

# 行政院國家科學委員會專題研究計畫成果報告

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† 八十六年度及以前的一般國科會專題計畫亦可選擇適用，唯較特殊的計畫如產學計畫，國科會規劃案等，請先洽得國科會各學術處同意。

## 一、中文摘要

本研究以黑白室 (Black and white chambers) 及舉高型十字迷宮 (Elevated plus-maze) 之動物焦慮模式探討黃連甲醇粗抽物及其活形成分小蘗鹼一次給藥和一星期給藥之抗焦慮作用，並用 buspirone 為正對照。

結果顯示，於黑白室之動物焦慮模式實驗中，黃連甲醇粗抽取物及其活形成分小蘗鹼在 0.1 及 0.5 g/kg 劑量，一次給藥及一星期給藥均可增加小鼠在白室的滯留時間、運動量、由白室第一次進入黑室的時間及二室間的穿梭次數，並減少了在黑室的滯留時間。於舉高型十字迷宮之動物焦慮模式實驗中，一次給藥組及一星期給藥組均可增加小鼠在開放臂之滯留時間及進入開放臂的次數，減少小鼠在封閉臂之滯留時間及進入封閉臂的次數。黃連甲醇粗抽取物及小蘗鹼在 0.5 g/kg 劑量，一次給藥及一星期給藥均可增加 hexobarbital 誘發睡眠時間及降低大鼠自發運動量。小蘗鹼 0.5 g/kg 可拮抗 APO, LD+BEZ 及 PCPA 誘發之運動量增加；並增強  $\alpha$ -MT 及 5-HTP 誘發之運動量減少。

高速液相層析儀檢測大鼠腦內單胺濃度顯示，小蘗鹼 0.1 及 0.5 g/kg 可降低大鼠皮質 NE, DA 濃度及腦幹中 NE, DA, 5-HT 濃度，並增加大鼠皮質中 5-HT 濃度。

綜合以上結果顯示黃連甲醇粗抽取物及其活性成分小蘗鹼一次給藥及一星期給藥於較低劑量

具有抗焦慮作用，於較高劑量具有鎮靜作用，其抗焦慮及鎮靜作用機轉與降低大鼠皮質 NE, DA 濃度及腦幹中 NE, DA, 5-HT 濃度，並增加大鼠皮質中 5-HT 濃度有關。

關鍵詞：黃連、小蘗鹼、抗焦慮

## Abstract

In this study, we investigated the anxiolytic effect of Coptis rhizome and berberine after acute and one-week administration by black/white test and elevated plus-maze, the sedative effect of Coptis rhizome and berberine after acute and one-week administration by hexobarbital-induced hypnosis and locomotor activity detection. Furthermore, we investigated the sedative mechanism of berberine by combining APO,  $\alpha$ -MT, LD+BEZ, PCPA and 5-HTP, and the anxiolytic and sedative mechanisms of the berberine by detecting the changes of monoamines in the rats' brain.

In the black/white test, the methanol extract of Coptis rhizome and berberine after acute and one-week administration prolonged the first time entry, time spent in the white compartment and the total changes between the two compartments, shorten the time spent in the black compartment. In the

elevated plus-maze test, the methanol extract of Coptis rhizome and berberine after acute and one-week administration prolonged the entries and time spent in the open arms. The methanol extract of Coptis rhizome and berberine at higher dose after acute and one-week administration prolonged the hexobarbital-induced sleeping time in mice and decreased the locomotor activity in the rats. The hypermotilities induced by APO, LD+BEZ and PCPA were decreased by the berberine and the hypomotilities induced by HAL and 5-HTP were augmented by the berberine. The berberine decreased the levels of NE and DA in the cortex, the NE, DA and 5-HT in the brain stem, and increased the levels of 5-HT in the cortex.

These results suggested that the methanol extract of Coptis rhizome and berberine after acute and one-week administration possessed anxiolytic and sedative effects. The sedative mechanism of the berberine was related to the decrease in the catecholaminergic system activity and the increase in the serotonergic system activity. The anxiolytic mechanism of the berberine was related to the decrease in the levels of NE, DA in the cortex and NE, DA, 5-HT in the brain stem, and the increase in the levels of 5-HT in the cortex.

**Keywords:** Coptis rhizome, Berberine,  
Antianxiety

## 二、緣由與目的

黃連，始載於本經，為毛茛科多年生草本植物黃連 *Coptis* spp. 的根莖。具有瀉心

火之功效，治心熱煩擾不寐，如內外傷辨惑論之硃砂安神丸；治心火盛而又陰血不足以致心煩不眠者，宜與阿膠、白芍等伍用，如傷寒論之黃連阿膠湯。日華子本草記載：「治五勞七傷，益氣，止心腹痛。驚悸煩躁……」。傳統中醫師有用於治療焦慮症(1)。其主要有效成分是原小蘗生物鹼(protoberberine type alkaloids; berberine, palmatine) (2)。現代藥理研究證實黃連具有抗菌 (3)、抗高血壓(4)、抗潰瘍(5)、中樞抑制 (6)、鹼性磷酸酯 興奮 (7)等作用。小蘗鹼有較強的廣譜抗菌作用、小蘗鹼有解熱及興奮呼吸作用。小蘗鹼的降壓作用與直接擴張血管、抗膽鹼酯酶、抗腎上腺素及抑制血壓反射、抑制血管運動中樞有關。黃連及小蘗鹼有利膽作用，能增加膽汁形成，使膽汁變稀。然有關黃連止煩躁、治鬱熱在中煩燥之焦慮症狀的藥理作用並未見有探討。因此本研究擬以黑白室(Black & White)及舉高型十字迷宮 (Elevated plus-maze) 等動物焦慮實驗模式，來探討黃連甲醇萃取物及有效分離物小蘗鹼之抗焦慮作用，又現今臨床所用之抗焦慮藥，一般在治療劑量或比治療劑量稍高之劑量下，會有鎮靜安眠之作用，如 diazepam；因此本研究除探討黃連甲醇萃取物及有效分離物小蘗鹼之抗焦慮作用外，亦擬探討黃連甲醇萃取物及有效分離物小蘗鹼鎮靜安眠劑量與抗焦慮劑量之關係。並以現今臨床常用的抗焦慮藥物 buspirone 為正對照組，以評估其治療效價。

## 三、實驗材料與方法

### (一) 實驗藥材之製備

本實驗所用之黃連之基原鑑定如下：

1. 黃連：為毛茛科植物黃連 *Coptis chinensis* Franch. 的根莖。

將黃連以甲醇浸潤之，在 50°C 下迴流抽取四~五次，每次約四小時，合併抽取液，經減壓濃縮乾燥後，即得黃連甲醇粗萃取物。

### (二) 實驗方法

<1>、對明暗室測定 ( Light/dark test ) 之影響：

明暗室測定已用於鋸齒類動物焦慮狀態的測定，此儀器不須實驗者親自觀察，為全自動化儀器，數據的取得較為容易且較為可靠<sup>(8)</sup>。此儀器由大小不同的二實驗箱所組成，一個實驗箱塗成白色 ( 27X27 cm )，另一個塗成黑色 ( 27X18 cm ) 兩實驗箱間由47cm高的壁隔開，此壁底部有一開口 ( 7X7 cm )，每實驗箱底部均有其相同大小及顏色的可移動地板，地板上用線畫出9X9cm格子。每實驗相各有其獨立照明，明室用100W白光燈泡 ( 燈光強度4400lux )，暗室用40W紅光燈泡，兩燈泡離地板37cm高。每隻動物測試5分鐘，並於每隻動物測試完後將地板擦拭乾淨，實驗從下午二點至下午五點。

實驗分一次給藥組及一週給藥組，一次給藥組於黃連甲醇粗萃取物及小蘗鹼以不同劑量分別經由口服投藥給與小鼠60分鐘後；一週給藥組每天給藥一次，連續給

七天，於第七天口服給予小鼠不同劑量黃連甲醇粗萃取物及小蘗鹼 60 分鐘後，再將小鼠至於洞口處，小鼠頭部面向暗室，記錄小鼠停留在每一室的總時間、於明暗室間的穿梭次數、第一次變換至另一實驗箱的時間、於兩室內越過線 ( line crossings ) 的次數<sup>(8)</sup>。本實驗以 buspirone(2 mg/kg, i.p.) 作正對照，於給藥 20 分鐘後進行試驗<sup>(9)</sup>。

<2>、對舉高型十字迷宮 (Elevated plus-maze) 誘發焦慮之影響：

本實驗主要是依 Pellow 氏於 1985 年發表用於評估焦慮的儀器(Elevated plus-maze)，是是目前最廣泛被使用的動物焦慮模式，其原理主要是依據鋸齒類動物厭惡高及開放空間的天性<sup>(10)</sup>。本儀器由兩個開放臂(40×10 cm)及兩個封閉臂(40×10×40 cm)，中以開放之正方形 (10×10 cm)連接。

實驗分一次給藥組及一週給藥組，一次給藥組於黃連甲醇粗萃取物及小蘗鹼以不同劑量分別經由口服投藥給與大鼠60分鐘後；一週給藥組每天給藥一次，連續給七天，於第七天口服給予大鼠不同劑量黃連甲醇粗萃取物及小蘗鹼 60 分鐘後，將大鼠放入實驗箱中央，實驗動物頭部面對其中一個封閉臂，記錄大鼠進入開放臂及封閉臂總次數、滯留開放臂之時間為評估標準<sup>(11)</sup>。實驗者連續觀察 5 分鐘，每隻大鼠只測定一次，每組 20 隻。實驗時間從上午八點至下午五點。本實驗以 buspirone(2 mg/kg, i.p.)作正對照，於給藥 20 分鐘後進

行試驗。

<3>、對 hexobarbital 誘發睡眠時間之影響：

實驗分一次給藥組及一週給藥組，一次給藥組於黃連甲醇粗萃取物及小蘗鹼以不同劑量分別經由口服投藥給與小鼠 60 分鐘後；一週給藥組每天給藥一次，連續給七天，於第七天口服給予小鼠不同劑量黃連甲醇粗萃取物及小蘗鹼 60 分鐘後，再分別腹腔注射 hexobarbital ( 100 mg/kg )，誘發睡眠，觀察記錄從注射 hexobarbital 後至小鼠之翻正反射 ( righting reflex ) 消失時間 ( onset ) 及從翻正反射消失至恢復的時間 ( sleeping time, duration ) (12)。對照組給 vehicle。

<4>、對大鼠運動量之影響：

運動量之測定是使用「動物運動量測定裝置」(1-Opto-Varimex-3 Animal Activity Meter, USA)。實驗分一次給藥組及一週給藥組，一次給藥組於黃連甲醇粗萃取物及小蘗鹼以不同劑量分別經由口服投藥給與大鼠 55 分鐘後；一週給藥組每天給藥一次，連續給七天，於第七天口服給予大鼠不同劑量黃連甲醇粗萃取物及小蘗鹼 55 分鐘後，放入此裝置內適應 5 分鐘，再開始記錄大鼠之各種活動行為的變化 (包括垂直、水平走動，站立，及移動總距離等)。使用雄性大鼠，每組 6 隻，觀察並連續記錄 1 小時 (13)。對照組均給予 vehicle。

<5>、對改變腦內 catecholaminergic system

之物質所引起運動量之影響：

小蘗鹼以不同劑量分別經由口服投藥給與大鼠 60 分鐘後，再與下述物質分別併用，依前法 (方法 <2>)，于測定前 5 分鐘將大鼠移入運動量測定裝置適應，待 5 分鐘後開始記錄，觀察並連續記錄 1 小時。對照組給 vehicle。

本實驗所使用於改變腦內 catecholaminergic system 物質的劑量及時間分別為：APO ( 0.3 mg/kg, s.c. ) 10 分鐘前給于，需於使用前新鮮配製<sup>(14)</sup>； $\alpha$ -MT ( 50 mg/kg, i.p. ) 2 小時前給藥<sup>(15)</sup>；L-dopa ( 200 mg/kg, i.p. ) 50 分鐘前給藥，加上 benserazide ( 50 mg/kg, i.p. ) 80 分鐘前給藥，二者均需於使用前新鮮配製<sup>(16)</sup>。

<6>、對改變腦內 serotonergic system 之物質所引起運動量之影響：

小蘗鹼以不同劑量分別經由口服投藥給與大鼠 60 分鐘後，再與下述物質分別併用，依前法 (方法 <2>)，于測定前 5 分鐘將大鼠移入運動量測定裝置適應，待 5 分鐘後開始記錄，觀察並連續記錄 1 小時。對照組給 vehicle。

本實驗所使用於改變腦內 serotonergic system 物質的劑量及時間為：5-HTP ( 50 mg/kg, i.p. ) 30 分鐘前給藥<sup>(17)</sup>；PCPA ( 200 mg/kg, i.p. ) 24 小時前給藥<sup>(18)</sup>。

<7>、對大鼠腦內單胺及其代謝物濃度之影響：

黃連甲醇粗抽物之活性成分小蘗鹼口

服給予大鼠，一小時後，將大鼠斷頭，取出全腦，將此腦組織置於碎冰中保持冷凍。然後將腦組織分成 cortex 與 brain stem 二部分，在 5ml 0.01N HCl 與 500 $\mu$ l 0.1M EDTA 下以均質機研勻之，加入 4 g NaCl，並以 12 ml n-butanol 抽取，經振盪離心後，取得 n-butanol 層，再加入 17 ml n-heptan 與 400 $\mu$ l 0.025N HCl 酸性溶液振盪之，則單胺移入酸性水溶液層。上述餘留 n-heptane 層續以 200 $\mu$ l 0.2M tris-HCl 鹼性緩衝溶液 (pH8.5) 振盪抽取並離心，可得單胺代謝物<sup>(19)</sup>。分離所得腦內單胺 (NE, DA, 5-HT)，以外標準法測定之。使用高速液態層析儀 (HPLC model 510, Solvent Delivery system M45) (Waters Associates) 及檢出器 (Electrochemical Detectors LC-4C) (Bioanalytical system Inc.) 測定之。分離所用 Column 為 Lichrospher 100 (RP-18 endcapped, 4mm $\times$ 125mm) (E.Merck 50734)，移動相為加有 PIC B7(Waters Associates) 之 methanol/water，其流速為 2.0 ml/min。分離溶出物面積係使用 Data module M746 型記錄之。

#### <8> 統計學分析：

本實驗所得之數據，hexobarbital 誘發睡眠及自發運動量實驗，均以 one-way ANOVA 分析其變異數，再以 Duncan's multiple range test 檢定，統計並分析其間差異之顯著性，凡 P 值小於 0.05 以下時，則認為有統計意義。黑白室實驗及舉高型十字迷宮，則以 non-parameter 統計，先以 Kruskal-Wallis 單

因子變異數分析變異數，再以 Mann-Whitney U-test 檢定其間差異之顯著性，凡 P 值小於 0.05 以下時，則認為有統計意義。

#### 四、結果

##### 1、對明暗室測定 (Light/dark test) 之影響：

由圖一至圖三結果顯示，黃連甲醇抽取物及小蘗鹼 (0.1, 0.5 g/kg) 對小鼠在黑白室中第一次由明室跑至暗室的時間、停留在明室的時間均有延長的作用，縮短在暗室的時間，增加在兩室間的穿梭次數。

由圖四至圖六結果顯示，黃連甲醇抽取物及小蘗鹼 (0.1, 0.5 g/kg) 每天給藥一次，連續給七天，對小鼠在黑白室中第一次由明室跑至暗室的時間、停留在明室的時間均有延長的作用，縮短在暗室的時間，增加在兩室間的穿梭次數。

##### 2、對舉高型十字迷宮 (Elevated plus-maze) 誘發焦慮之影響：

圖七、圖八結果顯示，黃連甲醇抽取物及小蘗鹼 (0.1, 0.5 g/kg) 一次給藥及一星期給藥均對小鼠在舉高型十字迷宮中開放臂進入次數及時間均有增加的作用，關閉臂的進入次數及時間均有減少的作用。

##### 3、對 hexobarbital 誘發睡眠時間之影響：

如圖九、圖十結果顯示，黃連甲醇抽取物及小蘗鹼 (0.1, 0.5 g/kg) 一次給藥及

一星期給藥均對 hexobarbital 誘發的睡眠時間有延長作用。

#### 4、對大鼠運動量之影響：

如圖十一結果顯示，黃連甲醇提取物及小蘗鹼 ( 0.1 , 0.5 g/kg ) 一次給藥及一星期給藥均對大鼠自發運動量有抑制作用。

#### 5、對改變腦內 catecholaminergic system 之物質所引起運動量之影響：

如圖十二結果顯示，小蘗鹼( 0.5 g/kg ) 對 APO 及 LD+BEZ 誘發大鼠自發運動量增加有抑制作用；對 $\alpha$ -MT 誘發大鼠自發運動量抑制有更抑制作用。

#### 6、對改變腦內 serotonergic system 之物質所引起運動量之影響：

如圖十三結果顯示，小蘗鹼( 0.5 g/kg ) 對 p-chlorophenylalanine 誘發大鼠自發運動量增加有抑制作用；對 5-hydroxytryptophan 誘發大鼠自發運動量抑制有更抑制作用。

#### 7、對大鼠腦內單胺及其代謝物濃度之影響：

如圖十四結果顯示，小蘗鹼 ( 0.1 , 0.5 g/kg ) 均可降低大鼠皮質 NE, DA 濃度及腦幹中 NE, DA, 5-HT 濃度，並增加大鼠皮質中 5-HT 濃度。

### 五、討論

首先，在黑白室誘發小鼠焦慮模式之實驗中，黑白室是利用老鼠在遇到明亮環境時，其本身會去探索陌生環境的驅動力

而設計出來的模式<sup>(20)</sup>。Crawley & Goodwin 之研究發現，由老鼠不喜歡開放且明亮環境的習性，因此提出老鼠在黑白室中會誘發兩室間穿梭次數減少、第一次由白室至明室的時間較短及停留在黑室的時間較白室長之探索行為，則此行為可用來當焦慮評估指標<sup>(21)</sup>。已有研究指出 benzodiazepine 類，如 chlordiazepoxide 及 diazepam 會延長小鼠在明室之滯留時間而具有抗焦慮活性，此結果在 Crawley & Goodwin、Costall 及 Onaivi & Martin、Young & Johnson 等之研究中被證實<sup>(22-24)</sup>。另外，buspirone 在 Costall、Carli、Lopez-Rubalcava、Misslin 及 Sanchez 等之研究發現，老鼠停留在明室之滯留時間延長，而在暗室滯留時間會縮短<sup>(23, 25-27)</sup>。本研究之正對照組 buspirone 之實驗結果均與先前 Crawley 及 Costall 等之研究結果一致。而黃連粗提取物及小蘗鹼於 0.1 , 0.5 g/kg 劑量下，發現可延長小鼠第一次由明室跑至暗室的時間、於明室之滯留時間及兩室間穿梭次數，且減少小鼠於暗室之滯留時間。此結果與本研究之正對照組之 diazepam 及 buspirone 一致，顯示黃連粗提取物及小蘗鹼應具有抗焦慮之作用。

其次，在舉臂式十字形迷宮誘發焦慮模式之實驗中，Montgomery 在其研究中指出，此實驗是利用老鼠對處在較高的地方及開放的空間，會引起恐懼因而誘發焦慮而設計出來的模式<sup>(28)</sup>。Pellow 等之研究發現，舉臂式十字形迷宮是一個簡單且迅速，可用來測定藥物有無抗焦慮或致焦慮作用，且此模式對小鼠及大鼠均有作用

(<sup>29</sup>)。在Pellow、Lister及Rodgers等之研究指出，以評估老鼠於開放臂之進入次數及滯留時間之百分率，當作焦慮作用的指標(<sup>29-31</sup>)。先前在Pellow等之研究已經證實舉臂式十字形迷宮可用來測定benzodiazepine類的抗焦慮活性；而在Rodgers和Cole同樣指出，給予diazepam及benzodiazepine受體作用劑，如chlordiazepoxide及bretazenil，可明顯增加進入開放臂次數及延長在開放臂滯留時間百分率(<sup>32</sup>)(<sup>33</sup>)。而buspirone在Dunn、Soderpalm、Lee & Rodgers、Kostowski及Luscombe等人之研究一致發現，可增加小鼠停留在開放臂滯留時間及進入開放臂次數百分率，顯示buspirone在舉臂式十字形迷宮焦慮模式有抗焦慮作用(<sup>34-36</sup>)。本研究所用之正對照組diazepam及buspirone，均可增加小鼠在開放臂之滯留時間及進入次數百分率，作用結果與上述多位學者之研究結果一致。而黃連粗提取物及小蘗鹼於0.1, 0.5 g/kg劑量下，可增加小鼠在開放臂之滯留時間及進入次數百分率，顯示黃連粗提取物及小蘗鹼應具有抗焦慮之作用。

因黑白室及舉臂式十字型迷宮誘發小鼠焦慮模式均牽涉動物之運動行為，故進行動物之運動活性測定及hexobarbital誘發睡眠實驗，結果發現黃連粗提取物及小蘗鹼於0.5 g/kg劑量下一次及一週給藥具降低大鼠自發運動量及延長hexobarbital誘發之睡眠時間，而黃連粗提取物及小蘗鹼於0.1 g/kg劑量下一次及一週給藥對大鼠自發運動量及hexobarbital誘發之睡眠時間則無

明顯影響，顯示黃連粗提取物及小蘗鹼於0.5 g/kg無論一次給藥或一週給藥均具有鎮靜作用。且小蘗鹼0.5 g/kg可降低APO，LD+BEZ及PCPA所誘發之運動量興奮，並增強 $\alpha$ -MT及5-HTP所誘發之運動量抑制，顯示小蘗鹼0.5 g/kg之鎮靜作用機轉與降低中樞catecholaminergic system活性及增強serotonergic system活性有關。

由於情緒與位於腦幹中邊緣系統內的神經化學物質(如5-HT、NE、DA等)及其代謝物的含量有密不可分的關係(<sup>37</sup>)。有文獻指出將5-HT直接注入腦幹中會誘發焦慮產生，而減低腦幹中5-HT之濃度則會產生抗焦慮作用(<sup>38-40</sup>)；其次，腦幹中DA過多或locus coeruleus之NE神經被活化時亦會引起焦慮之產生(<sup>41</sup>)。此外，1990年Golembiowska指出ipsapirone之抗焦慮作用是經由減少5-HT的turnover rate，增加NE、DA之turnover rate所引起(<sup>42</sup>)；由於locus coeruleus中NE的神經元會受到raphe神經核的控制(<sup>43</sup>)(<sup>44</sup>)，而ipsapirone會經由活化5-HT<sub>1A</sub> autoreceptors及減少神經終端5-HT的turnover rate，進而減弱raphe對locus coeruleus之控制，使得NE神經細胞產生去抑制(disinhibition)作用，並增加NE之turnover rate(<sup>45</sup>)(<sup>46</sup>)；又substantia nigra中的DA神經亦受raphe神經核的控制，當此控制被Ipsapirone抑制時，substantia nigra中的DA神經細胞亦會產生去抑制作用，且DA之turnover rate亦有增加之現象。由此可知腦幹中5-HT、NE、DA等單胺神經傳遞物質與其代謝物濃度之變化在

焦慮的產生與治療上扮演一重要性之角色<sup>(38-41)</sup><sup>(47)</sup>。再者，本所謝明村教授等研究指出，當皮質中 NE、DA 之濃度減少及 NE、DA 之 turnover rate 增加時，腦幹中 5-HT、NE、DA 之濃度減少及 5-HT、NE、DA 之 turnover rate 增加時，會產生鎮靜作用<sup>(48)</sup>。因此，本研究進一步利用生物化學方法(高速液相層析暨電化學法)探討小蘗鹼 0.1, 0.5 g/kg 對實驗動物皮質及腦幹中單胺及其代謝物之濃度變化之影響，實驗結果顯示小蘗鹼 0.1, 0.5 g/kg 口服給予均可減少皮質及腦幹內 NE、DA 之濃度及腦幹中 5-HT 之濃度，並增加皮質 5-HT 的濃度。故可推知小蘗鹼 0.1 g/kg 劑量下之抗焦慮作用，可能降低大鼠皮質 NE, DA 濃度及腦幹中 NE, DA, 5-HT 濃度，並增加大鼠皮質中 5-HT 濃度有關。

綜合以上結果，顯示黃連粗提取物及小蘗鹼均具明顯之抗焦慮及鎮靜作用，其作用機轉可能與降低大鼠皮質 NE, DA 濃度及腦幹中 NE, DA, 5-HT 濃度，並增加大鼠皮質中 5-HT 濃度有關。

## 六、參考文獻

1. Tang W, Eisenbrand G: Chinese Drugs of Plant Origin. Pp.362-371, Springer Verlag, Berlin, Heidelberg, New York, 1992.
2. Lee MK, Kim HS: Inhibitory effects of protoberberine alkaloids from the roots of *Coptis japonica* on catecholamine biosynthesis in PC12 cells. *Planta Medica* 1996; 62: 31-34.
3. Franzblau SG, Cross C: Comparative in vitro antimicrobial activity of Chinese medicinal herbs. *Journal of Ethnopharmacology* 1986; 15: 279-288.
4. Chen HC, Hsieh MT: Hemodynamic effects of "san-huang-hsieh-hsin-tang" in patients with essential hypertension. *American Journal of Chinese Medicine* 1986; 14: 51-58.
5. Takase H, Imanishi K, Miura O, Yumioka E: A possible mechanism for the gastric mucosal protection by oren-gedoku-to (OGT), a traditional herbal medicine. *Japanese Journal of Pharmacology* 1989; 51: 17-23.
6. Yamahara J: Behavioral pharmacology of berberine-type alkaloids. (1) Central depressive action of *Coptidis rhizoma* and its constituents. *Nippon Yakurigaku Zasshi* 1976; 72; 899-908.
7. Kumazawa N, Ohta S, Tu SH, Kamogawa A, Shinoda M: Protective effects of various methanol extracts of crude drugs on experimental hepatic injury induced by alpha-naphthylisothiocyanate in rats *YakugakuZasshi* 1991; 111: 199-204.
8. Griebel G, Moreau JL, Jenck F, Misslin R, Martin JR Acute and chronic treatment with 5-HT reuptake inhibitors differentially modulate emotional responses in anxiety models in rodents. *Psychopharmacology* 1994; 113: 463-470.
9. Ogawa N, Hara C, Takaki S: Anxiolytic Activity of SC-48274 compared with those of buspirone and diazepam in experimental anxiety models. *Japan J Pharmacol* 1993; 61: 115-21.



10. Montgomery KC The relation between fear induced by novelty stimulation and exploratory behaviour. *J Comp Physiol Psychol* 1955; 48: 254-260.
11. Pellow S, File SE: Anxiolytic and axiogenic drug effects on exploratory activity in an elevated plus-maze: A novel test of anxiety in the rat. *Pharmacol Biochem Behav* 1986;24:525-9.
12. Fujomori H Porentiation of barbital hypnosis as an evaluation method for central nervous system depressant. *Psychopharmacology* 1965; 7:374-377.
13. Itoh T, Murai S, Yoshida Y, Asito H and Ching HC Effects of methamphetamine and morphine on the vertical and horizontal motor activities in mice. *Pharmacol Biochem Behav* 1987; 27: 139-197.
14. Montanaro N, Vaccheri A, Dall OR, Gandolfi O Time course of rat motility response to apomorphine: A simple model for studying preferential blockade of brain dopamine receptors mediating seddation. *Psychopharmacology* 1983; 81:214-9.
15. Widerlov E, Lewander T Inhibition of the in vivo biosynthesis and changes of catecholamine levels in rats brain after alpha-methyl-p-tyrosine; Time-and dose-response relationships. *Naunyn-Schmiedeberg's Arch Pharmacol* 1978; 304:111-123.
16. Shibuya T, Takhashi N Pharmacological studies of L-dopa andd dopa decarboxylase inhibitor especially effect on central nervous system combination of L-dopa and benserazide HCl. *Tokyo IKA Daigaku Zasshii* 1977; 35:715-30.
17. Everett GM Effect of 5-hydroxytryptophan on brain levels of dopamine, norepinephrine and serotonin in mice. *Adv Biochem Psychopharmacol* 1974; 10: 261-262.
18. Kenneth KB, Weissman A p-Chlorophenylalanine: a specific depletor of brain serotoin. *J Pharmacol Exp Ther* 1966; 154: 499- 516.
19. Shibuya T, Sato K, Saiafsky B (1982) Simulaneous measurement of biogenic amines and related compounds by high performance liquid chromatograghy (HPLC). *Int J Clin Pharmacol* 20:297-301.
20. Crawley JN, Goodwin FK: Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. *Pharmacol Biochem Behav* 1980; 13:167-70.
21. Young R, Johnson DN: A fully automated light/dark apparatus useful for comparing anxiolytic agents. *Pharmacol Biochem & Behav* 1991; 40:739-43.
22. Sanchez C: Serotonergic mechanisms involved in the exploratory behaviour of mice in a fully automated two-compartment black and white test box. *Pharmacol Toxicol* 1995; 77:71-8.
23. Costall B, Jones BJ, Kelly ME, Naylor RJ, Tomkins DM: Exploration of mice in a black and white test box: validation as a

- model of anxiety. *Pharmacol Biochem & Behav* 1989; 32(3):777-85.
24. Lopez-Rubalcava C, Saldivar A, Fernandez-Guasti A: Interaction of GABA and serotonin in the anxiolytic action of diazepam and serotonergic anxiolytics. *Pharmacol Biochem & Behav* 1992; 43(2):433-40.
  25. Onaivi ES, Martin BR: Neuropharmacological and physiological validation of a computer-controlled two-compartment black and white box for the assessment of anxiety. *Progress in NeuroPsycho-pharmacology & Biological Psychiatry* 1989; 13(6):963-76.
  26. Carli M, Prontera C, Samanin R: Evidence that central 5-hydroxytryptaminergic neurones are involved in the anxiolytic activity of buspirone. *Br J Pharmacol* 1989; 96(4):829-36.
  27. Misslin R, Griebel G, Saffroy-Spittler M, Vogel E: Anxiolytic and sedative effects of 5-HT<sub>1A</sub> ligands, 8-OH-DPAT and MDL 73005EF, in mice. *Neuroreport* 1990; 1(3-4):267-70.
  28. Montgomery KC: The relation between fear induced by novelty stimulation and exploratory behaviour. *J Comp Physiol Psychol.* 1955; 48:254-60.
  29. Pellow S, Chopin P, File SE, Briley M: Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Meth.* 1985; 14(3):149-67.
  30. Lister RG: The use of a plus-maze to measure anxiety in the mouse. *Psychopharmacology* 1987; 92(2):180-5.
  31. Rodgers RJ, Cole JC, Cobain M, Daly P, Doran PJ, Eells JR, Wallis P: Anxiogenic-like effects of fluprazine and eltoprazine in the mouse elevated plus-maze: profile comparisons with 8-OH-DPAT, CGS 12066B, TFMPP and mCPP. *Behav Pharmacol* 1992; 3:621-34.
  32. Cole JC, Rodgers RJ: An ethological analysis of the effects of chlordiazepoxide and bretazenil (Ro 16-6028) in the murine elevated plus- maze. *Behav Pharmacol* 1993; 4: 573-80.
  33. Rodgers RJ, Cole JC: The elevated plus-maze: Pharmacology, methodology and ethology. In: Cooper SJ, Hendrie CA, eds. *Ethology and psychopharmacology.* Chichester: John Wiley and Sons 1994; 9-44.
  34. Dunn RW, Corbett R, Fielding S: Effects of 5-HT<sub>1A</sub> receptor agonists and NMDA receptor antagonists in the social interaction test and the elevated plus maze. *Eur J Pharmacol* 1989; 169(1):1-10.
  35. Kostowski W, Dyr W, Krzascik P, Jarbe T, Archer T: 5-Hydroxytryptamine<sub>1A</sub> receptor agonists in animal models of depression and anxiety. *Pharmacol & Toxicol* 1992; 71(1):24-30.
  36. Soderpalm B, Hjorth S, Engel JA: Effects of 5-HT<sub>1A</sub> receptor agonists and L-5-HTP in Montgomery's conflict test. *Pharmacol, Biochem & Behav* 1989;

- 32(1):259-65.
37. Vander AJ, Sherman JH, Luciano DS: Consciousness and behavior. In: Human physiology: The mechanisms of body function. Vander AJ, Sherman JH, Luciano DS(eds) , USA, Mc Graw-Hill, 1994; 369-82.
38. Thiebot MH, Hamon M, Soubrie P: Attenuation of induced-anxiety in rats by chlordiazepoxide: role of raphe dorsalis benzodiazepine binding sites and serotonergic neurons. *Neuroscience*. 1982; 7(9):2287-94.
39. Thiebot MH, Soubrie P, Hamon M, Simon P: Evidence against the involvement of serotonergic neurons in the anti-punishment activity of diazepam in the rat. *Psychopharmacology* 1984; 82(4):355-9.
40. Higgins GA, Jones BJ, Oakley NR, Tyers MB: Evidence that the amygdala is involved in the disinhibitory effects of 5-HT<sub>3</sub> receptor antagonists. *Psychopharmacology* 1991; 104(4):545-51.
41. Stahl SM: Essential psychopharmacology: neuroscientific basis and practical applications. New York, Cambridge university press, 1996;167-215.
42. Golembiowska K: Ipsapirone, a new anxiolytic drug, stimulates catecholamine turnover in various regions of the rat brain. *Pol J Pharmacol Pharm* 1990; 42:143-50.
43. Segal M: Serotonergic innervation of the locus coeruleus from the dorsal raphe and its action on responses to noxious stimuli. *J Physiol* 1979; 286:401-15.
44. Verge D, Daval G, Patey A, Gozlan H, el Mestikawy S, Hamon M: Presynaptic 5-HT autoreceptors on serotonergic cell bodies and/or dendrites but not terminals are of the 5-HT<sub>1A</sub> subtype. *Eur J Pharmacol* 1985; 113(3):463-4.
45. Beninger RJ, The role of dopamine in locomotor and learning. *Brain Res Rev* 1983; 6:173-96.
46. Hsieh MTT, Peng WH, Hsieh CC: Effects of DL-tetrahydropalmatine on motor activity and the brain monoamine concentration in rats. *Chin J Physiol* 1994; 37:79-82.

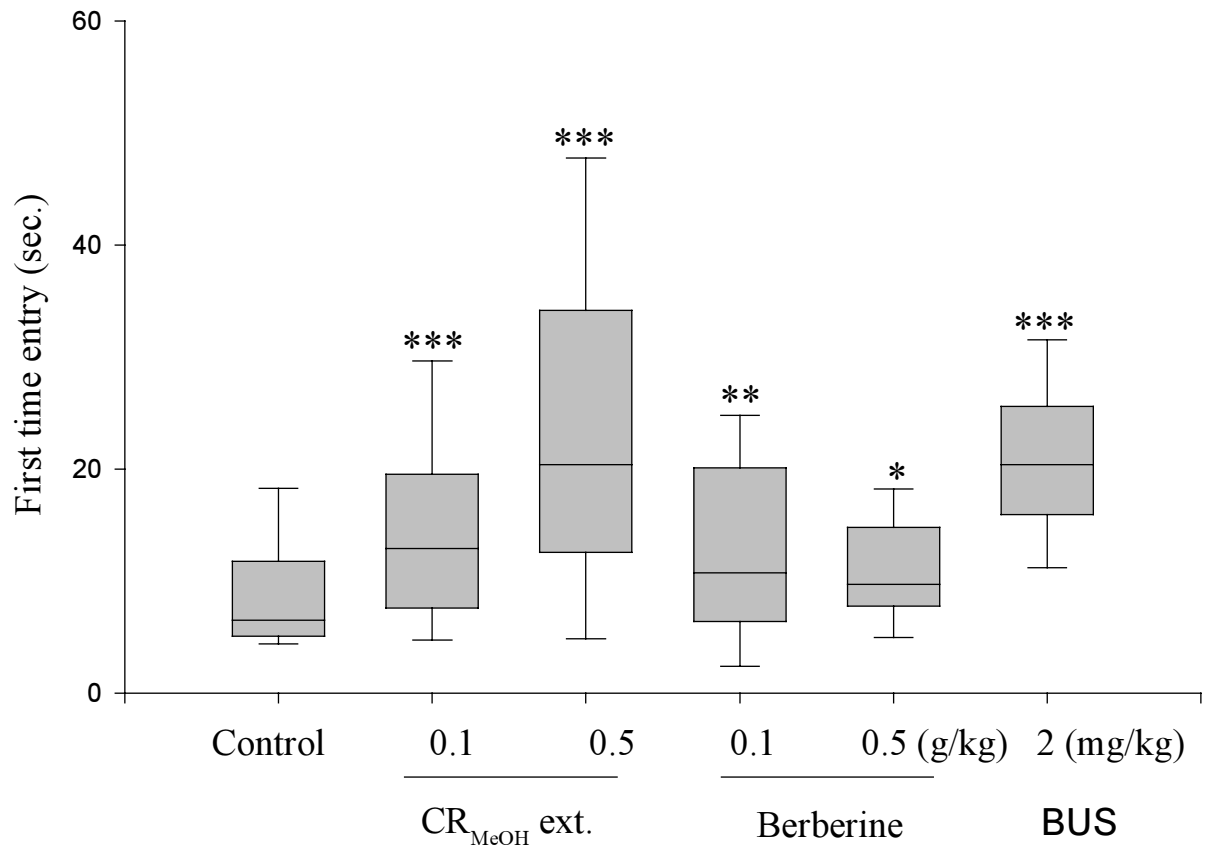


Fig 1. Effects of methanol extract of *Coptis rhizome* (CR<sub>MeOH</sub> ext.), berberine and buspirone (BUS) after acute treatment on the first time entry in black and white compartment in mice. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 as compared with the control group (Kruskal-Wallis H following by Mann-Whitney U test).

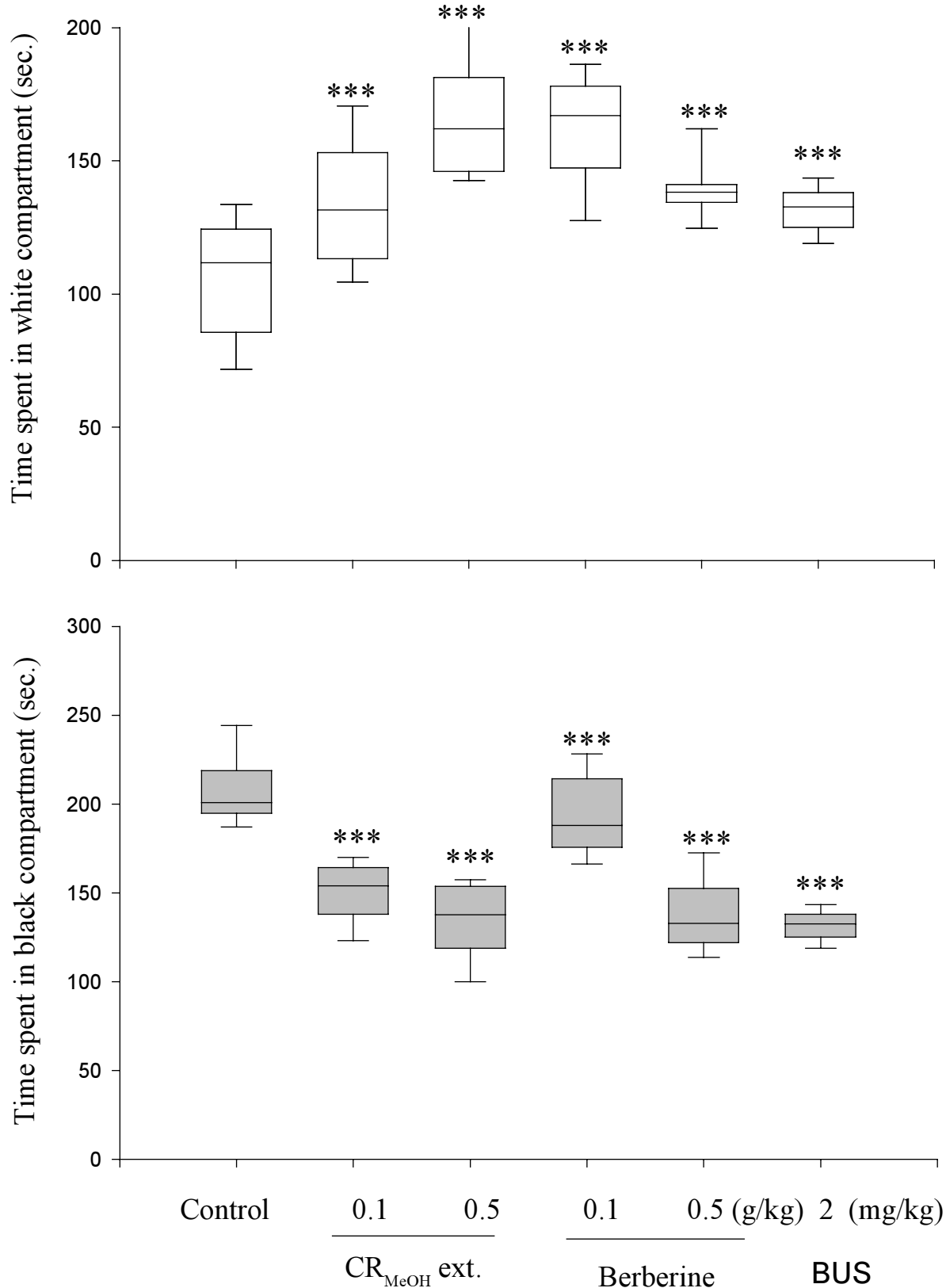


Fig 2. Effects of methanol extract of *Coptis rhizome* (CR<sub>MeOH</sub> ext.) berberine, and buspirone (BUS) after acute treatment on the time spent in white and black compartments of black and white test in mice. \*\*\*P < 0.001 as compared with the control group (Kruskal-Wallis H following by Mann-Whitney U test).

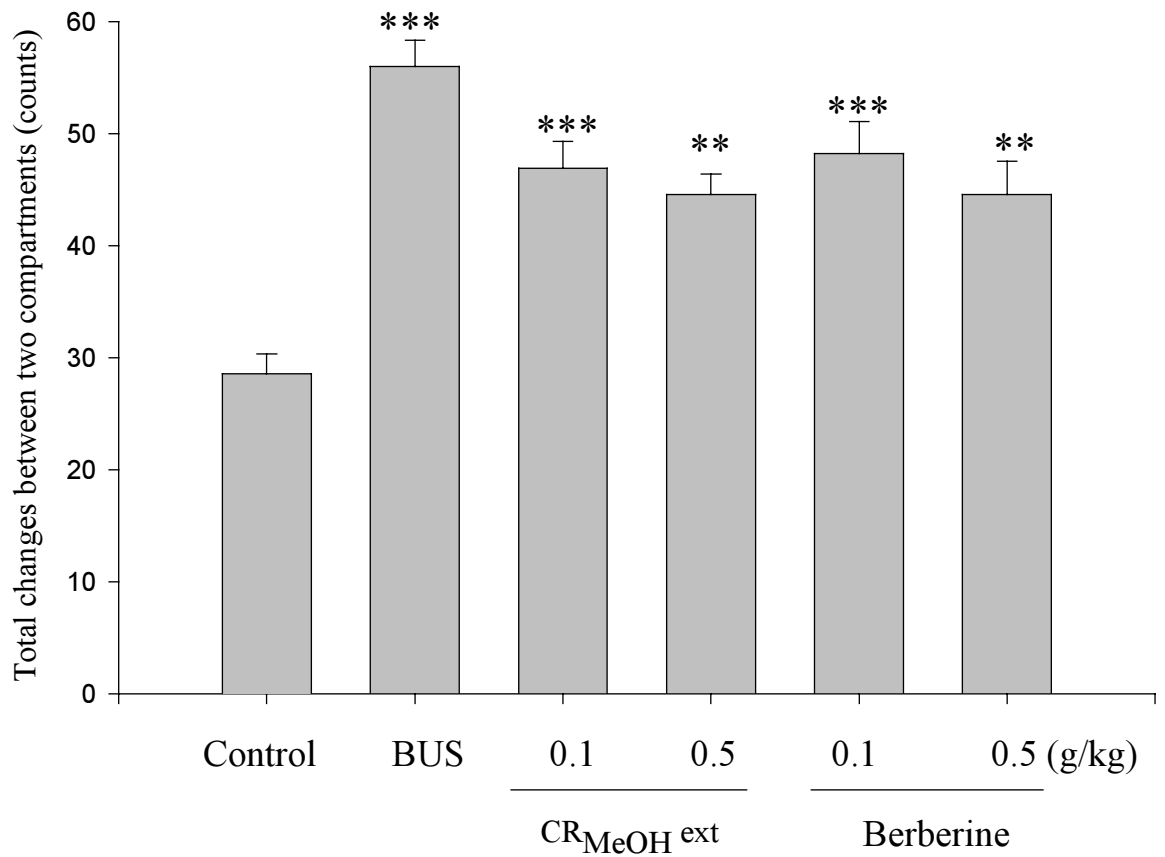


Fig 3. Effects of buspirone (BUS), methanol extract of *Coptis rhizome* (CR<sub>MeOH</sub> ext) and berberine on the total changes between two compartments in the Black & white test in mice. \*\*P < 0.01, \*\*\*P < 0.001 as compared with the control group (Oneway ANOVA following by Scheffe test).

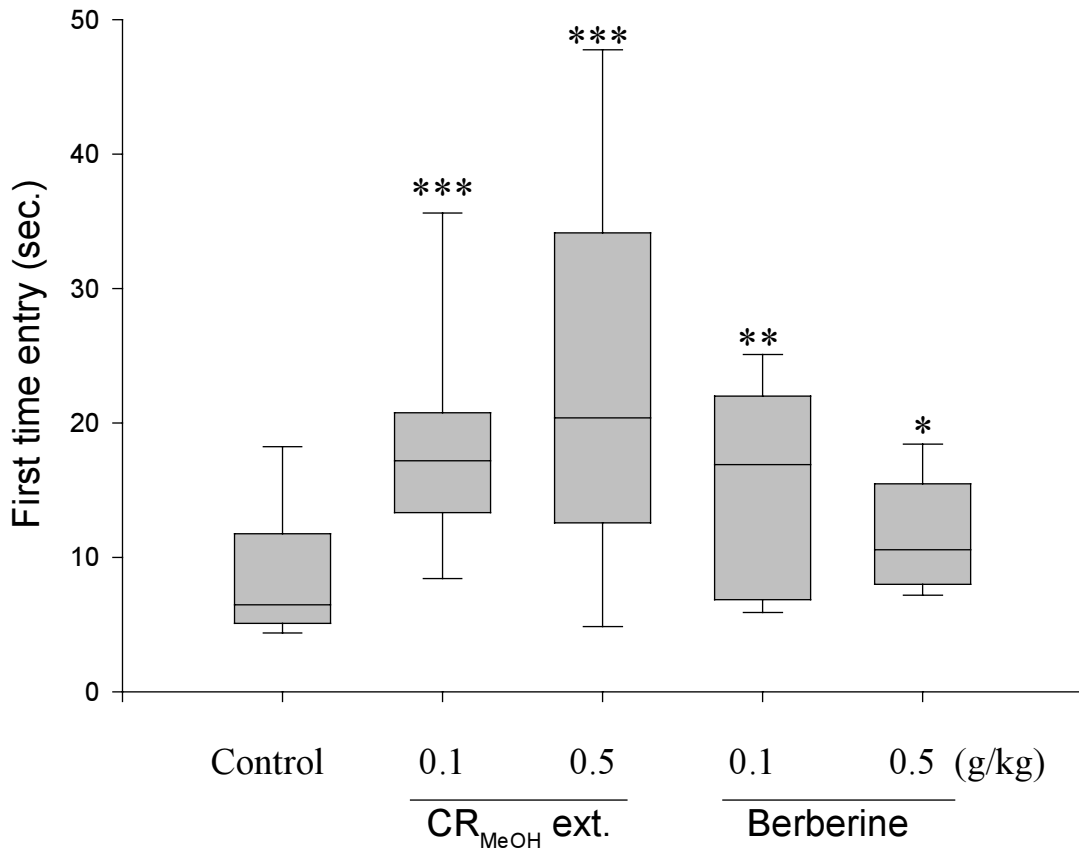


Fig 4. Effects of methanol extract of Coptis rhizome (CR<sub>MeOH</sub> ext.) and berberine after one-week administration on the first time entry from white to black in black & white test in mice. \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001 as compared with the control group. (Kruskal-Wallis H following by Mann-Whitney U test).

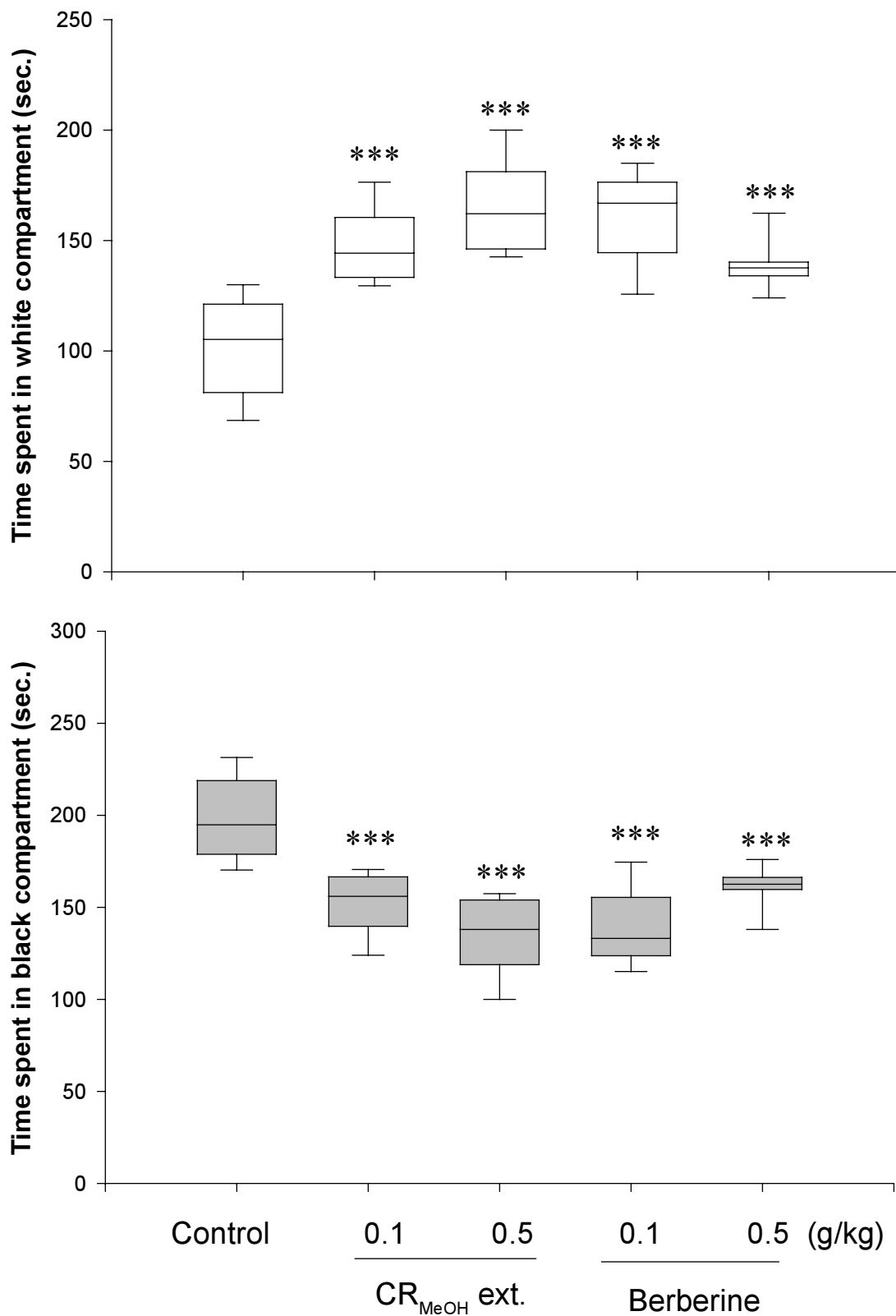


Fig 5. Effects of methanol extract of Coptis rhizome (CR<sub>MeOH</sub> ext.) and berberine after one-week administration on the time spent in the white and black compartments in black & white test in mice. \*\*\*P < 0.001 as compared with the control group (Kruskal-Wallis H following by Mann-Whitney U test).



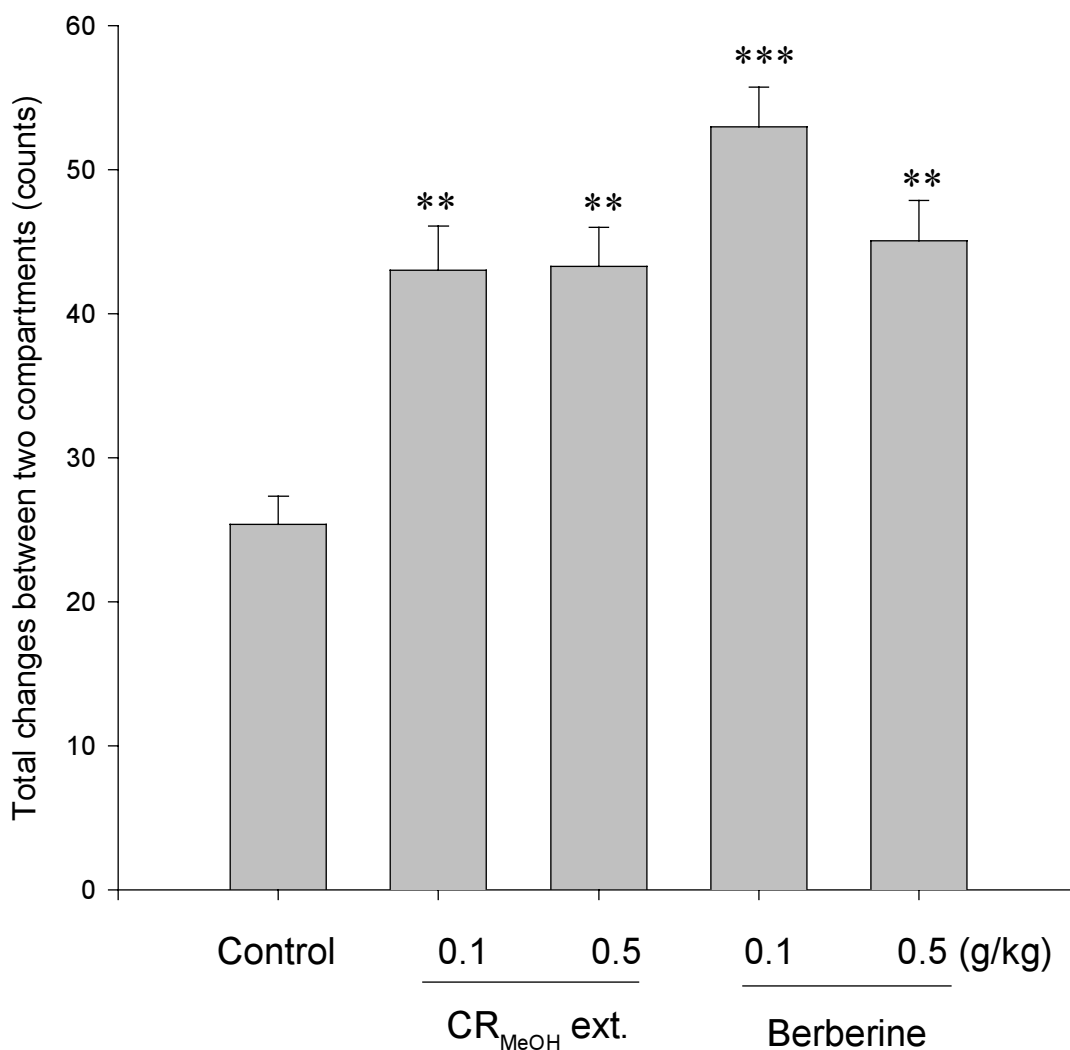


Fig 6. Effects of methanol extract of Coptis rhizome (CR<sub>MeOH</sub> ext.) and berberine after one week administration on the total changes between two compartments in black & white test. \*\*P < 0.01, \*\*\*P < 0.001 as compared with the control group (One-way ANOVA following by Scheffe test).

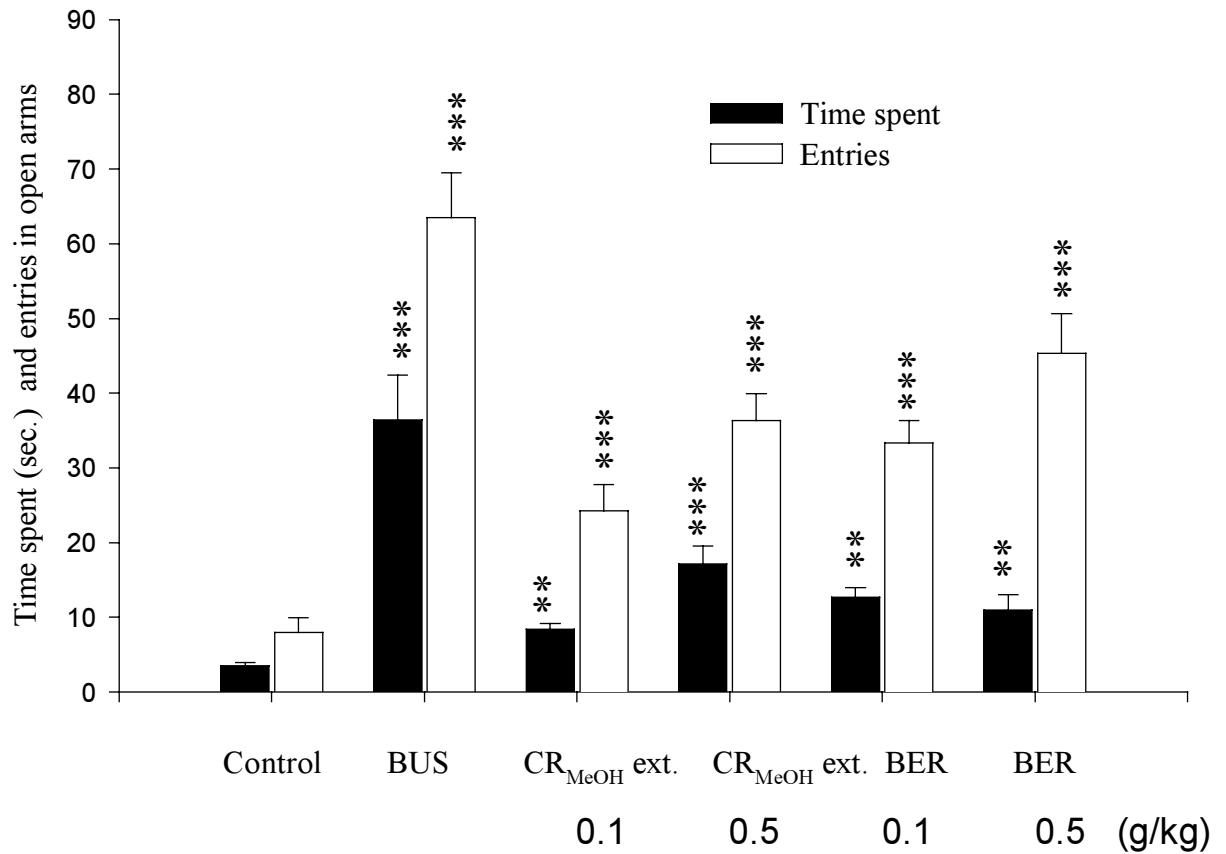


Fig 7. Effects of buspirone (BUS, 2 mg/kg), methanol extract of Coptis rhizome (CRMeOH ext.) and berberine (BER) on the time spent and entries in open arms during 5 mins in mice. \*\*P < 0.01, \*\*\*P < 0.001 as compared with the control group, respectively (One-way ANOVA following by Scheffe test).

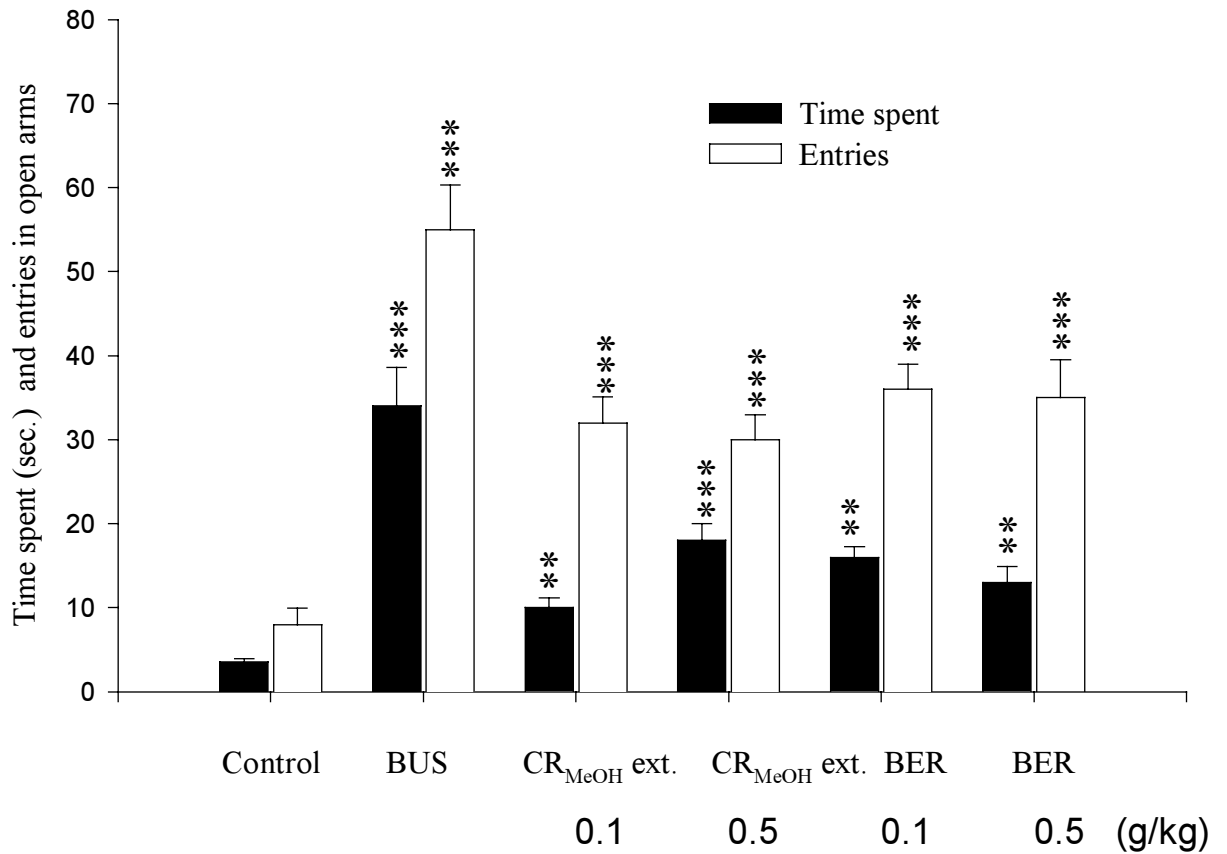


Fig 8. Effects of buspirone (BUS, 2 mg/kg), methanol extract of Coptis rhizome (CRMeOH ext.) and berberine (BER) after one-week administration on the time spent and entries in open arms during 5 mins in mice. \*\* $P < 0.01$ , \*\*\* $P < 0.001$  as compared with the control group, respectively (One-way ANOVA following by Scheffe test).

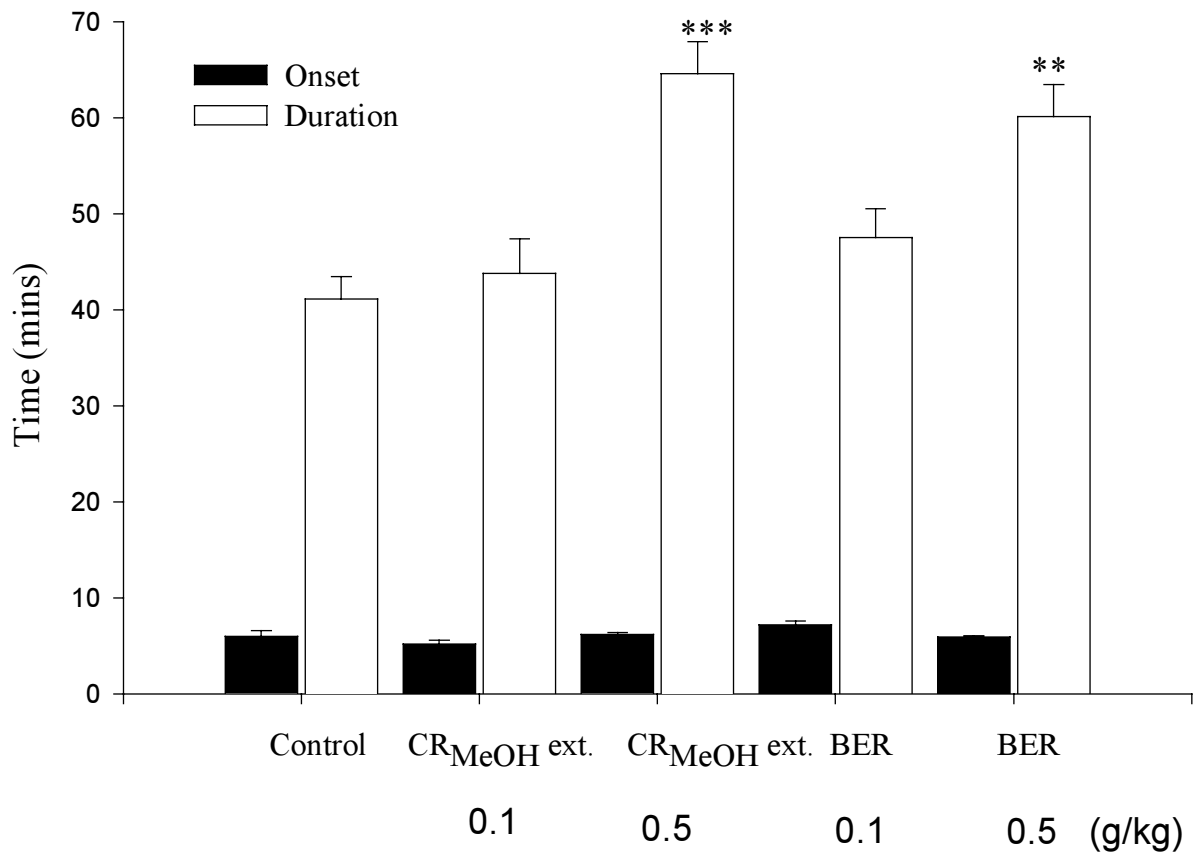


Fig 9. Effect of methanol extract of Coptis rhizome (CR<sub>MeOH</sub> ext.) and berberine (BER) on the hexobarbital-induced hypnosis in mice. \*\*P < 0.01, \*\*\*P < 0.001 as compared with the control group. (One-way ANOVA following by Scheffe test).

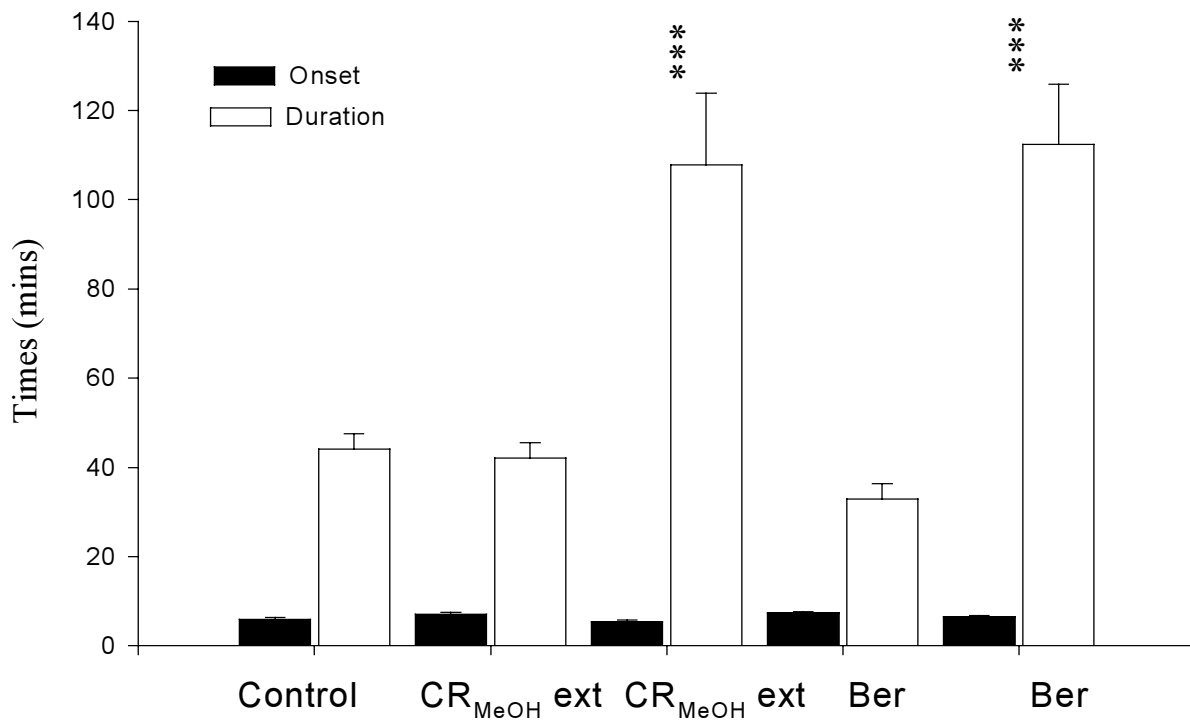


Fig 10. Effect of methanol extract of Coptis rhizome (CR<sub>MeOH</sub> ext) and berberine after one-week administration on the hexobarbital-induced hypnosis in mice. \*\*\*P < 0.001, as compared with the control group. (One-way ANOVA followed by Scheffe test).

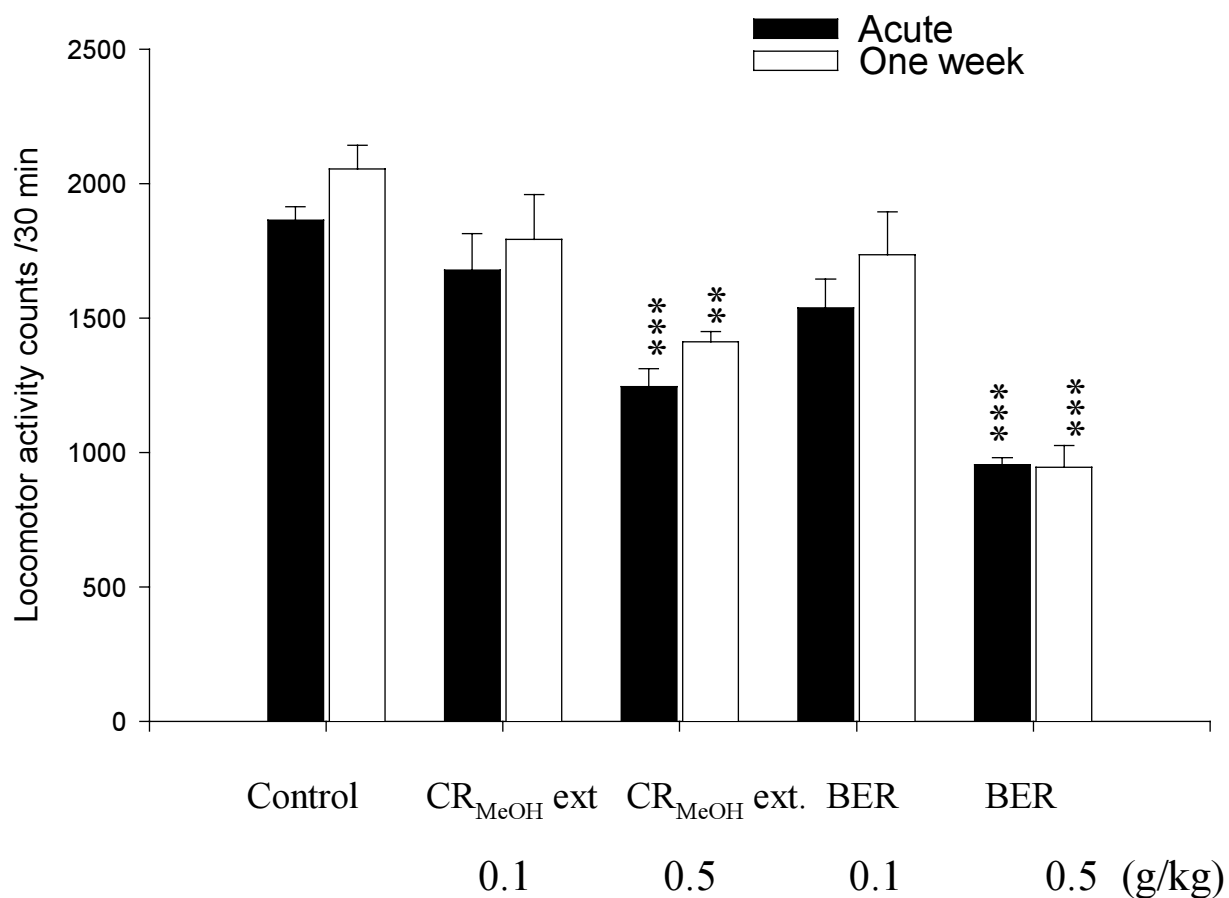


Fig 11. Effect of methanol extract of Coptis rhizome (CR<sub>MeOH</sub> ext.) and berberine (BER) after acute or one-week administration on the locomotor activity in rats. \*\*P < 0.01, \*\*\*P < 0.001 as compared with the control group, respectively. (One-way ANOVA following by Scheffe test).

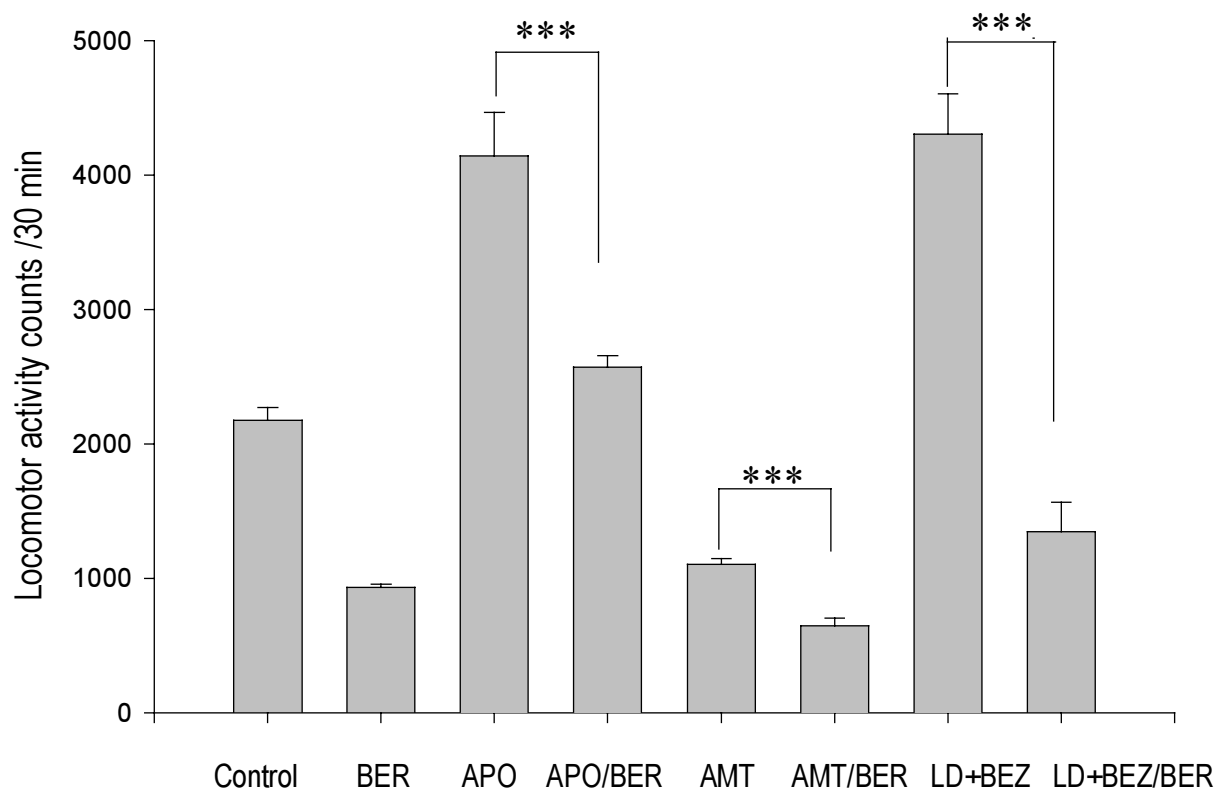


Fig 12. Effects of berberine (BER, 0.5 g/kg) on the changes of apomorphine (APO, 3 mg/kg)-,  $\alpha$ -methyl-p-tyrosine (AMT, 100 mg/kg)-, and l-dopa plus benserazide (LD+BEZ)-induced locomotor activities. \*\*\*P < 0.001 as compared with the APO and AMT group, respectively. (One-way ANOVA following by Scheffe test).

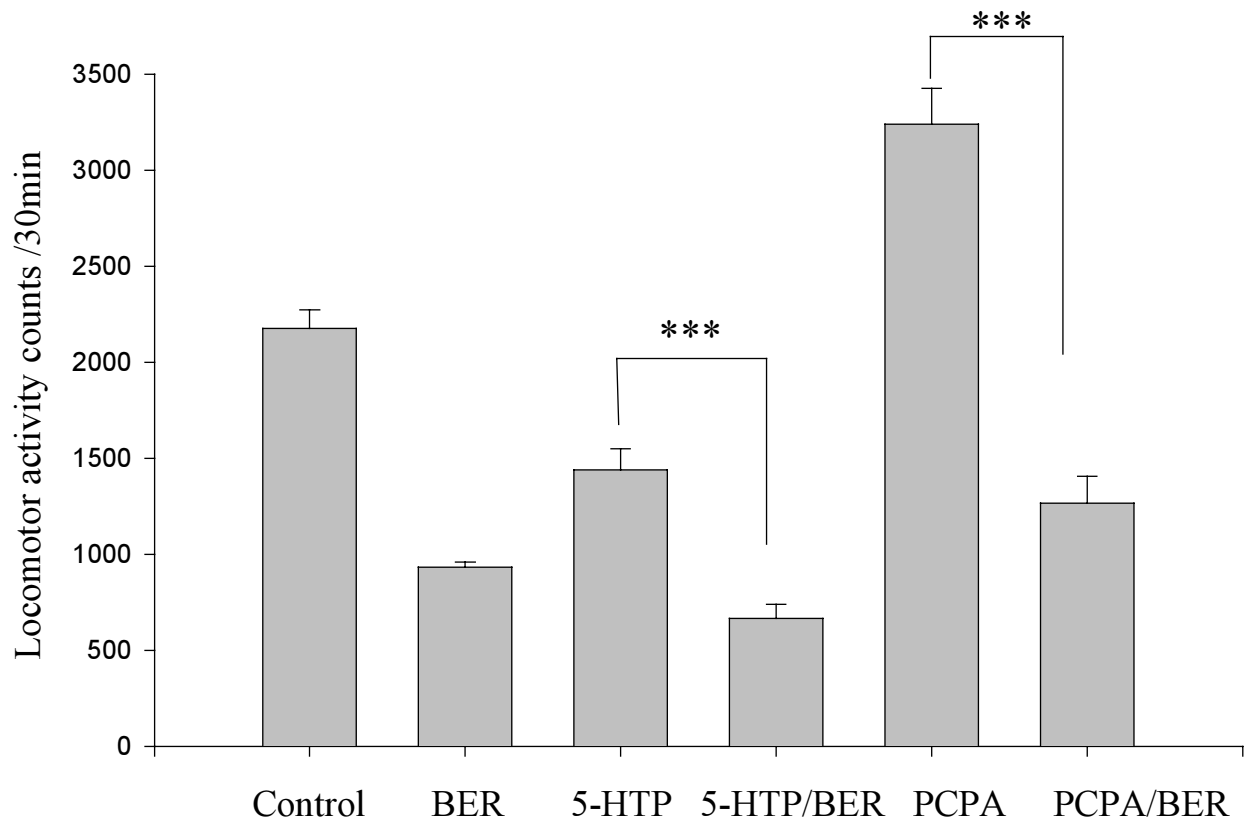


Fig 13. Effects of berberine (BER, 0.5 g/kg) on the changes of 5-hydroxytryptophan (5-HTP, 50 mg/kg)- and p-chloro-phenylalanine (PCPA, 200 mg/kg)-induced locomotor activities in rats. \*\*\* $P < 0.001$  as compared with 5-HTP and PCPA group, respectively. (Oneway ANOVA following by Scheffe test).



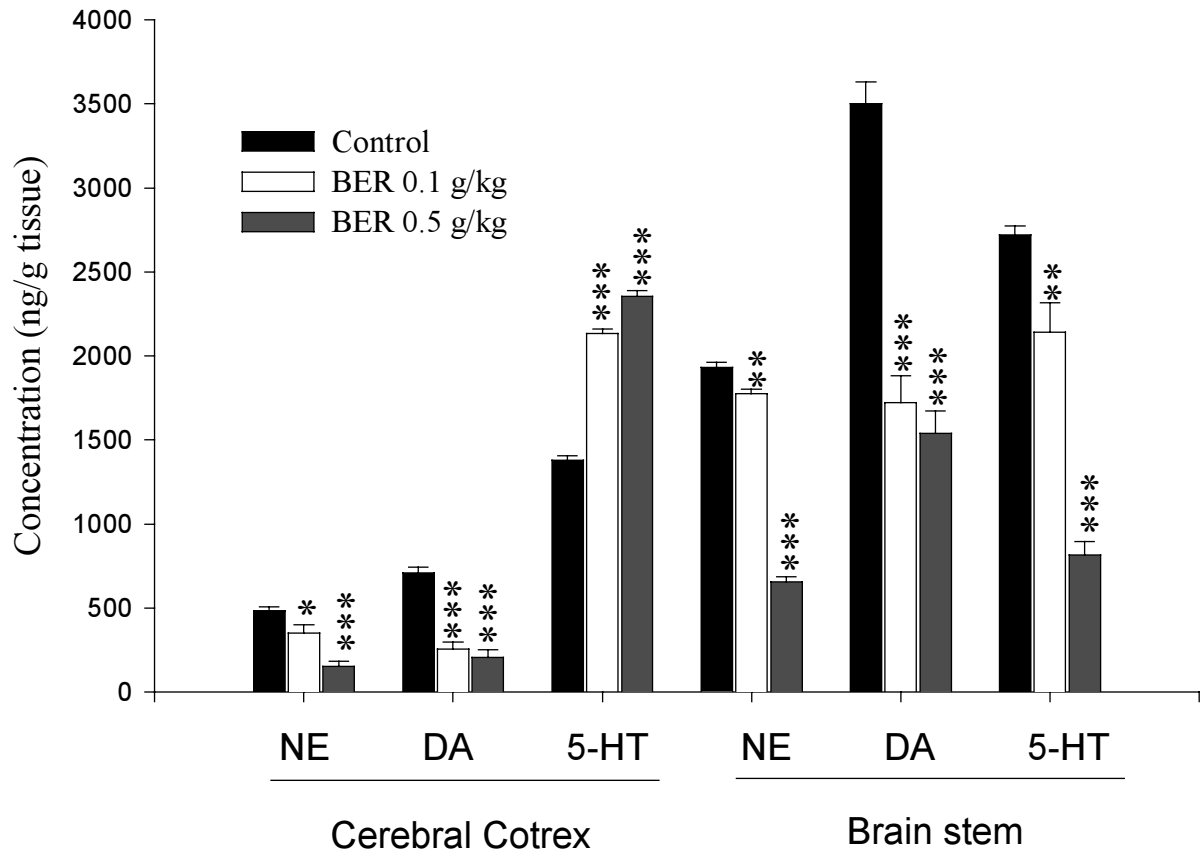


Fig 14. Effect of BERBERINE (0.1, 0.5 g/kg) on the monoamines' levels in the cortex and brain stem of rats. \*\*P < 0.01, \*\*\*P < 0.001 as compared with the control group, respectively. (unpaired Student's t-test).