行政院國家科學委員會補助專題研究計畫成果報告

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※ **知母及其主要活性成分mangiferin對熱源性發燒大鼠** ※
※ **與急性熱中暑大鼠體溫調節作用之研究** ※
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行政院國家科學委員會專題研究計畫成果報告

知母及其主要活性成分 mangiferin 對熱源性發燒大鼠與急性熱中暑大鼠體溫調節作用之研究

The thermoregulatory effects of *Anemarrhena asphodeloides* and its main active substance, mangiferin, on pyrogenic fever and acute heatstroke in rats

計畫編號：NSC89-2320-B039-033

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一、中文摘要

知母為百合科植物知母（*Anemarrhena asphodeloides*）之乾燥根莖，具抗菌、抗腫瘤、抗血小板凝集和解熱等作用；mangiferin （芒果素）為知母的活性成分之一，具抗發炎、抗自由基、抗菌、抗病毒以及降血糖等作用。但有關知母及 mangiferin 解熱作用之作用機轉至今仍未見有研究報告提出，因此本研究擬針對知母及 mangiferin 於室溫下對熱原性發燒大鼠解熱作用之機轉進行進一步之探討，並利用急性熱中暑大鼠實驗模式評估知母及 mangiferin 對熱中暑之療效。

實驗結果顯示，知母乙醇抽取物 (0.1-2.0g/kg , i.p) 與其主要活性成分 mangiferin (5-40 mg/kg, i.p.)均可明顯降低室溫下正常清醒大鼠之體溫，且具劑量依存性；此外，知母乙醇抽取物(0.1-1.0 g/kg , i.p)或 mangiferin (1-10 mg/kg, i.p.)對致熱原 lipopolysaccharide 及 interluckin-1β誘發高溫之大鼠亦具明顯之降溫作用；知母乙醇抽取物 (0.1-1.0 g/kg, i.p) 或 mangiferin (1.0-10 mg/kg, i.p.) 對 PGE_2 或 cAMP 誘發之體溫升高現像亦具抑制作用；但腹腔給予知母乙醇抽取物或 mangiferin 對 SNAP、SNP 或 cGMP 誘發之體溫升高現像則不具抑制作用。而在急性熱中暑大鼠實驗方面，不論升溫前或升溫後腹腔給予知母乙醇抽取物或 mangiferin 對熱中暑大鼠之中暑產生時間或存活時間均無作用。

綜合以上結果，可推知母乙醇抽取物或 mangiferin 具解熱降溫作用，其作用機轉可能與抑制中樞 prostaglandin 等與體溫調節相關系統之活性有關。

關鍵詞：知母、芒果素、熱原性發燒

Abstract

Anemarrhena asphodeloides was an ancient Chinese herb to cure infection-induced fever. It was reported to have the effects of antitumor and anti-agglutination. Mangiferin is a major C-glucosylxanthine purified from *Anemarrhena asphodeloides*. It was reported to have anti-virus, anti-inflammatory and anti-diabetic effects. However, the effects and mechanism of *Anemarrhena asphodeloides* and mangiferin on the antipyretic effects are not investigated even till now. On this account, the purpose of the present study was intended to investigate the thermoregulatory mechanism of *Anemarrhena asphodeloides* and mangiferin on pyrogenic fever and heatstroke in rats.

In unanesthetized rats, the ethanol extracts of *Anemarrhena asphodeloides* (AA, 0.1-2.0g/kg, i.p.) and mangiferin (5-40 mg/kg, i.p.) caused a dose-related fall in colonic temperature at room temperature. The fever induced by either lipopolysaccharide (LPS, 100μg/kg, i.p.) or interluckin-1β (10ng/10μl, i.c.v.) was attenuated by treatment with (AA,

0.1-1.0g/kg, i.p.) or mangiferin (1-10 mg/kg, i.p.). The hyperthermia induced by PGE2 (200ng/10μl, i.c.v.) or 8-Bromo-cAMP (cAMP analogue, 40μg/10μl, i.v.c.) was attenuated by treatment with AA (0.1-1.0g/kg, i.p.) and mangiferin (1-10mg/kg, i.p.) in rats. But, AA and mangiferin could not inhibit the hyperthermia induced by S-Nitroso-N-acetylpen icillamine (nitric oxide donor, 10μg/10μl, i.c.v.), sodium nitroprusside (NO releaser, 20μg/10μl, i.c.v.) or 8-Bromo-cGMP (cGMP anologue, 100μg/10μl, i.c.v.) in unanesthetized rats. Both AA and mangiferin could not prolong the onset and survival time on the rats with heatstroke.

These results indicate that AA and mangiferin its antipyretic effects mainly through the central nervous prostaglandin mechanisms.

Keywords: *Anemarrhena asphodeloides,* mangiferin, pyrogenic fever

二、緣由與目的

知母為百合科植物知母（*Anemarrhena asphodeloides*）之乾燥根莖，始載於神農本草經列為中品，「味苦、寒。主消渴，熱中，除邪氣，補不足，益氣。」[1]。具清熱瀉火、生津潤燥之功，中醫臨床上常用于外感熱病及高熱煩渴等熱症之治療；現代藥理學研究證明知母具明顯之抗菌、抗腫瘤等作用[2]；1935 年，大陸學者經利彬等報告指出知母浸膏皮下注射，能防止和治療大腸桿菌對家兔引起之高熱，且作用持久[3]。1982 年，陳銳群等發現知母根莖所含之皂﹖具明顯降低甲狀腺造成之耗養率增高及抑制 Na^+-K^+‥‥之活性作用，據此推論解釋知母清熱瀉火之功效[4]。

Mangiferin （芒果素、芒果‥‥） 屬 xanthone glycoside[5,6]，為知母的活性成分之一，並廣泛存在於漆樹科芒果（*Mangifera indica*）[7]、龍膽科肺形草（*Trichomanes reniforme*）[8]與當藥（*Swertia punicea*）[5]等具清熱瀉火、生津潤燥作用之藥用植物或中藥中。實驗証明 mangiferin 除具抗發炎[9]、抗自由基[10,11]等作用外，尚具有抗菌、抗病毒[12,13]以及降血糖[14]等作用。

然有關知母及 mangiferin 解熱降溫作用之機轉至今仍並未見有研究報告提出，因此，本研究乃先針對 puerarin 對致熱原 lipopolysaccharide (LPS) 或 interluckin-1β (IL-1β)誘發高溫大鼠之解熱作用進行測定，再利用 prostaglandin E2 (PGE2)、cAMP 之類似物 (8-Bromo-cAMP)、NO donor (S-Nitroso-N-acetylpenicillamine, SNAP)、NO 釋放劑(sodium nitroprusside, SNP) 或 cGMP 之類似物(8-Br-cGMP)等，進行更深入之作用機轉探討。

由於臺灣地處亞熱帶，夏季高溫多雨，加上全球性之溫室效應，更使得氣溫連創新高，中暑患者日益增加，輕則影響日常生活與工作，重則導致死亡，不可不慎。因此本研究最後擬利用陽明大學生理學研究所林茂村博士所開發之急性熱中暑大鼠研究模式[15]，進行知母及 mangiferin 對實驗性急性熱中暑大鼠之療效評估，期有助於中藥新用途之開發。

三、結果與討論

實驗結果發現腹腔給予知母乙醇抽取物 (0.1-2.0g/kg, i.p) 與其主要活性成分 mangiferin (5-40 mg/kg, i.p.)均可明顯降低室溫下正常清醒大鼠之體溫，且具劑量依存性，給藥後約 80 分鐘體溫降至最低，其後則慢慢回復，持續時間則會隨劑量增加而延長 (Fig.1,2)。

由於傳統上把能引起人體或動物發熱的物質，通稱為致熱原(pyrogen)，一般又可分為外生性致熱原及內生性致熱原[16]。而外生性致熱原(如：endotoxin)可促使白血球與巨噬細胞等釋放內生性致熱原(如：interleukin-1, tumor necrosis factor, interferon等)，後者再作用於體溫調節中樞而產熱[17-19]。1923年Seibert 指出引起動物體發燒的物質主要是來自葛蘭氏陰性菌細胞壁受破壞所游離出的毒素，稱為細菌內毒素，其會造成動物畏寒、發燒等症狀，甚至導致敗血性休克(septic shock)而死

亡；1943年Shear根據細菌內毒素結構上特性，另命名為lipopolysaccharide[20]。Interleukin-1(IL-1)為細菌內毒素誘發發燒機制中，所媒介之最主要內生性熱原[21]，IL-1源自於巨噬細胞、免疫性T、B淋巴細胞和血管內皮細胞等，隨著血液循環分布全身[21]，亦可由中樞星狀細胞(astrocyte)和微小神經膠細胞(microglia)合成[22]；IL-1β由側腦室給予大鼠可誘發一具劑量依存性、明顯持久之高溫[23]。Aspirin可抑制cyclooxygenase，進而減少prostaglandins之合成，為臨床上常用之解熱劑[24]。本研究利用腹腔給予LPS (100μg/kg)或側腦室給予IL-1β (10ng/10μl)誘發一較符合實際引發疾病發熱之動物模式，並以aspirin作為正對照組。實驗結果顯示，腹腔給予知母乙醇抽取物(0.1-1.0g/kg)或mangiferin (1-10 mg/kg)對致熱原LPS及IL-1β誘發高溫之大鼠亦具明顯之降溫作用(Table 1)。

此外，近年來報告指出cytokines或LPS能刺激腦內astroglia，經由精胺酸路徑合成大量之cyclic GMP [25]；而最近之研究更進一步發現LPS或IL-1β可藉由tyrosine kinases路徑將訊息傳入細胞內，並增加inducible nitric oxide synthase (iNOS)及cyclooxygenase II (COX-2)之量，促使NO及PGE等致炎介質合成增加[26,27]。將PGE_2直接注入大鼠之側腦室可引起一快速之升溫之現象[28]；其次，1982年林茂村教授研究指出PGE_2誘發之升溫作用可能是經由下視丘之NE-cAMP徑路而達成[29]。NO能活化腦細胞製造cGMP作為次級神經傳遞物質，產生各種生理或病理反應[30]，早在1983年Kandasamy等發現腦室給予cGMP之類似物可誘發發燒之反應[31]。因此本研究併用PGE_2 (200ng/rat, i.c.v.)、cAMP之類似物(8-Bromo-cAMP, 40μg/rat, i.c.v.)、NO donor SNAP (10μg/10μl, i.c.v.)、NO釋放劑SNP (20μg/10μl, i.c.v.)和cGMP之類似物8-Bromo-cGMP (100μg/10μl, i.c.v.)等藥物，以了解知母乙醇抽取物或mangiferin解熱作用之作用機轉，實驗結果發現腹腔給予知母乙醇抽取物(0.1-1.0g/kg)或mangiferin (1.0-10 mg/kg)對PGE_2或cAMP誘發之體溫升高現像具抑制作用(Table 2)；但腔給予知母乙醇抽取物或mangiferin即使在較高劑量下對SNAP、SNP或cGMP誘發之體溫升高現像均不具抑制作用(Table 3)。

而在急性熱中暑大鼠實驗方面，動物長期暴露於高溫之環境下（>42℃），當熱量之產生超過熱量之散失時，會導至高溫，動物不斷藉喘氣來散熱，因而表現出過度換氣(hyperventilation)現象，使得Pco_2下降，動脈中大量二氧化碳喪失，血液之酸鹼失調，pH值上升，由於體溫不斷增高，可能直接造成各器官組織嚴重受傷，或是熱刺激增加靜脈壓(venous pressure)和週邊血管擴張(peripheral vasodilatation)、心跳加速，進而造成心衰竭、心輸出量減少，血壓下降（亦即熱中暑產生），各器官血流不足，sGOT、sGPT、k^+、BUN和glucose等增加，腎、肝、肺、肌肉等組織壞死。其中循環之改變以及直接高溫傷害腦組織，會導致腦血管充血(vasocongestion)、腦水腫(cerebral edema)和其他神經傷害(neuron damage)。而腦水腫和腦充血則會引起顱內壓增加(intracranial hypertension,) [32]。平均血壓下降和顱內壓增加，則會使腦通流壓(cerebral perfusion pressure)減少，進而造成腦血流量減少或腦缺血，並促使下視丘IL-1β、5-HT和DA濃度的增加，中樞神經系統受到傷害，一系列複雜之作用導致熱中暑病症於焉產生，甚或死亡[32,33]。實驗結果發現，不論升溫前、升溫後或中暑時腹腔給予知母乙醇抽取物或mangiferin對熱中暑大鼠之中暑產生時間或存活時間均無影響。

綜合以上結果，顯示知母乙醇抽取物或mangiferin具明顯之解熱降溫作用，其解熱作用之機轉可能與抑制中樞prostaglandin等與體溫調節相關系統之活性有關。

四、計畫成果自評

本研究計畫案計畫書中擬進行之各項實驗除部分藥物給藥方式與劑量，依實際實驗狀況略有修正更動外，均已完成。

五、參考文獻

[1] **孫星衍、孫馮翼：神農本草經卷二。昭人出版社。台北；**1977**；**pp.220.

[2] **蕭培根、陰健，郭力弓：中藥現代研究與臨床應用** I**。學苑出版社。北京。**1994;pp.444-7**。**

[3] **經利彬等：國立北平研究院生理學研究所中文報告匯刊，**1935, (2), 39**。**

[4] **陳銳群等：生物化學與生物物理學報，**1982, 14(2), 159**。**

[5] Tan P, Liu YL and Hou CY: Structure of swertiapuniside from Swertia punicea Hems. *Acta Pharmaceutica Sinica*, 1992; 27(6):476-9.

[6] Aritomi M and Kawasaki T: A new xanthone C-glucoside, position isomer of mangiferin, from Anemarrhena asphodeloides Bunge. *Tetrahedron Letters*, 1969; 12: 941-4.

[7] Saito S, Nagase S and Ichinose K: New setroidal saponins from the rhizomes of Anemarrhena asphodeloides Bunge (Liliaceae), *Chemical & Pharmaceutical Bulletin,* 1994; 42(11):2342-5.

[8] Wada H, Shimizu Y, Tanaka N, Cambie R.C. and Braggins JE: Chemical and chemotaxonomical studies of ferns. LXXXVII. Constituents of Trichomanes reniforme. *Chemical & Pharmaceutical Bulletin*, 1995; 43(3): 461-5.

[9] **沙世炎、劉愛如：中草藥有效成分分析法。人民衛生出版社。北京。**1984; 696-7.

[10] Sato T, Kawamoto A, Tamura A, Tatsumi Y and Fujii T: Mechanism of antioxidant action of pueraria glycoside (PG)-1 (an isoflavonoid) and mangiferin (a xanthonoid).; *Chemical & Pharmaceutical Bulletin*, 1992; 40(3): 721-4.

[11] Hsu MF, Raung SL, Tsao LT, Lin CN, Wang JP: Examination of the inhibitory effect of norathyriol in formylmethionyl-leucyl- phenylalanine-induced respiratory burst in rat neutrophils; *Free Radical Biology & Medicine*, 1997, 23(7):1035-45.

[12] Zheng MS and Lu ZY: Antiviral effect of mangiferin and isomangiferin on herpes simplex vurus.; *Chinese Medical Journal*, 1990; 103(2): 160-5.

[13] Guha S, Ghosal S and Chattopadhyay U**：** Antitumor, immunomodulatory and anti-HIV effect of mangiferin, a naturally occurring glucosylxanthone; *Chemotherapy*, 1996, 42(6):443-51.

[14] Ichiki H, Miura T, Kubo M, Ishihara E, Komatsu Y, Tanigawa K and Okada M**：**New antidiabetic compounds, mangiferin and its glucoside; *Biological & Pharmaceutical Bulletin*, 1998, 21(12):1389-90.

[15] Kao TY, Chio CC and Lin MT: Hypothalamic dopamine release and local cerebral blood flow during onset of heatstroke in rats. *Stroke*, 1994; 25:2483-7.

[16] Mackowiak PA: Fever :basic mechanisms and ma-nagement. *Raven Press, New York,* 1991; 1-70.

[17] Saigusa T: Participation of interleukin-1 and tumor necrosis factor in the responses of the sympathetic nervous system during lipopolysaccharide-induced fever. *Pflugers Archiv*, 1989; 416: 225-9.

[18] Bouchama A, Parhar RS, Yazigi AE, Sheth K and Sedairy SA: Endotoxin and release of tumor necrosis factor and interleukin-1α in acute heat stroke. *J Appl Physiol*, 1991; 70: 2640-4.

[19] Cannon JG, Tompkins RG and Gelfand JA: Circulating interleukin-1 and tumor necrosis factor in septic shock and experiment endo-toxin fever. *J Infect Dis*, 1990; 161:79-81.

[20] Rietschel ET and Brade H**：**Bacterial Endotoxins. *Scientific Americant*, 1992; 8: 54-61.

[21] Kluger MJ: Fever: Role of pyrogen and cryogens. *Physiol Rev*, 1991; 71:93-127.

[22] Lechan RM, Toni R, Clark BD, Cannon, J G, Shaw AR, Dinarello CA and Reichlin S. Immu-noreactive interleukin-1β localization in the rat forebrain. *Brain Res*, 1989; 514:135-40.

[23] Morimotm A, Murakami N, Sakata Y, Watanabe T and Yamaguchi K: Functional and structural diffe-rences in febrile mechanism between rabbits and rats. *J Physiol*, 1990; 427**：**227-39.

[24] Susan JA, Keith EC and Warren LV: Blockade of prostaglandin E_1 hyperthermia by sodium salicyl-ate given into the ventral septal area of the rat brain. *J Physiol (London)*, 1987; 384: 223-31.

[25] Simmons ML and Murphy S: Cytokines regulate L-arginine-dependent cyclic GMP production in rat glial cells. *Eur. J. Neurosci*, 1993; 5, 825-31.

[26] Sautebin L, Ialenti A, Ianaro A and Dirosa M:Mo-dulation by nitric oxide of prostaglandin biosyn-thesis in the rat. *Br J Pharmacolol*, 1995;14,323-8.

[27] Swierkosz TA, Mitchell JA, Warner TD, Botting RM and Vane JR:Co-induction of nitric oxide syn-thase and cyclooxygenase: interactions betweem nitric oxide and prostanoids. *Br J Pharmacolol,* 1995; 114, 1335-42.

[28] Akio M, Naotoshi M and Yoshiyuki S: Functional and structural difference in febrile mechanism between rabbits and rats. *J Physiol (London)*, 1990; 427: 227-39.

[29] Lin MT,Wu JJ,Chandra A and Tsay BL: A nore-pinephrine-cyclic AMP link in the hypothalamic pathways which mediate fever induced by endoto-xin and prostaglandin E_2 in the rat. *J Pharmacol Exp Ther,* 1982; 222: 251-7.

[30]Bruhwtler J, Chleide E, Liegeos JF and Carreer F: Nitric oxide: new messenger in the brain. Neurosci. Biobehavior. Rev., 1993, 17, 373-84.

[31] Kandasamy SB and Williams BA: Central effects of dibutyryl cyclic AMP and GMP on the tempera-ture in conscious rabbits. *Brain Res*, 1983; 227, 311-20.

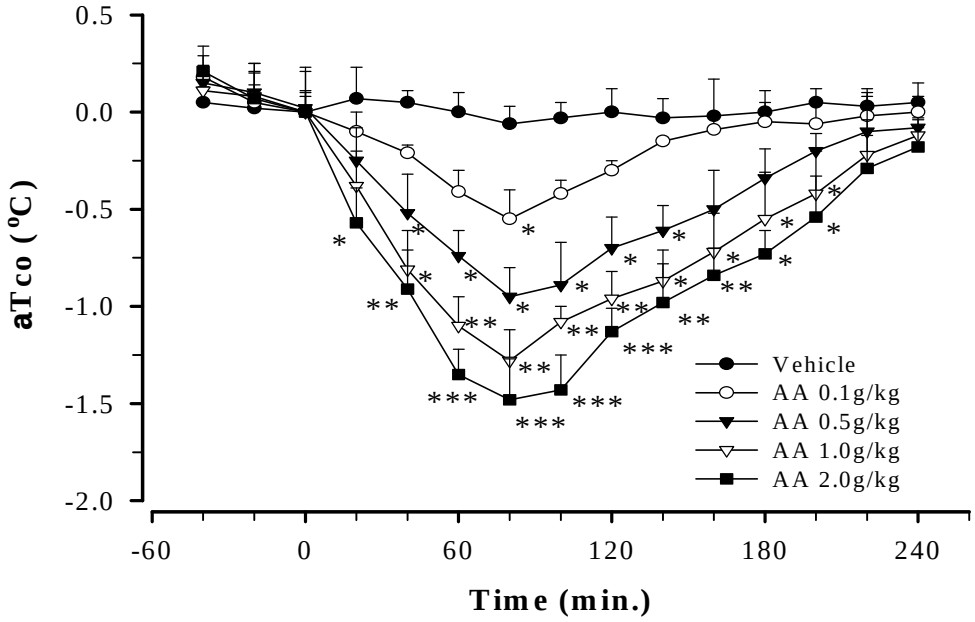

Fig. 1 Time course of the effects of the extracts of *Anemarrhena asphodeloides* (AA) on colonic temperature in rats. AA (0.1, 0.5, 1.0, 2.0g/kg, i.p.) was injected at 0 min. The colonic temperature of vehicle-injected rats was 37.82±0.20°C at time 0 min. Δ, denote the difference between the control value before injected and exchange after injected. The values are mean±SEM of 8-12 rats per group. *P<0.05, **P<0.01, ***P<0.001, significantly different from corresponding control value (vehicle group), ANOVA.

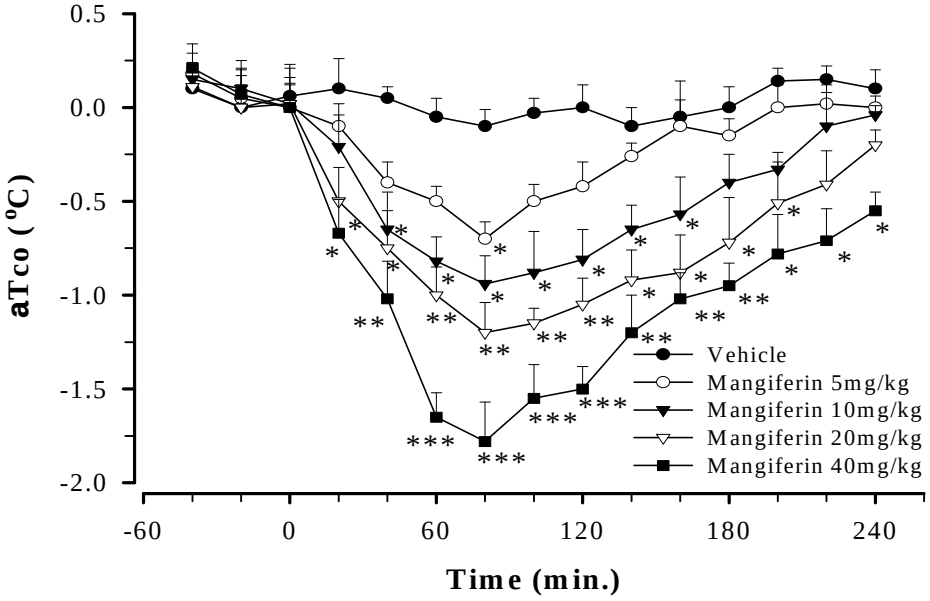

Fig. 2 Time course of the effects of mangiferin on colonic temperature in rats. Mangiferin (5, 10, 20, 40mg/kg, i.p.) was injected at 0 min. The colonic temperature of vehicle-injected rats was 38.12±0.15°C at time 0 min. Δ, denote the difference between the control value before injected and exchange after injected. The values are mean±SEM of 8-12 rats per group. *P<0.05, **P<0.01, ***P<0.001, significantly different from corresponding control value (vehicle group), ANOVA.

Table 1 Effects of the extracts of *Anemarrhena asphodeloides* (AA) and mangiferin on the colonic temperature in the hyperthermia rats induced by lipopolysaccharide (LPS) and interleukin-1β (IL-1β).

Treatment	Change in colonic temperature (Δ°C)		
	Normal	LPS	IL-1β
Vehicle (i.p.)	0.11±0.15	1.51±0.10	1.98±0.22
AA			
0.1 g/kg (i.p.)	-0.55±0.10	0.58±0.13*	1.04±0.14*
0.5 g/kg (i.p.)	-0.95±0.15*	-0.40±0.07**	0.67±0.23**
1.0 g/kg (i.p.)	-1.28±0.16**	-0.95±0.20***	-0.37±0.21***
Mangiferin			
1.0 mg/kg (i.p.)	-0.41±0.15	0.56±0.13*	1.12±0.10*
5.0 mg/kg (i.p.)	-0.70±0.09*	-0.33±0.21**	0.75±0.08**
10.0 mg/kg (i.p.)	-0.94±0.20*	-0.70±0.17***	-0.26±0.16***
Aspirin			
75 mg/kg (i.p.)	-0.12±0.13	0.42±0.17*	1.02±0.12*
150 mg/kg (i.p.)	-0.21±0.21	0.14±0.09**	0.72±0.21**

AA and mangiferin were injected 40 min after LPS (100μg/kg) intraperitoneal injected (i.p.) or 120 min after IL-1β (10ng/rat) intracerebroventricular injected (i.c.v.). The values are mean±SEM of 8 rats per group. Δ, Denote the difference between the control values before injected and maximum exchange after injected. *P<0.05, **P<0.01, ***P<0.001, significantly different from the corresponding control values (vehicle group), ANOVA.

Table 2 Effects of the extracts of *Anemarrhena asphodeloides* (AA) and mangiferin on the hyperthermia induced by intracerebroventricular injection (i.c.v.) of prostaglandin E2 (PGE2) and 8-Bromo-cAMP.

Treatment	Change in colonic temperature (Δ°C)		
	Normal	PGE_2	8-Bromo-cAMP
Vehicle (i.p.)	0.11±0.15	1.33±0.29	0.75±0.17
AA			
0.1 g/kg (i.p.)	-0.55±0.10	0.68±0.13*	0.14±0.10*
0.5 g/kg (i.p.)	-0.95±0.15*	-0.58±0.07**	-0.42±0.19**
1.0 g/kg (i.p.)	-1.28±0.16**	-0.97±0.15***	-0.67±0.21***
Mangiferin			
1.0 mg/kg (i.p.)	-0.41±0.15	0.45±0.13*	0.08±0.11*
5.0 mg/kg (i.p.)	-0.70±0.09*	-0.17±0.20**	-0.59±0.18**
10.0 mg/kg (i.p.)	-0.94±0.20*	-0.88±0.12***	-0.96±0.15***

AA and mangiferin were injected 30 min before PGE_2 (500ng/rat, i.c.v.) injected and 10 min after 8-Bromo-cAMP (40μg/rat, i.c.v.) injected. The values are mean±SEM of 8 rats per group. Δ, Denote the difference between the control values before injected and maximum exchange after injected. *P<0.05, **P<0.01, ***P<0.001, significantly different from the corresponding control values (vehicle group), ANOVA.

Table 3. Effects of the extracts of *Anemarrhena asphodeloides* (AA) and mangiferin on the hyperthermia induced by intracerebroventricular injection of S-nitroso-N-acetylpenicillamine (SNAP), sodium nitroprusside (SNP) or 8-Bromo-cGMP

Treatment	Change in colonic temperature (Δ°C)			
	Normal	SNAP	SNP	8-Bromo-cGMP
Vehicle (i.p.)	0.11±0.15	1.81±0.20	1.35±0.21	0.75±0.13
AA				
0.1 g/kg (i.p.)	-0.55±0.10	1.79±0.11	1.30±0.15	0.76±0.18
0.5 g/kg (i.p.)	-0.95±0.15*	1.52±0.20	1.18±0.15	0.60±0.21
1.0 g/kg (i.p.)	-1.28±0.16**	1.45±0.16	1.00±0.10	0.43±0.14
2.0 g/kg (i.p.)	-1.42±0.20**	1.36±0.13	0.95±0.20	0.21±0.20
Mangiferin				
5.0 mg/kg (i.p.)	-0.70±0.09*	1.82±0.08	1.34±0.21	0.74±0.11
10.0 mg/kg (i.p.)	-0.94±0.20*	1.71±0.17	1.25±0.18	0.62±0.16
20.0 mg/kg (i.p.)	-1.20±0.16**	1.50±0.10	1.15±0.04	0.54±0.22
40.0 mg/kg (i.p.)	-1.78±0.21***	1.44±0.21	0.10±0.17	0.43±0.20

AA and mangiferin were injected 120 min after SNAP (10μg/10μl, i.c.v.), 120 min after SNP (20μg/10μl, i.c.v.) or 90 min after 8-Bromo-cGMP (100μg/10μl, i.c.v.) injected. The values are mean±SEM. of 8 rats per group. Δ, Denote the difference between the control values before injected and maximum exchange after injected. *P<0.05, significantly different from the corresponding control values (saline group), ANOVA.