gastrodin or HBA and which one could be a potential candidate for developing a natural antidepressant in the future.

2010-2011 The project:Color-stability characterization of the juice and pigments derived from *Basella alba* L. fruits

The methanolic extract was subjected to SPE (solid phase extract) to separation, semi-preparative HPLC fractionation and MS, three major pigments were detected. Then, the major pigment (Gomphernin I) were purified and collected through HPLC. The structure of Gomphernin I was confirmed through NMR.

2009-2010 The project:Isolation, structure identification and property characterization of xanthine oxidase inhibitors in vinegars

Gout is a common arthritis caused by disorder of purine metabolism to from urate crystal deposition in and around the joints. The vinegars was subjected to SPE and got some fractions. Some of them have inhibitory effects of xanthine oxidase. Then, the highest inhibitory effect of fraction were analyzed by HPLC and LC/MS. Two active compound

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were 5-hydroxymethylfurfural and 1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid.

2008-2009 **The project: To increase the production of aglycone isoflavones by optimizing lactic acid bacteria-fermented condition from soy milk**

Genetic engineering and biotechnology program (undergraduate program)

2007-2008 The basic animal (rats and mice) raise and sacrifice operation (Blood collection by hart, tail vein, eyeball and jaw; organs collection (except brain)

CURRRENT RESEARCH TOPIC

1. **To explore the therapeutic potential and molecular mechanism of antidepressant effects exerting by gastrodin and HBA**
2. **The neuroprotective effect of coffee with different roast degree against the cell damage induced by glucocorticoid**
3. **To explore the mechanism of antidepressant effects of water extracts of** *Gastrodia elata* Blume **on neural growth and differentiation**
4. **Color-stability characterization of the juice and pigments derived from *Basella alba* L. fruits**

AWARDS and HONORS

1. **2016 國立臺灣大學生物資源暨農學院博士班學生學術論文獎**
2. **2015 國立臺灣大學生物資源暨農學院劉古雄先生傑出研究獎**

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PUBLICATION

1. Panyod, S., Wu, W.K., Ho, C.T., Lu, K.H., Liu, C.T., Chu, Y.L., Lai, Y.S., **Chen, W.C.,** Lin, Y.E., Lin, S.H., Sheen, L.Y. ***2016.** Diet supplementation with allicin protects against alcoholic fatty liver disease in mice by improving antioxidative and anti-inflammation functions. *Journal of Agricultural and Food Chemistry*. (SCI, Revised)
2. Lu, K.H., Weng, C.Y., **Chen, W.C.,** and Sheen, L.Y.***2016.** Ginseng essence, a medicinal and edible herbal formulation, ameliorates carbon tetrachloride-induced oxidative stress and liver injury in rats. *Journal of Ginseng Research.* (SCI, Accepted)
3. Huang, F.L., Chiou, R. Y.-Y., **Chen, W.C.,** Ko, H.J., Lai, L.J., and Lin, S.M.* **2016.** Dehydrated *Basella alba* fruit juice as a novel natural colorant: pigment stability, *in vivo* food safety evaluation and anti-inflammatory mechanism characterization. *Plant Foods for Human Nutrition.* (SCI, Accepted)
4. Lin**,** Y.E., Lin, S.H., **Chen, W.C.,** Ho, C.T., Lai, Y.S., Panyod, S., and Sheen, L.Y.* **2016.** Antidepressant-like effects of water extract of *Gastrodia elata* Blume in rats exposed to unpredictable chronic mild stress via modulation of monoamine regulatory pathways. *Journal of Ethnopharmacology* 187: 57-65. (SCI)
5. **Chen, W.C.,** Lai, Y.S., Lin, S.H., Lu, K.H., Panyod, S., Ho, C.T., and Sheen, L.Y.***2016.** Anti-depressant effects of *Gastrodia elata* Blume and its compounds, gastrodin and 4-hydroxybenzyl alcohol, involve the monoaminergic system and neuronal cytoskeletal remodeling processes. *Journal of Ethnopharmacology* 182: 190-199. (SCI)
6. Lin, S.H, Chou, M.L., **Chen, W.C.,** Lai, Y.S., Lu, K.H., Hao, C.W., Chu, Y.L., and Sheen, L.Y.* **2015**. Antidepressant-like activity of the water extract of *Melissa officinalis* L. in forced swimming test via regulation of the serotonergic neurotransmitter. *Journal of Ethnopharmacology* 175: 266-272. (SCI)
7. Lai, Y.S., **Chen, W.C.,** Kuo, T.C., Ho, C.T., Kuo, C.H., Tseng, Y, Lu, K.H., Lin, S.H. Panyod, S., and Sheen, L.Y.* **2015.** Mass spectrometry-based serum metabolomics of a C57BL/6J mouse

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model of high-fat diet induced nonalcoholic fatty liver disease development. *Journal of Agricultural and Food Chemistry* 63(35): 7873-7884. (SCI)

8. **Chen, W.C.,** Lai, Y.S., Lu, K.H., Lin, S.H., Liao, L.Y., Ho, C.T., Sheen, L.Y.* **2015**. Method development and validation for the HPLC assay of gastrodin in water extracts from different sources of *Gastrodia elata* Blume. *Journal of Food and Drug Analysis* 23(4): 803-810. (SCI)
9. Lai, Y.S., **Chen, W.C.,** Ho, C.T., Lu, K.H., Lin, S.H., Tseng, H.C., and Sheen, L.Y.* **2014.** Garlic essential oil protects against obesity-triggered nonalcoholic fatty liver disease through modulation of lipid metabolism and oxidative stress. *Journal of Agricultural and Food Chemistry* 62(25): 5897-5906. (SCI)
10. Chu, Y.L., Raghu, R., Lu, K.H., Liu, C.T., Lin, S.H., Lai, Y.S., **Chen, W.C.**, Lin, S.H., and Sheen, L.Y.* **2013**. Autophagy Therapeutic Potential of Garlic in Human Cancer Therapy. *Journal of Traditional and Complementary Medicine* 3(3): 159-162.
11. Wu, J.Y., **Chen, W.C.,** Wu, Y.Y., Chen, J.T., L.-G. Chen, L.G., and Chiou, R.Y.-Y.* **2013.** NMR-based elucidation of the positional C6-O-Glucopyranosyl substitution of GomphreninI isolated from *Basella alba* fruits. *Journal of Agricultural Science and Application* 2(1): 8-12.

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POSTER

1. **Chen, W.C.,** Lu, K.H., Ho, C.T., Huang, C.C.*, and Sheen, L.Y.* **2016.** Development of a rapid microscopic Raman spectroscopic screening method to determine *Gastrodia elata* Blume varieties. 4th Taiwan International Symposium on Raman Spectroscopy (TISRS 2016). Jun. 29-30, Taipei, Taiwan.
2. Panyod, S., Wu, W.K., Ho, C.T., Jan, T.R., Lu, K.H., Liu, C.T., Chu, Y.L., **Chen, W.C.,** and Sheen, L.Y.* **2015.** Effect of allicin on cecum microbiota in alcoholic fatty liver disease mice. 2015 Asian Pacific Digestive Week (APDW). Dec. 3-6, Taipei, Taiwan.
3. Lai, Y.S., **Chen, W.C.,** Ho, C.T., Lu, K.H., Lin, S.H., Panyod, S., and Sheen, L.Y.* **2015.** An animal model in C57BL/6J mice fed a high-fat diet induced non-alcoholic fatty liver disease development. 2nd International Conference of Traditional and Complementary Medicine on Health (ICTCMH 2015). Oct. 24-27, Taipei, Taiwan.
4. **Chen, W.C.,** Lai, Y.S., Chou, Y.Y., Lin, S.H., Lu, K.H., Panyod, S., Lin, Y.E., and Sheen, L.Y.* **2015.** Water extract of *Gastrodia elata* Blume ameliorates depression symptom via neuroplasticity enhance. 2nd International Conference of Traditional and Complementary Medicine on Health (ICTCMH 2015). Oct. 24-27, Taipei, Taiwan.
5. Panyod, S., Wu, W.K., Ho, C.T., Lu, K.H., Chu, Y.L., Lai, Y.S., **Chen, W.C.,** and Sheen, L.Y.* **2015.** The liver-protective effects of allicin against alcoholic fatty liver disease and liver inflammation. 6th International Conference on Nutrition and Physical Activity in Aging, Obesity, and Cancer (NAPA 2015). Oct. 21-24, Taipei, Taiwan.
6. Lai, Y.S., **Chen, W.C.,** Kuo, T.C., Ho, C.T., Kuo, C.H., Tseng, Y.J., Lu, K.H., Lin, S.H., Panyod, S., and Sheen, L.Y.* **2015.** Serum metabolomic profiling of *C57BL*/6J mice from high-fat diet induced nonalcoholic fatty liver disease development. 6th International Conference on Nutrition and Physical Activity in Aging, Obesity, and Cancer (NAPA 2015). Oct. 21-24, Taipei, Taiwan.
7. **Chen, W.C.,** Huang, P.S., Kuo, Y.J., Lin, P.Y., Wang, R., Lu, T.J.*, Lo, Y.C.* **2015.** Biotransformation of Ginsenosides in *Saccharomyces cerevisiae*. 6th International Conference on Nutrition and Physical Activity in Aging, Obesity, and Cancer (NAPA 2015). Oct. 21-24, Taipei, Taiwan.

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8. **Chen, W.C.,** Lai, Y.S., Lin, S.H., Lu, K.H., Ho, C.T., and Sheen, L.Y.* **2015.** Monoaminergic system regulation and neuroplasticity enhancement effects of water extract of *Gastrodia elata* Blume and its compounds, gastrodin and 4-hydroxybenzyl alcohol, on forced swimming test in rats. 6th International Conference on Nutrition and Physical Activity in Aging, Obesity, and Cancer (NAPA 2015). Oct. 21-24, Taipei, Taiwan.
9. Panyod, S., Wu, W.K., Ho, C.T., Jan, T.R., Lu, K.H., Liu, C.T., Chu, Y.L., **Chen, W.C.**, and Sheen, L.Y.* **2015.** Effect of allicin on cecum microbiota in alcoholic fatty liver disease mice. 2015 Asian Pacific Digestive Week (APDW). Dec. 3-6, Taipei, Taiwan.
10. Lin, Y.E., **Chen, W.C.**, Lin, S.H., and Sheen, L.Y.* **2015.** The monoamine regulatory effects of water extract of *Gastrodia elata* Blume and gastrodin *in vivo* and *in vitro*. 2015 Institute of Food Technologists (IFT) Annual Meeting. Jul. 11-14, Chicago, America.
11. Lin, S.H., Liu, Y.T., **Chen, W.C.**, Lin, Y.E., Ho, C.T., and Sheen, L.Y.* **2015**. The neuroprotective and antidepressive activities of coffee with different roast degrees. 2015 Institute of Food Technologists (IFT) Annual Meeting. Jul. 11-14, Chicago, America.
12. **Chen, W.C.**, Lai, Y.S., Lin, S.H., Chou, Y.Y. Lu, K.H., Ho, C.T., and Sheen, L.Y.* **2015.** The molecular mechanism of the anti-depressive effects exerted by gastrodin and 4-hydroxybenzyl alcohol, the active compounds of *Gastrodia elata* Blume. 2015 Institute of Food Technologists (IFT) Annual Meeting. Jul. 11-14, Chicago, America.
13. Huang, F.L., **Chen, W.C.**, Ko, H.J., Lin, S.M., Chang, J.C., and. Chiou, R.Y.-Y.* **2014.** Mature *basella* alba L. fruits as a novel source of natural colorant bearing safe toxicological and antioxidant properties. The XXVII th International Conference on Polyphenols, Sep. 2-9, Nagoya, Japan.
14. **Chen, W.C.**, Lu, K.H., and Sheen, L.Y.* **2013.** Determinations of gastrodin in water and ethanolic extracts from different sources of *Gastrodia elata* Bl. (tiān má) by a validated HPLC method. 2013 International Society for Nutraceuticals and Functional Foods (ISNFF) Conference. Nov. 5-9, Taipei, Taiwan.
15. Lin, S.H., **Chen, W.C.**, and Sheen, L.Y.* **2013.** Brain health and traditional Chinese herbal

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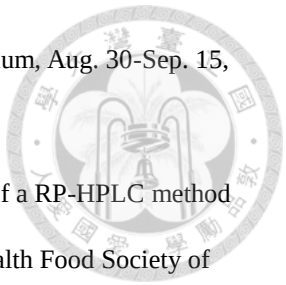

medicine: *Gastrodia elata* Blume. 2013 KNS-Cheonan International Symposium, Aug. 30-Sep. 15, Korea.

16. **Chen, W.C.**, Lu, K.H., and Sheen, L.Y.* **2013.** Development and validation of a RP-HPLC method for the determination of gastrodin in water extracts of *Gastrodia elata* Bl. Health Food Society of Taiwan. Mar. 8, Taichung, Taiwan. (In Chinese)

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