甲苯达唑 吡喹酮 细粒棘球蚴 GPI GAPDH

3 Bradford MN. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding.
Anal Biochem 1976; 72: 265 - 8
4 McManus DP, Smyth JD.
Isoelectric focusing of some enzymes from *Echinococcus granulosus* (horse and sheep strains) and *E multilocularis*
Trans R Soc Trop Med Hyg 1979; 73: 259 - 65

75~77

甲苯达唑和吡喹酮对小鼠细粒棘球蚴囊壁的葡萄糖磷酸异构酶和甘油醛磷酸脱氢酶的影响

尤纪青、肖树华、郭惠芳、焦佩英、梅静艳、姚民一（中国预防医学科学院寄生虫病研究所、世界卫生组织疟疾、血吸虫和丝虫病合作中心，上海 200025，中国）

关键词 棘球属；葡萄糖磷酸异构酶；甘油醛磷酸脱氢酶；甲苯达唑；吡喹酮

目的：测定抗包虫药物对细粒棘球蚴囊壁的葡萄糖磷酸异构酶（GPI）和甘油醛磷酸脱氢酶（GAPDH）的影响。方法：感染细粒棘球蚴囊达 8 - 10 个月的小鼠 ig 甲苯达唑（Meb）或吡喹酮（Pra）治疗，然后剖杀取囊，用生化方法测定 GPI 和 GAPDH 活力。结果：感染小鼠用 Meb 25 - 50 mg·kg^{-1}·d^{-1}治疗，连续 7 - 14 d，未见对 GAPDH 活力有明显影响。若用 Pra 500 mg·kg^{-1}·d^{-1} ig 14 d，囊壁的 GAPDH 活力被抑制 26.5 %。至于 GPI，仅 Meb 25 mg·kg^{-1}·d^{-1} × 14 d 组的癌囊示该酶活力被抑制 33.2 %。结论：GPI 和 GAPDH 不是有效的抗包虫药的主要作用靶。

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1997 Jan; 18 (1): 77 - 80

Antioxidative and chelating activities of phenylpropanoid glycosides from *Pedicularis striata*[1]

LI Ji, GE Rui-Chang, ZHENG Rong-Liang[2], LIU Zi-Min[3], JIA Zhong-Jian[3] (*Department of Biology and* [3]*Institute of Organic Chemistry, Lanzhou University, Lanzhou 730000, China*)

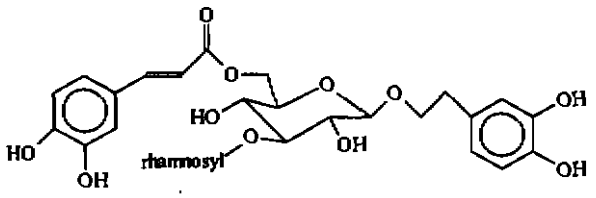
KEY WORDS phenylpropanoid glycosides; verbascoside; isoverbascoside; *Pedicularis striata*; antioxidants; chelating agents

AIM: To study the antioxidative and iron chelating activities of phenylpropanoid glycosides (PPG) isolated from a Chinese herb *Pedicularis striata*. METHODS: Antioxidative effects of PPG on lipid peroxidation induced by $FeSO_4$-edetic acid in linoleic acid were measured by thiobarbituric acid method. Chelating activities of PPG for Fe^{2+} were tested by differential spectrum method. RESULTS: The reaction rates (A_{532} · min^{-1}) of lipid peroxidation were 0.0046 in the control, 0.0021 in verbascoside group, and 0.0008 in isoverbascoside group. The chelating activity of isoverbascoside was 2-fold stronger than that of verbascoside. Permethyl verbascoside showed neither antioxidative nor chelating activities. CONCLUSION: The inhibitory effects of PPG with phenolic hydroxy groups on lipid peroxidation are owing to their chelating properties. Under physiological condition PPG-Fe^{2+} chelates are sufficiently stable. Thus PPG are able to inhibit the Fe^{2+}-dependent lipid peroxidation *in vivo* through chelating Fe^{2+} and exhibit their therapeutic potential by the same mechanism *in vitro*.

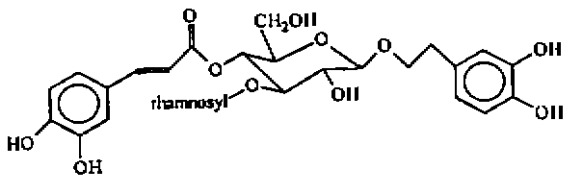
Phenylpropanoid derivatives existing in plants were used as antibiotics, ultraviolet protectants, and insect repellents[1]. Phenylpropanoid glycosides (PPG) exhibited antibiotic[2], antiviral[3], antiplatelet aggregation[4] and inhibition of leutriene B_4 formation[5]. We found PPG possessed scavenging effects on superoxide[6], inhibition on lipid peroxidation[7], and protection against oxidative

[1] Project partly supported by the National Natural Science Foundation of China, No 38970238, and partly by the State Key Laboratory of Applied Organic Chemistry
[2] Corresponding to Prof ZHENG Rong-Liang.
Received 1994-06-16 Accepted 1996-07-08

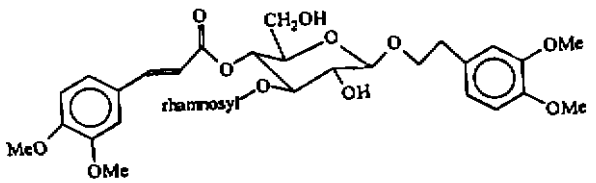
hemolysis[8]. Fe^{2+}-dependent lipid peroxidation is thought to play a central role in pathologically revelant oxy-radical-induced tissue damage *in vitro*[9]. Thus agents that inhibit Fe^{2+}-dependent lipid peroxidation may represent a rational approach to the management of oxy-radical related diseases. The present paper was to study the antioxidative and Fe^{2+} chelating activities of 2 PPG, with 4 phenolic hydroxy groups, isolated from a traditional Chinese herb *Pedicularis striata* which medicinal functions have been described in reference 6, and one modified PPG without phenolic hydroxy group.



Isoverbascoside



Verbascoside



Permethyl verbascoside

MATERIALS AND METHODS

Drugs and chemicals Verbascoside and isoverbascoside were extracted from *Pedicularis striata*, permethyl verbascoside was prepared from verbascoside[10]. 2-Thiobarbituric acid (TBA) was obtained from Sigma. Linoleic acid was obtained from Beijing Shijingshan Chemical Co, lot № 8308155. All other reagents were of AR.

Determination of antioxidative activity of PPG 0.1 mL of linoleic acid 2 $mol \cdot L^{-1}$ dissolved in 95 % ethanol was added into every 1.3 mL of KH_2PO_4 buffer (20 $mmol \cdot L^{-1}$, pH 6.8) followed by adding 1.5 $mmol \cdot L^{-1}$ PPG 0.1 mL or buffer 0.1 mL for control. After incubation at 37 ℃ for 5 min, $FeSO_4$-edetic acid 0.5 mL was added. At intervals of 30 min aliquots were taken out, 20 % trichloroacetic acid 0.5 mL and 0.67 % TBA 1.0 mL were added and simultaneously added 2 % butylated hydroxytoluene 0.05 mL to prevent from further peroxidation of linoleic acid during heating. The mixture was heated for 10 min in a boiling water bath and the tubes were then cooled and centrifuged. The absorption of supernatant was read at 532 nm. The antioxidative acitivity was expressed by reaction rate: $A_{532} \cdot min^{-1}$.

Measurement of chelating activity of PPG Chelating activity for Fe^{2+} by PPG was measured using differential spectrum[11]. Firstly, the absorption spectrum of PPG in buffer was recorded from 190 nm to 450 nm, then $FeSO_4$ was added into PPG solution and differential spectrum was recorded again by Shimadzu UV-240 spectrometer.

RESULTS

Antioxidative activity of PPG Both verbascoside and isoverbascoside can greatly inhibit lipid peroxidation in linoleic acid (Fig 1).

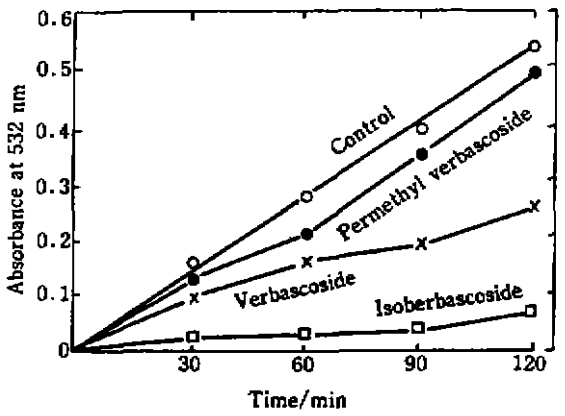


Fig 1. Inhibition of formation of TBA reactive substance by phenylpropanoid glycosides 0.1 $mmol \cdot L^{-1}$.

The reaction rates ($A_{532} \cdot min^{-1}$) were 0.0046 in the control, 0.0021 in verbascoside group and 0.0008 in isoverbascoside group. Thus antioxidative activity of isoverbascoside was stronger than that of verbascoside. The longer the incubation time, the stronger the inhibition appeared, while permethyl verbascoside had little effect on lipid peroxidation (0.0041). The inhibition on lipid peroxidation by various concentrations (10 − 40 $\mu mol \cdot L^{-1}$) of verbascoside or isoverbascoside was concentration-dependently

(data not shown).

Chelating activity of PPG Neither $FeSO_4$ nor PPG showed spectral peak at 365 nm, but Fe^{2+}-PPG chelate gave a peak at 365 nm when it was recorded against $FeSO_4$ or against PPG solution (Fig 2, 3).

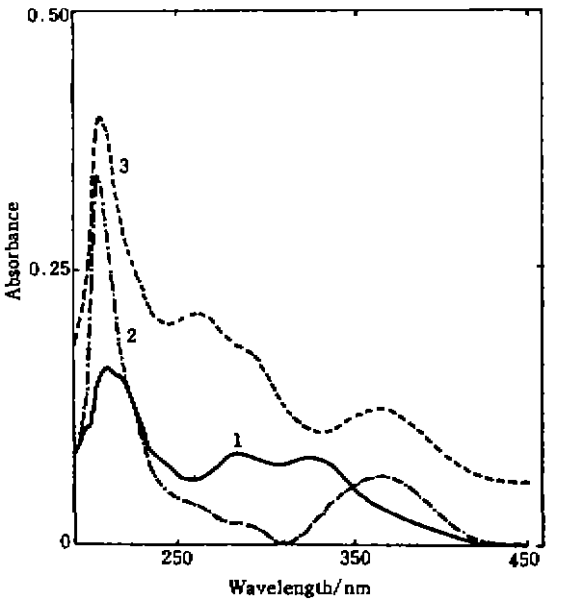


Fig 2. Absorption spectra of isoverbascoside and Fe^{2+}-isoverbascoside chelate. ① Absorption spectrum of isoverbascoside; ② differential spectrum of Fe^{2+}-isoverbascoside chelate against the $FeSO_4$ solution; ③ differential spectrum of Fe^{2+}-isoverbascoside chelate against the isoverbascoside solution.

According to the inference by Afanas'ev[11], the most interesting new peak should be a Fe^{2+}-phenol chelate. The intensity of this new peak of isoverbascoside-Fe^{2+} chelate was 2-fold higher than that of verbascoside-Fe^{2+} chelate. Therefore, in the light of the chelating activity for Fe^{2+}, isoverbascoside was stronger than verbascoside. However, when $FeSO_4$ was added to permephyl verbascoside in buffer there was no peak at 365 nm, thus permethyl verbascoside had no Fe^{2+} chelating activity (Fig 4).

The isoverbascoside-Fe^{2+} chelate and verbascoside-Fe^{2+} chelate were sufficiently stable, which spectra did not change as long as 5 h under pH 7.2.

DISCUSSION

Natural phenols such as PPG may possess a

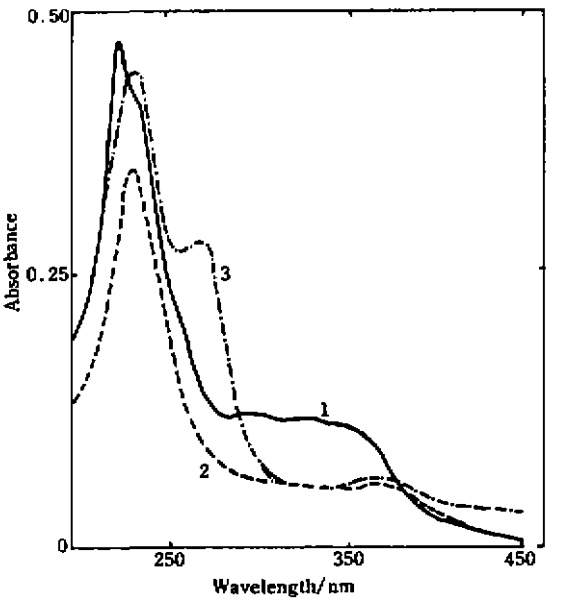


Fig 3. Absorption spectra of verbascoside and Fe^{2+}-verbascoside chelate. ① Absorption spectrum of verbascoside; ② differential spectrum of Fe^{2+}-verbascoside chelate against the $FeSO_4$ solution; ③ differential spectrum of Fe^{2+}-verbascoside chelate against the verbascoside solution.

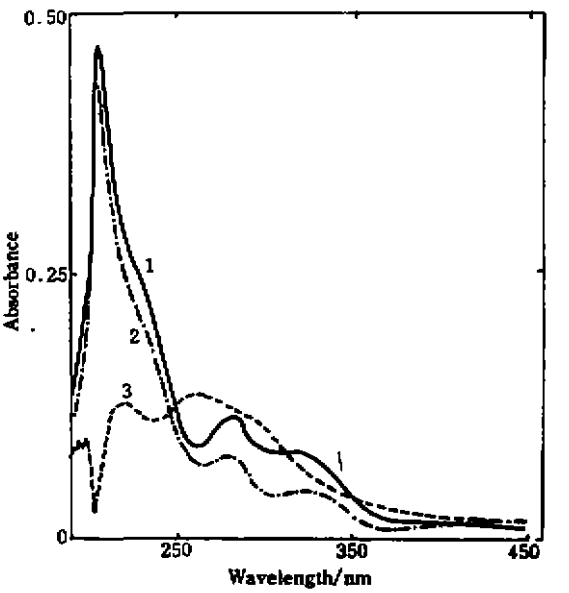


Fig 4. Absorption spectra of permethyl verbascoside and Fe^{2+}-permethyl verbascoside chelate. ① Absorption spectrum of permethyl verbascoside; ② differential spectrum of Fe^{2+}-permethyl verbascoside chelate against $FeSO_4$ solution; ③ differential spectrum of Fe^{2+}-permethyl verbascoside chelate against permethyl verbascoside solution.

unique activity to inhibit lipid peroxidation under at least 2 different stages: initiation by chelating iron ions and propagation by scavenging peroxyl radicals [12], and both activities related to their molecular structure, ie the number of phenolic hydroxy groups. Both isoverbascoside and verbascoside possess 4 phenolic hydroxy groups, but permethyl verbascoside possesses no phenolic hydroxy group, so the formers exhibit strong antioxidative and chelating activities, which of isoverbascoside were the strongest, while permethyl verbascoside was hardly any. This rank is also the same as that of inhibiting lipid peroxidation in microsomes[6], in micelles[7] and in oxidative hemolysis[8]. Therefore inhibiting effects of PPG with phenolic hydroxy groups on lipid peroxidation are really explained by their chelating properties.

Under physiological condition PPG-Fe^{2+} chelates are sufficiently stable. Thus PPG are able to inhibit the Fe^{2+}-dependent lipid peroxidation *in vivo* through chelating Fe^{2+} and exhibit their therapeutic potential by the same mechanism *in vitro*.

REFERENCES

1 Hahlbrock K, Scheel D.
Physiology and molecular biology of phenylpropanoid metabolism.
Annu Rev Plant Physiol Plant Mol Biol 1989; **40**: 347 - 69

2 Shoyama Y, Matsumoto M, Nishioka I Four caffeoyl glycosides from callus tissue of *Rehmannia glutinosa*.
Phytochemistry 1986; **25**: 1633 - 6.

3 König B, Dustmann JH.
The caffeoylics as a new family of natural antiviral compounds.
Naturwissenschaften 1985; **72**: 659 - 61

4 Cano E, Veiga M, Jiménez C, Riguera R. Pharmacological effects of three phenylpropanoid glycosides from *Mussatia*.
Planta Med 1990; **56**: 24 - 6.

5 Kimura Y, Okuda H, Nishibe S, Arichi S. Effects of caffeoylglycosides on arachidonate metabolism in leukocytes.
Planta Med 1987; **53**: 148 - 53.

6 Li J, Zheng RL, Liu ZM, Jia ZJ. Scavenging effects of phenylpropanoid glycosides on superoxide and its antioxidation effect. Acta Pharmacol Sin 1992; **13**: 427 - 30.

7 Zheng RL, Wang PF, Li J, Liu ZM, Jia ZJ.
Inhibition of the autoxidation of linoleic acid by phenylpropanoid glycosides from *Pedicularis* in micelles.
Chem Phys Lipids 1993; **65**: 151 - 4.

8 Li J, Wang PF, Zheng RL, Liu ZM, Jia ZJ. Protection of phenylpropanoid glycosides from *Pedicularis* against oxidative hemolysis *in vitro*. Planta Med 1993; **59**: 315 - 7.

9 Halliwell B, Gutteridge JMC Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts.
Arch Biochem Biophys 1984; **246**: 501 - 14

10 Liu ZM, Jia ZJ Phenylpropanoid and iridoid glycosides from *Pedicularis striata* Phytochemistry 1991; **30**: 1341 - 4.

11 Afanas'ev IB, Dorozhko AI, Brodskii AV, Kostyuk VA, Potapovitch AI. Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. Biochem Pharmacol 1989; **38**: 1763 - 9.

12 Mora A, Paya M, Rios JL, Alcaraz MJ.
Structure-activity relationships of polymethoxyflavones and other flavonoids as inhibitors of non-enzymic lipid peroxidation.
Biochem Pharmacol 1990; **40**: 793 - 7.

77-80

红纹马先蒿中苯丙素苷抗氧化和络合作用[1]

李 忌, 葛瑞昌, 郑荣梁[2], 刘自民[3], 贾忠建[3]
(兰州大学生物系, [3]兰州大学有机化学研究所, 兰州 730000, 中国)

关键词 苯丙素苷; 毛蕊花苷; 异毛蕊花苷; 红纹马先蒿; 抗氧化剂; 螯合剂

目的: 研究从中草药红纹马先蒿提取的 PPG 的抗氧化和络合作用. **方法**: 用 $FeSO_4$-依他酸引发亚油酸过氧化, 用硫代巴比妥酸法测过氧化产物. 用差示光谱法测对 Fe^{2+} 的络合作用. **结果**: 对照组脂类过氧化速率($A_{532}\cdot min^{-1}$)为 0.0046, 毛蕊花苷组为 0.0021, 异毛蕊花苷组为 0.0008. 异毛蕊花苷络合 Fe^{2+} 的能力 2 倍于毛蕊花苷. 多甲基毛蕊花苷既无抗氧化力(氧化速率 0.0041)也无络合作用. **结论**: 具有酚羟基的 PPG 的抗氧化作用正是通过它们的络合特性起作用的. 在生理条件下, PPG-Fe^{2+} 络合很稳定, 因此 PPG 在整体中也能像离体时那样抑制 Fe^{2+} 催化的脂类过氧化.