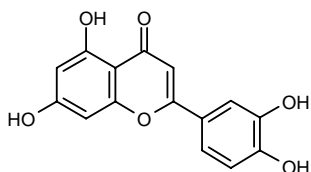


# Luteolin

## Treatment of Chronic Obstructive Bronchitis

2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4H-1-benzopyran-4-one  
3',4',5,7-Tetrahydroxyflavone



$C_{15}H_{10}O_6$

Mol wt: 286.2380

CAS: 000491-70-3

EN: 244028

### Isolation

Luteolin was isolated from several plants, including *Ajuga decumbens* Thunb. (1), *Dracocephalum integrifolium* Bge. (1), *Ixeris denticulata* f. *pinnatipartita* Kitag. (2), *Veronica peregrina* L. (3) and *Pteris multifida* (4), some of which were used for the treatment of bronchitis in Chinese folk medicine. Luteolin was also semisynthesized in China by using phloroglucinol or hesperidin as starting materials (5-7).

### Introduction

The search for effective drugs for the treatment of bronchitis continues to be the major focus of many researchers throughout the world. In China, some substances including natural products and traditional medicines have been screened and recommended for the clinic treatment of bronchitis (1). However, the high recurrence rates of bronchitis have been disappointing and the search for new bioactive compounds against bronchitis with improved therapeutic efficacy and reduced side effects is ongoing. Luteolin is a compound of natural origin that has been experimentally proven to exert marked antiinflammatory, spasmolytic and immunoregulatory activities (8) which may contribute to its therapeutic effects in bronchitis as assessed by preliminary clinical trials. Moreover, some of its actions are quite different from those of conventional drugs. Thus, luteolin has the

potential to be developed as a new drug for the treatment of bronchitis. In addition, recent studies indicated that luteolin exhibits antitumor activities, but has only weak antiproliferative effects on normal human cell lines *in vitro* (9-13).

### Pharmacological Actions

#### Antiinflammatory effects

Luteolin (80 and 160 mg/kg i.m.) significantly inhibited rat ankle joint swelling induced by carrageenin and yeast. The agent also exhibited marked antiinflammatory effects against croton oil-induced rat air pouch granuloma when injected i.m. (80 and 120 mg/kg/day) for 5 days (14). A linear, dose-proportionate relationship was obtained for the rapid and sustained inhibitory action of luteolin (i.m.) on dimethylbenzene-induced ear edema in mice, with an  $ED_{50}$  value of 106 mg/kg (Table I) (15). The efficacy of luteolin was also demonstrated against acetic acid-induced acute pleurisy in rats (16), with results showing a significant reduction in pleural exudate volume with no

Table I: Inhibitory effects of luteolin on dimethylbenzene-induced ear edema in mice.

Dose (mg/kg)	Route	Time <sup>a</sup> (h)	% Inhibition
65	i.m.	1.0	25.1
81	i.m.	1.0	35.2
102	i.m.	1.0	45.3
127	i.m.	1.0	54.7
159	i.m.	1.0	73.5
158	i.m.	0.5	49.9
158	i.m.	1.0	72.1
158	i.m.	3.0	61.5
158	i.m.	7.0	29.5

<sup>a</sup>Time before dimethylbenzene was given.

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Table II: Effects of luteolin on  $H_2O_2$  release of rat peritoneal macrophages *in vitro*.

Dose ( $\mu\text{mol/l}$ )	Culture time (h)	% Inhibition
0.4	4	27.3
2	4	36.4
10	2	26.9
10	4	61.8
10	6	16.7

obvious changes in total exudate leukocyte number. Overall, these results indicate that luteolin has significant inhibitory effects on acute inflammatory responses.

Luteolin (0.4-10  $\mu\text{mol/l}$ ) was shown to concentration-dependently reduce  $H_2O_2$  release of peritoneal macrophages stimulated by opsonized zymosan *in vitro* using fluorescent techniques in rats. Maximum inhibitory effects of the agent were observed when macrophages were incubated with luteolin for 4 h (Table II) (17).

Since nitric oxide (NO) produced by inducible NO synthase (iNOS) is a mediator of inflammation, the effects of luteolin on NO production in a lipopolysaccharide-activated macrophage cell line (RAW 264.7) were recently evaluated *in vitro*. Results indicated that luteolin suppressed NO production possibly through reduction of iNOS enzyme expression (18). In addition, luteolin was shown to have dose-dependent inhibitory effects on interleukin (IL)-5 activity. IL-5 is a chemotactic factor which promotes eosinophil growth and survival and is known to play an important role in eosinophilia-associated allergic inflammation (19).

#### Spasmodic effects

Luteolin significantly antagonized acetylcholine- and histamine-induced contraction of smooth muscle in the guinea pig model of modified air overflow (8, 20). The Schultz-Dale response of isolated guinea pig ileum was also inhibited by 48.2 and 100% following *in vitro* treatment with 109 and 218  $\mu\text{mol/l}$  luteolin, respectively (21). Luteolin antagonized the contraction of isolated guinea pig ileum induced by slow response substance-A (SRS-A) with an  $IC_{50}$  value of 27.6  $\mu\text{mol/l}$ . Moreover, the cumulative dose-response curves of SRS-A were shifted to the right with a  $pA_2$  value of 4.72 obtained, suggesting that luteolin might act as a competitive antagonist of SAS-A. The agent also dose-dependently inhibited electrically induced contractions of rat vas deferens ( $IC_{50}$  = 43.4  $\mu\text{mol/l}$ ). However, this action was not associated with alpha, muscarinic or opioid receptors, indicating that luteolin might also have direct spasmodic effects. In addition, the histamine-induced contraction of isolated guinea pig ileum was suppressed by luteolin *in vitro*.

In studies using isolated guinea pig ileum to investigate the pharmacodynamics of luteolin-histamine and receptor responses, values of 5.6, 0.6 and 4.4 were

obtained for the  $pA_2$ , slope and  $pD_2'$ , respectively (22). The dissociation constants and the maximum responses were calculated as follows:  $EA_{\text{max}} \neq EAB_{\text{max}}$ ;  $\neq \alpha K_A$  and  $K_B \neq \alpha K_B$ . The results indicate both noncompetitive and competitive antagonism by luteolin. The estimated Hill coefficient approached 1, according to the Michaelis-Menten formula. Luteolin (40-100  $\mu\text{mol/l}$ ) was also found to decrease the release of histamine and SRS-A from guinea pig lungs by 61.5 and 86.5%, respectively (23).

#### Immunomodulatory effects

Luteolin exhibited immunomodulatory effects in immunodeficient animals and in patients with bronchitis (24). The results from studies examining plaque- and rosette-forming cell activity revealed that luteolin attenuated cyclophosphamide-induced decreases in humoral immunity in mice while specific immune functions in normal mice were unaffected. These results indicate that luteolin may have beneficial immunomodulatory activity (Table III) (25).

A change in cellular immunity before and after luteolin treatment was also observed in 64 patients with chronic bronchitis as assessed by PHA-induced lymphocyte proliferation and PHA skin tests. The results showed that luteolin treatment (120 mg/day p.o.) for 10 days increased the cellular immunity of patients with chronic bronchitis (Table IV) (26).

Table III: Effects of luteolin (180 mg/kg p.o.) on specific humoral immunity in mice.

Group	PFC activity ( $A_{413}$ )			%RFC
	1:4	1:8	1:16	
Normal mice				
Control	0.67	0.56	0.39	3.3
Luteolin	0.66	0.54	0.38	2.9
Cy-treated mice				
Control	0.27	0.12	0.05	1.6
Luteolin	0.49	0.30	0.18	2.2

PFC = plaque forming cells. RFC = rosette forming cells. Cy = cyclophosphamide.

Table IV: Effects of luteolin on cellular immunity in patients with chronic bronchitis.

Group	PHA skin test (cm)		PHA-induced lymphocyte proliferation	
	Males	Females	cpm	RI
Normal	1.57	1.82	15,857	32.2
Patients <sup>a</sup>				
Before	1.12	1.28	8623	20.3
After	1.45	1.84	11,402	23.3

<sup>a</sup>Before or after oral administration of luteolin. cpm = count per minute. RI = response index.

Table V: Pharmacokinetic parameters of luteolin.

$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	$K_{12}$ (h)	$K_a$ (h)	$K_{10}$ (h)	$V_d$ (l/kg)	CL (mg·kg/h)	F' (%)
0.181	1.66	1.495	0.465	1.124	1.432	588	6.9

$t_{1/2}$  = half-life.  $K_{12}$  = transferring rate constant.  $K_a$  = reabsorption rate constant.  $K_{10}$  = elimination rate constant.  $V_d$  = apparent volume of distribution. CL = clearance. F' = reabsorption percent.

#### Miscellaneous effects

Luteolin (30 mg/kg i.p.) exhibited antitussive and expectorant actions without having local anesthetic effects, as well as weak antibacterial activity *in vitro* (20). These pharmacological effects might also contribute to its clinical use in the treatment of bronchitis. Luteolin also had moderate effects on blood coagulation *in vitro* (3) and experiments in cats and dogs showed that luteolin (i.g. and i.v.) had significant effects on reducing blood pressure (27). Injection of luteolin into either the common carotid artery or the fourth ventricle and the pressor reflex of the carotid sinus in response to luteolin all resulted in a reduction in blood pressure, suggesting involvement of the central nervous system in these actions. Moreover, inderal attenuated the effects of luteolin on blood pressure, indicating that these actions may be associated with the  $\beta$ -receptor agonist. Experiments also showed that luteolin may have vasodilating effects. Finally, luteolin (10 mg/kg i.v.) markedly reduced coronary blood vessel resistance in dogs but had no significant effect on myocardial oxygen consumption (28).

#### Pharmacokinetics

Preliminary studies in rabbits and rats using [ $^3$ H]-luteolin and fluorescence spectrophotometry examined the absorption, distribution, metabolism and excretion of luteolin (29). In rats, oral administration of [ $^3$ H]-luteolin was rapidly absorbed and widely distributed in various tissues, with highest concentrations in liver and kidney. A comparison of the concentration-time curve of protocatechuic acid (PCA) with that of luteolin indicates that PCA is a product of luteolin. Luteolin was rapidly removed from the blood via the kidney and liver and 12-h urinary excretion in rabbits was 37.7% of the total i.v. dose. In rats, the 6-h biliary excretion was about 11.2% of the total i.v. dose. The blood concentration-time curve of luteolin fit a 3-compartment open model. The pharmacokinetic parameters of luteolin are shown in Table V.

#### Toxicology

The acute lethal doses ( $LD_{50}$ ) of luteolin in rats were 411 mg/kg i.p. and 592 mg/kg i.m. (14). There was no evidence of tolerance or physical dependence after drug administration.

#### Clinical Studies

Preliminary clinical studies with hundreds of bronchitis patients indicated that luteolin was effective for the treatment of chronic bronchitis, with a total response rate of more than 90% and a complete remission rate of 63.8% (14). The major symptoms of chronic bronchitis, including cough, asthma, sputum and wheezing, were effectively alleviated with luteolin treatment (Table VI). No liver, cardiac or renal toxicity was reported. Gastrointestinal reactions were the major adverse events but were well tolerated by most patients (30).

#### Conclusions

Bronchitis remains one of the most difficult and widespread problems in medicine today. Drugs currently used in clinical practice, such as antitussives, expectorants, spasmolytics and antiinflammatory agents, have some disadvantages, especially little efficacy during long-term therapy. In animal studies and preliminary studies in humans, luteolin has exhibited extensive pharmacological actions, including antiinflammatory, spasmolytic, immunomodulatory, antitussive, expectorant and antibacterial effects with little toxicity, which may be the reason for its good therapeutic response in patients with chronic bronchitis, although further investigation is still necessary. Several studies have shown that luteolin acts as a vasodilator and coagulant and, therefore, the agent should administered with caution in patients with cardiovascular diseases.

#### Sources

Anhui Provincial Institute of Medical Sciences (CN) and Institute of Materia Medica, Chinese Academy of Medical Sciences (CN).

Table VI: Therapeutic effect of luteolin against main symptoms of chronic bronchitis.

Group	No. of patients	Cough (%)		(Asthma (%))		Sputum (%)		Wheezing (%)	
		CR	PR	CR	PR	CR	PR	CR	PR
Control	31	19.3	22.7	25.0	12.5	19.4	29.0	12.5	25.0
Treated	33	48.5	42.4	93.3	0	84.9	12.0	93.3	0

CR = complete remission. PR = partial remission.

## References

1. Wei, J.X. *Studies on phytochemistry of Chinese herbal medicine preventing and treating bronchitis*. Chin Herb Med Commun 1976, 2: 39-46.
2. Ma, J.Y., Wang, Z.T., Qi, S.H., Xu, L.S., Xu, G.J. *Studies on chemical constituents of Ixeris denticulata f. pinnatifida*. J Chin Pharm Univ 1998, 29: 167-9.
3. Du, S.H., Jin, J.S. *Blood coagulant effect of main chemical constituents of Purslane speedwell (Veronica peregrina L.)*. Chin Herb Med J 1996, 27: 416-7.
4. Lu, H., Hu, J., Zhang, L.X., Tan, R.X. *Bioactive constituents from Pteris multifida*. Planta Med 1999, 65: 586-7.
5. Lu, Y.H., Ji, Z., Wu, S.C. *Synthesis of luteolin and kaempferol*. Acta Pharm Sin 1980, 15: 477-81.
6. Xing, Y.Q., Sun, Z.Z., Hao, W.H. *Semi-synthesis of luteolin*. Chin J Pharm Ind 1994, 25: 484-6.
7. Sun, Z.Z., Hao, W.H., Duan, S.H., Xing, Y.Q., Zhen, Q.B. *Partial synthesis of luteolin*. Chin J Mod Appl Pharm 1999, 16: 30-1.
8. Anhui Cooperation Group. *Preliminary experimental study of Ajuga decumbens Thunb. against chronic bronchitis*. Chin Herb Med Commun 1973, 2: 18-23.
9. Kawaii, S., Tomono, Y., Katase, E., Ogawa, K., Yano, M. *Antiproliferative activity of flavonoids on several cancer cell lines*. Biosci Biotechnol Biochem 1999, 63: 896-9.
10. Yin, F., Giuliano, A.E., Van Herle, A.J. *Growth inhibitory effects of flavonoids in human thyroid cancer cell lines*. Thyroid 1999, 9: 369-76.
11. Kawaii, S., Tomono, Y., Katase, E., Ogawa, K., Yano, M. *Effect of citrus flavonoids on HL-60 cell differentiation*. Anticancer Res 1999, 19: 1261-9.
12. Takahashi, T., Kobori, M., Shinmoto, H., Tsushida, T. *Structure-activity relationships of flavonoids and the induction of granulocytic- or monocytic- differentiation in HL-60 human myeloid leukemia cells*. Biosci Biotechnol Biochem 1998, 62: 2199-204.
13. Huang, Y., Kwang, J., Lee, P., Ke, F., Huang, J., Huang, C., Kandaswami, C., Middleton, E. Jr., Lee, M. *Effect of luteolin and quercetin, inhibitors of tyrosine kinase, on cell growth and metastasis-associated properties in A431 cells overexpressing epidermal growth factor receptor*. Br J Pharmacol 1999, 128: 999-1010.
14. Dai, L.M., Cheng, H., Li, W.P., Liu, S.Q., Chen, M.Z., Xu, S.Y. *The influence of luteolin on experimental inflammatory models in rats*. Acta Anhui Med Univ 1985, 20: 1-3.
15. Zhao, W.Z., Xu, L.H., Li, W.P., Chen, M.Z., Xu, S.Y. *Inhibitory effect of luteolin on ear inflammation induced by dimethylbenzene in mice*. Acta Anhui Med Univ 1985, 20: 11-3.
16. Jin, W.Z., Dai, L.M., Li, Y.F., Chen, M.Z. *The effect of luteolin on the inflammatory responses in rat acetic acid-induced acute pleurisy*. Acta Anhui Med Univ 1985, 20: 14-5.
17. Zheng, Y.W., Ma, D.L., Chen, M.Z. *Effect of luteolin on H<sub>2</sub>O<sub>2</sub> release of peritoneal macrophages in rats*. Chin Pharmacol Bull 1990, 6: 56-8.
18. Kim, H.K., Cheon, B.S., Kim, Y.H., Kim, S.Y., Kim, H.P. *Effects of naturally occurring flavonoids on nitric oxide production in the macrophage cell line RAW 264.7 and their structure-activity relationships*. Biochem Pharmacol 1999, 58: 759-65.
19. Park, K.Y., Lee, S.H., Min, B.K., Lee, K.S., Choi, J.S., Chung, S.R., Min, K.R., Kim, Y. *Inhibitory effect of luteolin 4'-O-glucoside from Kummerowia striata and other flavonoids on interleukin-5 bioactivity*. Planta Med 1999, 65: 457-9.
20. Zhou, Z.D., Wang, L.Y., Wang, P. *Pharmacological studies on semi-synthetic luteolin*. Chin Herb Med Commun 1979, 10: 35.
21. Gu, Y.Z., Xhao, W.Z., Wei, W., Chen, M.Z., Xu, S.Y. *The influence of luteolin on Schultz-Dale response in animals*. Acta Anhui Med Univ 1985, 20: 4-7.
22. Shen, Q.H. *Pharmacodynamics of luteolin-histamine and receptor response*. Acta Pharmacol Sin 1989, 10: 111-4.
23. Shen, Q.H. *Effect of luteolin on histamine and SRS-A in the lung tissues of guinea pigs*. Acta Pharm Sin 1980, 15: 36.
24. Chen, M.Z., Jin, W.Z., Dai, L.M., Xu, S.Y. *Effect of luteolin on inflammation and immune function*. Chin J Pharmacol Toxicol 1986, 1: 46-52.
25. Li, Y.F., Jin, W.Z., Dai, L.M., Wei, W., Chen, M.Z. *The influence of luteolin on humoral immunity in mice*. Acta Anhui Med Univ 1985, 20: 8-10.
26. Su, B.T., Wang, D.N., Deng, S.H., Dai, S.Y., Luo, C.M., Li, L.H. *The effect of luteolin on cellular immunity in patients with chronic bronchitis*. Acta Anhui Med Univ 1985, 20: 26-8.
27. Wang, L.Y., Han, C.H., Wang, P., Li, G.Y., Xu, S.Y. *Pharmacological study of semi-synthetic luteolin on reducing blood pressure*. Chin Pharmacol Bull 1986, 2: 34-6.
28. Wang, L.Y., Han, C.H., Wang, P. *Experimental studies on the effects of luteolin on coronary circulation dynamics*. Chin Pharmacol Bull 1992, 8: 388-90.
29. Chen, M.Z., Feng, R.J., Gu, Y.Z., Guang, L.X., Zhen, Y.W., Wang, G.B., Li, W.P., Xu, S.Y. *The pharmacokinetics of luteolin*. Chin Pharmacol Bull 1986, 2: 15-20.
30. Anhui Medical University Study Group. *Clinical observations of luteolin for the treatment of chronic bronchitis*. Acta Anhui Med Univ 1985, 20: 29-30.