

Anti-Oxidant, Anti-Inflammatory and Anti-Allergic Activities of Luteolin

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Abstract

Luteolin is a flavone which occurs in medicinal plants as well as in some vegetables and spices. It is a natural anti-oxidant with less pro-oxidant potential than the flavonol quercetin, the best studied flavonoid, but apparently with a better safety profile. It displays excellent radical scavenging and cytoprotective properties, especially when tested in complex biological systems where it can interact with other anti-oxidants like vitamins. Luteolin displays specific anti-inflammatory effects at micromolar concentrations which are only partly explained by its anti-oxidant capacities. The anti-inflammatory activity includes activation of anti-oxidative enzymes, suppression of the NFκB pathway and inhibition of pro-inflammatory substances. *In vivo*, luteolin reduced increased vascular permeability and was effective in animal models of inflammation after parenteral and oral application. Although luteolin is only a minor component in our nutrition (less than 1 mg/day) epidemiological studies indicate that it has the potential to protect from diseases associated with inflammatory processes such as cardiovascular disease. Luteolin often occurs in the form of glycosides in plants, but these are cleaved and the aglycones are conjugated and metabolized after nutritional uptake which has to be considered when evaluating *in vitro* studies. Some data for oral and topical bioavailability exist, but more quantitative research in this field is needed to evaluate the physiological and therapeutical potential of luteolin.

Introduction

Luteolin is a flavone contained in many medical plants and in vegetables. However, concentrations are generally low compared to some of the flavonols like quercetin or kaempferol. Consider-

Abbreviations

▼	
Akt:	serine/threonin protein kinase B
COX-2:	cyclooxygenase-2
CVD:	cardiovascular disease
DPPH:	2,2-diphenyl-1-picrylhydrazyl
EAE:	experimental autoimmune encephalomyelitis
EGCG:	epigallocatechin 3-gallate
fMLP:	fMetLeuPhe, formyl peptide
GSH:	glutathione
IFN-γ:	interferon γ
IκB:	Inhibitor of kappa B
IL:	interleukin
iNOS:	inducible nitric oxygen synthase
IP-10:	inducible nitric oxygen synthase
IRF:	interferon regulatory factor
LPS:	lipopolysaccharide
LysoPAF AcTF:	1-lysophospholipide acetyltransferase
MAPK:	mitogen-activated protein kinase
M-CSF:	macrophage colony-stimulating factor
NFκB:	nuclear factor kappa B
8-OHdG:	8-hydroxy-2'-deoxyguanosine
PGE ₂ :	prostaglandin E ₂
PMA:	phorbol myristate acetate
ROS:	reactive oxygen species
TEAC:	trolox equivalent anti-oxidant capacity
TNF-α:	tumor necrosis factor-α

able concentrations are found in some spices like thyme, parsley, sage, in wild carrots, artichokes and in peanut hulls. Celery, spinach, some varieties of peppers and lettuce are our major nutritional luteolin sources [1]. While luteolin is only a minor flavonoid component in food, high

amounts can be isolated from peanut hulls and *Reseda luteola* L. that has been used as a dyeing plant due to its high luteolin content since ancient times [2].

While quercetin has been studied most intensively among the flavonoids during the last decades, recent research has provided a plethora of anti-oxidant, immunological, anti-carcinogenic, anti-bacterial, cardiovascular and other pharmacological mechanisms which suggest luteolin to be a valuable compound for many medical applications. Meanwhile, it is already advertised and marketed as a food additive. Some epidemiological investigations indeed indicate that luteolin intake may protect from cardiovascular diseases or some cancer species, but prospective clinical studies are widely lacking. The present review gives an account of the pharmacological data for luteolin concerning its anti-oxidant, anti-inflammatory and anti-allergic functions, comparing it to other flavonoids.

When evaluating the effects of flavonoids, their administration form has to be considered as they are often highly metabolized when taken orally [3]. In this paper, "luteolin" generally applies to the aglycone; when glycosides were used, these are specified. The literature search was initiated to the end of February 2008 using "luteolin" as a key-word in the Pubmed database, yielding 1085 results, and in Medline, with 1077 citations. Restriction to title search in Medline gave 163 results, and 192 were obtained from Pubmed under restriction to the toxicology section. Both lists were checked for relevant literature. Additionally, "related articles" proposed by Pubmed for the selected citations were systematically searched. Articles relating to other fields like anti-carcinogenic or metabolic effects were not selected, although sometimes overlapping with anti-oxidant or anti-inflammatory effects.

Anti-Oxidant Effects

Anti-oxidant properties of a specific substance are complex, and relative efficacies of two substances in different assays can vary. An anti-oxidant action is therefore not characterized by a general "total anti-oxidant coefficient", but by its performance in different test systems [4]. This even applies to simple test assays with a minimal number of agents, since they determine specific characters like "peroxyl radical scavenging capacity" or "ferric ion reducing capacity". Numerous assays exist to determine the anti-oxidant capacity of anti-oxidants not only in a reduced chemical environment, but also in cellular systems or *in vivo*, where the preventive effect on oxidant-induced damage can be studied [5]. Anti-oxidant mechanisms and structure-activity relationships of flavonoids have been extensively described by Havsteen [6] and Heim et al. [7] in the context of comprehensive review articles. Specific data for the anti-oxidant effects of luteolin exist for a wide range of experimental conditions.

Cell-free systems

Belyakov et al. [8], using a chemiluminescence method, found flavonoids with two hydroxy groups in the B-ring to strongly inhibit autoxidation of diphenylmethane. Time constants (in $k/10^6 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$) of the reaction between flavonoids and diphenylmethane peroxy radical illustrate the influence of flavonoid structure on anti-oxidant capacity: luteolin 22, quercetin 21, dihydroquercetin 19, catechin 7, kaempferol 1 [8].

Jovanovic et al. [9] determined the redox properties of various flavonoid radicals generated by azide radical-induced one-electron

oxidation in aqueous solutions by a pulse radiolysis technique. Main electron-donating system is the B-ring as long as it is substituted with hydroxy groups; the A-ring is not a good electron donor and will scavenge alkyl peroxide radicals only when the B-ring is not substituted. Quercetin was the best electron donor of the flavonoids tested, due to the favourable electron-donating properties of the (flavonol-) 3-hydroxy group in the C-ring which is conjugated to the B-ring through the 2,3-double bond.

The conjugation of the A- and B-rings is minimal. All investigated radicals had reduction potentials lower than that of alkyl peroxy radicals, the parent flavonoids therefore being qualified as chain-breaking anti-oxidants in any oxidation process mediated by these radicals.

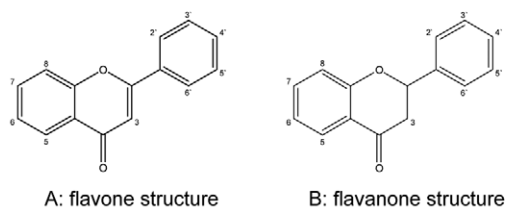
Rice-Evans et al. [8] systematically examined a variety of flavonoids for structure-activity relationships in the ABTS/trolox equivalent anti-oxidant capacity (TEAC) assay. Flavonoids with a high TEAC had many hydroxy groups and were characterized by:

1. An *ortho*-dihydroxy structure in the B-ring; dihydroxy groups in *meta*-positions, monohydroxylation, and a trihydroxy substitution were less effective.
2. A 2,3-double bond in conjunction with the 4-oxo function of the C-ring.
3. Hydroxy substitutions in positions 3 and 5 (see **Fig. 1** for terminology).

While the first two features apply to luteolin, it lacks a 3-hydroxy substitution. Accordingly, luteolin had a good, but not excellently high anti-oxidant capacity in this test. The TEAC for some flavonoids in aqueous phase were determined [10] as: epicatechin gallate: 4.9, epigallocatechin gallate (EGCG): 4.8, quercetin: 4.7, myricetin: 3.1, catechin: 2.4, rutin: 2.4, luteolin: 2.1, luteolin 4'-glucoside: 1.7, naringenin: 1.5, apigenin: 1.45, chrysin: 1.4, hesperitin: 1.4, kaempferol: 1.3, luteolin 3',7'-diglucoside: 0.8. (see **Fig. 1** for structures, and **Fig. 2** for basic anti-oxidative mechanism of flavonoids).

Metal ions bound to biological structures may function as catalytic centers for multiple radical formation. Flavonoids with *o*-di-OH groups in the B ring, like quercetin and luteolin, can chelate with the metal ion and are very effective protectives from oxidative damage. Brown et al. [11] used LDL-oxidation induced by either Cu^{2+} ions or by haem protein to compare the anti-oxidant capacity of these flavonoids. In the Cu^{2+} system, chelation was seen with both compounds, but quercetin was more effective, possibly due to its 3-OH moiety which gives additional sites for stable chelation. Luteolin has only the possibility for a further chelate complex between its 5-OH and 4-carbonyl, but which has a lower stability [12]. In the haem protein model, however, luteolin was more effective, probably because its higher lipophilicity allowed for better contact with the LDL or because of its higher stability. It was found that quercetin becomes oxidized during the chelation process, while luteolin reverted to its original form. This may explain the ability of quercetin and other flavonols to redox cycle under certain conditions. Stabilization (or elimination, as in luteolin) of the hydroxy group in position 3 is thought to disrupt this adverse effect.

Romanova et al. [13] investigated the DNA-protective effect of luteolin, quercetin and apigenin. With H_2O_2 as oxidizing agent, luteolin was the most effective. At low Fe^{2+} concentrations, apigenin was more effective, while at higher Fe^{2+} concentrations (10 mM) luteolin and quercetin were superior; apigenin misses a catechol group and a 3-OH moiety and thus can poorly form chelates.



Skeleton/Name	3	5	7	3'	4'	5'
A FLAVONE						
A Luteolin		OH	OH	OH	OH	
A Apigenin		OH	OH		OH	
A Chrysin		OH	OH			
A Diosmetin		OH	OH	OH	-OCH ₃	
A FLAVONOL						
A Fisetin	OH		OH	OH	OH	
A Kaempferol	OH	OH	OH		OH	
A Quercetin	OH	OH	OH	OH	OH	
A Myricetin	OH	OH	OH	OH	OH	OH
A Rutin	-O-Gluc-Rham	OH	OH	OH	OH	
B FLAVANONE						
B Naringenin		OH	OH		OH	
B Hesperitin		OH	OH	-OCH ₃	OH	

Fig. 1 Substitution patterns of flavonoids mentioned in this review. Flavonoids are characterized by aromatic ring skeletons and substitution of hydroxy groups or other moieties which influence their ability to act as anti- or pro-oxidants and their anti-inflammatory activity. Luteolin and apigenin are the main flavones from *Reseda luteola* L. (● = OH)

Luteolin was the most effective of 8 flavonoids in a rat liver cell membrane model with ascorbic acid and Fe²⁺, similar to (–)-epicatechin, but superior to quercetin and, e.g., kaempferol and apigenin. When lipid peroxidation was induced by arachidonic acid, the order was changed, quercetin and luteolin being only moderately active [14]. Luteolin also performed best among 12 flavonoids (200 μM) in a lipid peroxidation assay with thiobarbituric acid, using methyl lineolate and Fenton's reagent. The relative efficacy in this assay showed a good correlation with protection from γ-ray-induced DNA damage after gastric intubation of these flavonoids to mice at 5 μmol/kg [15].

Gal et al. [16] demonstrated, that only 2 of 13 tested flavonoids, luteolin and 3',4',7-trihydroxyflavone, showed "super-anti-oxidant potency" in an assay of Cu²⁺-induced liposome oxidation when phosphatidylserine was present in the liposomes. For liposomes made of palmitoyl-oleoyl-phosphatidylcholine (250 μM) and phosphatidylserine (25 μM) the "lag" preceding copper-induced peroxidation (5 μM copper) was doubled upon addition of 30–130 nM of the "super-active" antioxidants. This was due to replenishment of the anti-oxidant in a tertiary com-

plex of copper, phosphatidylserine and anti-oxidant. Other flavonoids were either moderately anti-oxidant or pro-oxidative. A change of relative anti-oxidant capacities may also result from interactions between anti-oxidants. Hirano et al. [17] tested 10 flavonoids in two different assays. Epigallocatechin 3-gallate (EGCG) was the most effective radical scavenger in an electron transfer test with 2,2-diphenyl-1-picrylhydrazyl (DPPH), luteolin the least effective. In a second experiment, the mutual anti-oxidant effect of these flavonoids with α-tocopherol was determined in an LDL-peroxidation assay with 2,2'-azobis-(2,4-dimethylvaleronitrile) (AMVN)-CH₃O. The lag time of peroxidation induced by 6 mg/100 mL α-tocopherol is shortened by increasing concentrations of the radical initiator, but restored by the flavonoids. In this experiment, luteolin was the most effective of ten flavonoids tested (including quercetin), apigenin being the least effective. Flavonoids act as hydrogen donors for α-tocopherol, thereby increasing their potential to delay LDL oxidation.

Cellular systems *in vitro*

Cellular systems are, on one hand, more biologically relevant than cell-free assays, because they account for some aspects of uptake, metabolism, and location of the test substances within cells. On the other hand, features specific for the organisms and cells are more involved so that results have to be generalized very carefully; e.g., rat liver has a high potential to cleave flavonoid glycosides, so that these substances appear more effective in relation to their aglycones than in human tissues.

Horváthová et al. [18] used the comet assay to determine the capacities of 4 flavonoids to protect DNA from H₂O₂ induced damage in murine leukemia L 1210 cells. At 600 μM quercetin had

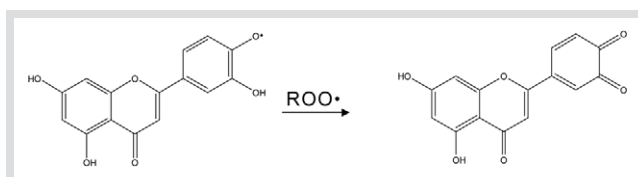


Fig. 2 Anti-oxidant mechanism of flavonoids (from Belyakov, [8])

the highest protective effect (45%), followed by luteolin (40%), while apigenin was only slightly and rutin was not protective. At high, not relevant concentrations of 1200 μM , apigenin induced single-strand breaks. In human myelogenous leukemia cells (K562) the results were similar, with luteolin (44%) and quercetin (42%) protective at 20–100 μM , rutin being only marginally effective at concentrations up to 1000 μM , and apigenin displaying no effect [19].

Horváthová et al. [20] demonstrated DNA-protective free radical scavenging potential of luteolin and quercetin in human HMB 2 melanoma cells. Both flavonoids reduced the damage caused by treatment with H_2O_2 in a dose-dependent manner, with 40% inhibition at 20 μM up to 80% at 100 μM . When combined with melphalan, a cytostatic substance which displays a variety of toxic side effects at high doses, 20 μM luteolin significantly reduced the frequency of chromosomal aberrations, whereas quercetin was less effective.

The comet assay was also used to investigate the effect of flavonoids and vitamin C on oxidative damage from 100 μM H_2O_2 in human lymphocytes [21]. Luteolin and quercetin were the most effective protectants, with ED_{50} concentrations of about 50 μM . At the high concentration of 279 μM , luteolin suppressed DNA damage almost completely by 91%. Apigenin and rutin or other flavone glycosides were more than 10 times less protective. Vitamin C was less effective than most of the flavonoids, but its effect was additive in combinations.

Luteolin and apigenin were isolated along with many other substances from the Indonesian traditional medicinal plant *Guazuma ulmifolia* [22]. Luteolin was among the strongest anti-oxidant compounds in the DPPH assay, while apigenin was ineffective. The authors find a good correlation of anti-oxidative and protective properties of different plant substances in the assays used. Incubation of H4IIE cells for 1 h with 50 μM luteolin reduced H_2O_2 -induced DNA breakage in the comet assay by 70–80%. At 100 μM , the effect of luteolin and kaempferol changed from protective to cytotoxic. The question arises whether this has any relevance, as these high levels of luteolin will never be reached *in vivo* (see also Section Bioavailability).

The effect of artichoke extract and some of its flavonoid constituents on reactive oxygen species was investigated in human leukocytes [23]. Three different agents were used to exhibit oxidative stress on the cells *in vitro*: H_2O_2 , phorbol myristate acetate (PMA), and formyl peptide (fMLP). H_2O_2 is a key compound in free radical metabolism since it can be transformed into other dangerous reactive oxygen species (ROS) in the cell [24]. PMA acts as a tumor promoter and fMLP is a chemotactic peptide released from bacteria during infections. Both stimulate ROS generation by different mechanisms. Artichoke extract and its constituents, caffeic acid, chlorogenic acid, luteolin and luteolin 7-glucoside, all showed a concentration-dependent inhibitory activity in the above models [23].

In rat hepatocytes incubated in 1 mM *tert*-butyl hydroperoxide malondialdehyde production was significantly reduced by flavonoids in concentrations from 5 to 100 μM , in the order of effectiveness luteolin = fisetin = quercetin > hesperitin > naringenin > apigenin. In contrast to cell-free assays, some glycosides were similarly effective compared to the aglycones, probably due to the high glucosidase activity in rat liver [25].

Wolfe et al. [26] used HepG2 human hepatocarcinoma cells with incorporated dichlorofluorescein which is easily oxidized to a fluorescent derivative. ABAP [2,2'-azobis(2-amidinopropane) dihydrochloride] was used to generate peroxy radicals. Selected

phytochemicals and fruits were used to test their anti-oxidant potential. Luteolin was active but inferior to quercetin, kaempferol, EGCG and myricetin in this test.

Harris et al. [27] hypothesized that differences in anti-oxidant activity between the structurally similar flavones, luteolin and chrysin (missing B-ring hydroxylation), would differentially affect inflammation-associated cyclooxygenase-2 (COX-2) expression and prostaglandin E_2 (PGE_2) formation in RAW 264.7 macrophage-like cells with 25, 50, or 100 μM . Luteolin and chrysin suppressed PGE_2 formation equally well although luteolin had stronger effects on COX-2 protein expression and on superoxide and hydroxyl radical scavenging. Different mechanisms may be involved in their anti-inflammatory activity. These results were confirmed later [28].

Psotová et al. [29] compared 5 flavones and flavonols in their capacity to protect isolated rat cardiomyocytes from doxorubicin-induced oxidative stress. Extracellular lactate dehydrogenase and cellular ADP and ATP protection was concentration-dependent for baicalein (= 5,6,7-trihydroxyflavone) > luteolin = apigenin > quercetin > kaempferol; all tested flavonoids were significantly better than dexrazosan, an agent currently used for adjuvant therapy during anthracycline antibiotic therapy. The cardioprotective effect did not correlate with the capacity to inhibit lipid peroxidation in microsomes and mitochondria, where the flavonols were superior to the flavones, especially to apigenin.

Kanazawa et al. [30] incubated HepG2 hepatic cells with various flavonoids (10 μM) to determine whether these substances can penetrate into the nuclei and protect DNA from oxidative damage. Most of the tested flavonoids were incorporated in the nuclei at 250–450 pMol/10(7) cells after 30 min incubation, 8% of the luteolin in form of the aglycone, a lower rate than for other flavonoids. When oxidative stress was induced, luteolin (–15%) and quercetin (–25%) significantly reduced DNA damage as measured by formation of 8-oxo-7,8-dihydrodeoxyguanosine (8-OHdG [30]).

In vivo

Shimoi et al. [15] investigated the anticlastogenic effect of 12 structurally different flavonoids in whole body gamma-ray irradiated ICR mice [single gastric intubation (5 $\mu\text{M}/\text{kg}$)] 6 h before irradiation (1.5 Gy). Luteolin had the most marked effect on reducing the frequencies of micronucleated reticulocytes in peripheral blood and also on inhibiting lipid peroxidation. A good correlation was observed between the anticlastogenic activity and the anti-oxidant activity of the 12 flavonoids (200 μM) determined by the thiobarbituric acid method with methyl lineolate and $\text{Fe}^{2+}/\text{H}_2\text{O}_2$.

Heroin, morphine and opiates are able to induce ROS formation in several cell types; they decrease the anti-oxidant defense system including enzymes: superoxide dismutase, catalase, and glutathione peroxidase; and anti-oxidants: glutathione (GSH), Se, and vitamins. Treatment of heroin-dependent mice with verbascoside or luteolin limited oxidative stress status and damage to DNA, proteins, and lipids in brain [31].

Anti-Inflammatory and Anti-Allergic Effects of Luteolin



In vitro

Flavonoids have multiple and specific effects on inflammatory processes. An example of synergistic actions by luteolin and oth-

er flavonoids was demonstrated by Ruiz et al. [32], who characterized the molecular mechanisms of flavonoids in inhibiting tumor necrosis factor- α (TNF- α) induced interferon-inducible protein (IP-10) gene expression in the murine non-carcinoma intestinal epithelial cell line Mode-K. They tested various flavonoids at a concentration of 100 μ M and demonstrated that 3'-hydroxyflavone blocked TNF- α induced nuclear-factor kappa B (NF κ B) transcriptional activity and IP-10 expression at the level of NF κ B/I κ B α phosphorylation/degradation by inhibiting the inhibitor of kappa B (I κ B) kinase activity. Luteolin, apigenin, 3'-hydroxyflavone and genistein also displayed inhibitory effects on the TNF-induced NF κ B signaling pathway, but with individual patterns on different steps of the signaling chain. Luteolin partially inhibited TNF-induced NF κ B DNA binding activity followed by the complete blockade of NF κ B transcriptional activity; EC₅₀ were 20 to 27 μ M.

In addition, apigenin and luteolin blocked protein kinase B (Akt) phosphorylation/activity; only luteolin caused long-lasting inhibition over 24 h, too. Luteolin and 3'-hydroxyflavone induced interferon regulatory factor (IRF)-1 degradation. Genistein blocked IP-10 but not IL-6 expression through NF κ B, IRF, and Akt independent mechanisms, demonstrating the functional diversity of flavonoids in inhibiting pro-inflammatory processes [32].

Essential mechanisms of luteolin's anti-inflammatory action are inhibition of inducible nitric oxygen synthase (iNOS) expression and NO production, scavenging of ROS, inhibition of ROS production and activation of anti-oxidant enzymes, inhibition of leukotriene production and release, suppression of pro-inflammatory cytokine expression, inhibition of the NF κ B pathway, Akt and the mitogen-activated protein kinase (MAPK) pathway, inhibition of adhesion molecule membrane binding, inhibition of hyaluronidase and elastase activity, stabilization of mast cells, reduction of vascular permeability and modulation of cell membrane fluidity (see **Table 1**). Differences in efficiency or in thresholds reported by different authors may be due to the models analyzed or methodology. There is no space for a complete account of these parameters in individual studies. The following overview gives some additional information on selected effects *in vitro* and comparisons of luteolin with other flavonoids.

Luteolin as the active component isolated from *Perilla frutescens* inhibited NO production with an IC₅₀ value of 6.9 μ M in LPS-stimulated BV-2 microglial cells [33]. Incubation correlated well with reduced levels of iNOS mRNA and protein in another cell line. The molecular mechanism appeared to involve inhibition of NF κ B activation which is not stimulus or cell specific as several flavonoids reduced macrophage colony-stimulating factor (M-CSF)-induced proliferation without affecting cellular viability, and some also inhibited TNF- α production as well as iNOS expression and NO production in lipopolysaccharide (LPS)-activated bone marrow-derived macrophages, an effect that has been associated with the inhibition of the NF κ B pathway [34]. Luteolin and quercetin were able to stimulate IL-10 expression at low concentrations (< 50 μ M). Analysis of the structure-activity relationship showed that four hydroxylations at positions 5, 7, 3' and 4', together with the double bond at C2-C3 and the position of the B ring at 2, seem to be necessary for the highest anti-inflammatory effect. This is exactly the structure of luteolin.

Luteolin, quercetin, and apigenin completely protected RINm5F (RIN) rat insulinoma cells against interleukin-1 β (IL-1 β)- and interferon- γ (IFN- γ)-mediated cytotoxicity [35].

In LPS-stimulated murine macrophages RAW 264.7 luteolin inhibited TNF- α and IL-6 release, tyrosine phosphorylation, NF κ B mediated gene expression, and Akt phosphorylation. Luteolin was more effective than luteolin 7-glucoside, quercetin, genistein, hesperitin and eriodictyol, with an IC₅₀ value of less than 1 μ M for TNF- α release [36].

In two examinations of 37 and 45 flavonoids and related compounds for inhibition of IL-4 and IL-13 production in basophils stimulated with anti-IgE antibody plus IL-3, ayanin (quercetin 3,4',7-trimethyl ether), luteolin and apigenin were the strongest inhibitors, with IC₅₀ values of 2–5 μ g/mL (1–2 μ M) [37], [38].

Flavonoids are the active principles for inhibition of IL-5 bioactivity in the Japanese plant *Kummerowia striata* Thunb. IL-5 is a chemotactic factor of eosinophils, and promotes the growth and survival of eosinophils, which play an important role in eosinophilia-associated allergic inflammation. Luteolin inhibited IL-5 bioactivity with an IC₅₀ value of 18.7 μ M, approximately equal to that of apigenin and stronger than that of kaempferol. The most effective compound, however, was luteolin 4'-O-glucopyranoside with IC₅₀ = 3.7 μ M, and 95% inhibition at 30 μ M [39].

Enhanced CD40 ligand expression in the human basophilic cell line KU812 in response to 12 h incubation with A23187 plus PMA was significantly suppressed by 10 or 30 μ M of luteolin, and less effectively by apigenin, fisetin and quercetin at 30 μ M, whereas myricetin failed to inhibit [40].

Hexosaminidase release from RBL-2H3 cells (degranulation marker) was employed as an estimate for anti-allergic actions. Among 22 flavonoid compounds tested, luteolin, apigenin, diosmetin, fisetin, and quercetin were found to be most active with IC₅₀ values below 10 μ M [41].

Apigenin, chrysin and luteolin dose-dependently inhibited both pro-inflammatory cytokine (TNF- α , IL-1 β and IL-6) production and metabolic activity of LPS-stimulated peripheral blood mononuclear cells (PBMC). Monocytes were specifically eliminated in PBMC at low concentrations (\approx 8 μ M), with apigenin appearing as the most potent, while quercetin and naringenin had virtually no effects [42].

A selection of 26 naturally occurring flavonoids was investigated for inhibition of NO production in LPS-activated RAW 264.7 cells. The most active were apigenin, wogonin, and luteolin, having IC₅₀ values of 23, 17, and 27 μ M, respectively, while AMT, a synthetic selective iNOS inhibitor, had an IC₅₀ value of 0.09 μ M [43]. Verbeek et al. [44] evaluated the inhibiting effects of various flavonoids on antigen-specific proliferation and IFN- γ production by human (multiple sclerosis) and murine experimental autoimmune encephalomyelitis (EAE) autoreactive T cells *in vitro*. Apigenin and luteolin were strong inhibitors of both murine and human T-cell responses while fisetin, quercetin, morin and hesperitin were ineffective. These promising *in vitro* results, however, were not confirmed by an *in vivo* investigation [45]. Luteolin was less effective than apigenin as a competitive inhibitor of hyaluronidase [46]. However, the relative potency depends not only on the structure of the flavonoid, but also varies for different kinds of hyaluronidases [47].

Luteolin (1 μ M) caused a significant elevation of the collagen content, alkaline phosphatase activity, and osteocalcin secretion in osteoblasts [48]. Yanoshita et al. [49] examined several flavonoids for inhibitory effects on the 2-lysophospholipid (lyso-PAF) acetyltransferase using homogenates of a rat mucosal-type mastocytoma cell line, RBL-2H3 as an enzyme source. Of the flavonoids tested, luteolin and quercetin exhibited significant inhibi-

Table 1 Anti-inflammatory mechanisms investigated with luteolin.

Target	Effect of luteolin	Publication source(s)
iNOS	Suppresses iNOS expression	[33], [35], [34], [43], [81], [82], [84]
NO	Suppresses NO production	[33], [35], [48], [34], [43], [56], [81], [82]
H ₂ O ₂	Suppresses H ₂ O ₂ production and scavenges H ₂ O ₂	[85]
SOD-1	Increases SOD-1 expression	[91]
PGE-2	Suppresses PGE-2 production	[48], [81]
PGD-2	Inhibits PGD-2 release from human mast cells	[51], [52]
Leukotrienes	Inhibits leukotriene release from human mast cells	[51], [52]
Leukotriene B4	inhibits LT B4 synthesis	[87]
Leukotriene C4	No effect	[37]
Thromboxane B2	Inhibits TX B2 synthesis	[87]
Histamine	Inhibits histamine release from human mast cells	[51], [52]
GM-CSF	Inhibits GM-CSF release from human mast cells	[51], [52]
TNF- α	Suppresses TNF- α expression	[48], [34], [36],[42], [53], [55], [56], [81], [93]
COX-2	Suppresses COX-2 expression	[28], [57], [81], [84]
mPGES-1	Suppresses mPGES-1 expression	[28]
IL-1 β	Suppresses IL-1 β expression	[42]
IL-4	Suppresses IL-4 expression	[37], [38]
IL-5	Inhibits bioactivity of IL-5	[39]
IL-6	Suppresses IL-6 expression	[48], [36], [42], [81], [91]
IL-8	Suppresses IL-8 expression	[91]
IL-10	Stimulates IL-10 expression	[34]
IL-13	Suppresses IL-13 expression	[37], [38]
MCP-1	Reduces elevated MCP-1 levels	[91]
CCL5 (RANTES)	Reduces elevated CCL5 levels	[91]
IFN- γ	Reduces IFN- γ production <i>in vitro</i> , not <i>in vivo</i>	[42], [45]
Complement	Inhibits classical pathway (esp. glycosides)	[89], [90]
Complement	No effect on classical pathway	[88]
MAPK	Reduces MAPK phosphorylation	[56], [82]
ERK	Suppresses IgE-stimulated activation of ERK	[51], [52]
JNK	Suppresses IgE-stimulated activation of JNK	[51], [52]
IKK	Inhibits IKK phosphorylation	[81]
I κ B α	Blocks I κ B α degradation	[33], [36], [81]
I κ B α	<i>Does not inhibit IκBα degradation</i>	[85]
NF κ B	Inhibits DNA binding and transcription	[32], [35], [36], [81], [82]
NF κ B	<i>Does not inhibit NFκB nuclear translocation, DNA binding or phosphorylation</i> but inhibits transcription	[85]
AP-1	Induces AP-1 DNA binding	[81], [83]
Akt	Inhibits phosphorylation and activity	[32], [36], [81], [82]
IRF	Induces IRF-1 transcription factor degradation	[32]
IP-10	Suppresses IP-10 expression	[32], [91]
CD40 ligand	Suppresses CD40 ligand expression	[38], [40]
ICAM-1	Inhibits LPS-stimulated ICAM-1 expression <i>in vivo</i> (mouse)	[55]
Rho GTPase	Reduces Rho GTPase activity and monocyte transepithelial migration	[54]
Tyrosine kinase	Suppresses tyrosyl phosphorylation	[36], [86]
Hyaluronidase	Inhibits native hyaluronidase more than recombinant	[46], [47]
Lyso-PAF-ActF	Inhibits Lyso-PAF-ActF specifically	[49]
PAF	Inhibits PAF production	[49]
Vascul. Permeabil.	Reduces increased vascular permeability	[50]
Lipid rafts	Blocks lipid raft accumulation	[56]
Mast cells	blocks mast cell activity and T-cell activation	[51], [52], [92]

tory effects (IC₅₀, 45 μ M and 80 μ M, respectively), whereas other structurally-related flavonoids were ineffective.

In vivo

Topical application of luteolin at concentrations of 20 and 100 μ g/site significantly reduced the number of scratching incidents associated with dinitrochlorobenzene-induced passive cutaneous anaphylaxis in mice, and a similar tendency was also observed in histamine-, serotonin- and compound 48/80-evoked

cutaneous reactions. Vascular permeability and ear thickness increase were also significantly reduced, attributed to inhibition of mediators and mediator release [50].

A preventive and ameliorating effect of luteolin on clinical symptoms in atopic dermatitis-prone mice was found after internal application [38]. Intraperitoneal injection of luteolin or baicalein doses of 0.1 mg/kg reduced antibody plus dinitrofluorobenzene-induced mouse ear edemas to 30% and 50%, respectively. Quercetin had only a small influence [51], [52].

Various flavonoids inhibited LPS-induced TNF- α production from macrophages *in vitro*, but only luteolin showed *in vivo* activity when administered orally. Serum TNF- α production was inhibited to about 20–25% in a highly significant manner by 0.1 and 1 mg/mouse luteolin (*p. o.*). Only luteolin or quercetin inhibited TPA-induced ear edema. The authors suggest that the structure of luteolin as 3',4',5,7-tetrahydroxyflavone is most suitable for oral anti-inflammatory activity [53].

Rats sensitized for acute and chronic EAE, an experimental model of multiple sclerosis, were treated with oral doses of luteolin and quercetin [54]. Luteolin substantially suppressed clinical symptoms and prevented relapse much stronger than quercetin when administered either before or after disease onset. Luteolin treatment reduced inflammation and axonal damage in the central nervous system (CNS) by preventing monocyte migration across the brain endothelium; this was due to modulation of the activity of Rho GTPases, signal transducers involved in trans-endothelial migration. Mice receiving LPS (*Salmonella enteritidis* LPS 32 mg/kg, *i. p.*) exhibited high mortality, with only 4.1% of the animals surviving seven days after LPS challenge. Mice that had received luteolin (0.2 mg/kg, *i. p.*) before LPS had an increased survival rate of 48%. Luteolin decreased LPS-induced tumor TNF- α production and ICAM-1 expression in the liver and abolished leukocyte infiltration in liver and lung [55]. These results were confirmed for oral application of luteolin and other flavonoids in the TNF- α triggered collagen-induced arthritis (CIA) mouse model of rheumatoid arthritis. Inhibitory effects were due to regulation of signaling pathways (NF κ B, MAPK, and FcRI expression). Accumulation of lipid rafts, which is the critical step for signaling, was blocked [56]. Oral administration of luteolin (10 and 50 mg/kg) to mice efficiently suppressed carrageenan-induced paw edema. Results derived from whole blood assay for COX and from the RT-PCR assay indicate that luteolin may be a potent selective inhibitor of COX-2 mRNA expression [57].

Bioavailability and Toxicological Aspects

Systemic bioavailability

The physiological role of an anti-oxidant is modulated in a complex way, depending on factors like resorption, its bioavailability at a specific site in the organism, partition coefficient between aqueous and lipophilic phases, its ability to chelate with metal ions, or its distribution between, and binding to certain cell compartments.

Zhou et al. [58] found that luteolin is passively resorbed in the jejunum and duodenum of the rat, and less in the colon and ileum. Application of identical amounts (14.3 mg/kg) of pure luteolin and in the matrix of a peanut hull extract revealed that absorption of the latter was higher (C_{\max} : 8.34 vs. 1.97 μ g/mL or 29.2 vs. 6.9 μ M).

Flavonoid glycosides are cleaved to their aglycones in the intestinal mucosa, and the aglycones are glucuronated by UDP-glucuronosyl transferases before release into blood serum [2]. Wittemer et al. [59] investigated pharmacokinetics of aqueous artichoke extracts containing 14.4 mg and 35.2 mg luteolin 7-*O*-glucoside in humans. Neither luteolin nor its glycosides were found in urine or plasma, but only their phase II conjugates. Maximal plasma levels of about 0.2 μ M were reached after 30–40 min. The time-concentration profile was biphasic with a fast distribution and a slow elimination phase. Terminal elimination half-

time was 2.5 h, and 2% of the applied dose were eliminated renally as luteolin conjugates.

Plasma concentrations observed in these experiments may suffice under certain conditions to display measurable anti-oxidant activity; luteolin inhibited Cu²⁺-induced oxidation of LDL-cholesterol in concentrations from 28 ng/mL (0.1 μ M) upward [60]. Anti-oxidant properties are to a large extent attributable to hydroxylation in positions 3' and 4'; it is there, where flavonoids are preferentially glucuronidized during resorption in the gut [61]. This might reduce the flavonoids' anti-oxidant potential. However, several human tissues, e.g., neutrophil granulocytes and CaCo-2 cells, are able to cleave luteolin glucuronides, and increase their activity when stimulated by pro-inflammatory substances [62]. Thus, these glucuronides may function as a depot and the full anti-oxidant capacity of luteolin may be re-established right at the site of inflammation.

Topical bioavailability

In vivo skin penetration studies of the flavones apigenin, luteolin, and apigenin 7-*O*- β -glucoside were carried out with nine healthy, female volunteers. During 7 hours the decline of flavonoid concentration in a saturated aqueous alcoholic solution filled in glass application chambers was repeatedly measured by spectrophotometry at fixed time periods. The maximal fluxes were calculated. From the observed time course it was concluded that apigenin and luteolin were not only adsorbed at skin surface but penetrated into deeper skin layers [63]. This indirect evidence of penetration should be confirmed by standard penetration studies such as the tape-stripping method. Skin penetration of luteolin and its glycosides is a prerequisite for their topical use as antiphlogistic agents in dermatology.

Toxicological aspects

LD₅₀ values of > 180 mg/kg *i. p.* were determined in mice [64], and 411 mg/kg in rats [65]. Oral LD₅₀ in mice was reported as >2500 mg/kg [65]. Luteolin was protective on H4IIE cells in concentrations up to 50 μ M, but displayed cytotoxic effects in the NRU test with an IC₅₀ of 100 μ M [22].

Both luteolin (IC₅₀ = 12.5 μ M) and quercetin (IC₅₀ = 45.5 μ M) displayed anti-leishmanial activity *in vitro*. Whereas quercetin displays non-specific toxic effects on normal human PBMC, luteolin was non-toxic. Further studies have to prove whether luteolin may serve as a lead for anti-leishmanial therapy [66].

Luteolin, in contrast to quercetin and rhamnetin, had no mutagenic effects in the Ames mutagenicity test [67], but luteolin (and 25 other flavonoids) inhibited mutagenicity induced by aflatoxin B in *Salmonella typhimurium* TA 100 by 70% [68], and in the comet assay [20]. Kawanishi et al. [69] investigated the capacity of phytal anti-oxidants to play a paradoxical role as pro-oxidant, and thus damage instead of protect DNA structure under certain experimental conditions. Some flavonoids like quercetin, genistein and catechins were indeed harmful in H₂O₂-generating systems or after metabolic activation, but luteolin was safe. For more information on earlier work on flavonoid safety and pharmacology the reader is referred to the extensive review by Middleton et al. [70].

Conclusions

Luteolin is a moderately effective anti-oxidant in simple standard *in vitro* tests, but may exert surprisingly high efficacy down

to the nanomolar range in more complex assays, cellular or *in vivo* settings. It is not always clear whether conditions modify the anti-oxidant capacity of the substance, or whether additional pharmacological activity is involved. The *o*-di-OH catechol group of the B ring is able to chelate metal ions and contributes strongly to the anti-oxidant capacities. Luteolin has only weak pro-oxidative capacities and will not undergo redox cycling, making it a safer anti-oxidant than quercetin.

The daily dietary intake of luteolin is very low in comparison to total flavonoid uptake (approx. 1%), so that the contribution to total anti-oxidant capacity in our nutrition may not be such a relevant factor. Epidemiological data nevertheless indicate a physiological role of flavones and especially of luteolin. This may be due to more specific mechanisms than just radical scavenging.

Luteolin exerts numerous effects *in vivo* and *in vitro*, which can reduce inflammatory processes in cells stimulated by pro-inflammatory factors. This includes inhibition of cytokine production, inhibition of enzymes and enzyme expression, suppression of regulatory proteins (especially of the NF κ B pathway), and radical scavenging. These effects may be related more or less to its anti-oxidant capacity, but some are very specific and distinct from effects of other flavonoids which have similar anti-oxidant properties.

When compared to other flavonoids, luteolin was usually among the most effective ones, or *the* most effective. For certain pharmacological effects its structure was even regarded as optimal. Some processes like TNF- α release from murine macrophages had IC₅₀ values as low as 1 μ M. This is higher than serum levels expected from everyday nutritional intake, but may be reached by supplementation. More quantitative investigations on luteolin pharmacokinetics, possible storage effects when bound to plasma or when biotransformed to glucuronides and bioavailability at specific action sites are needed to realistically estimate the pharmacological potential of luteolin. Results obtained with luteolin (or other flavonoid) glycosides *in vitro* have to be regarded sceptically, since these molecules are cleaved during uptake and will not be found in plasma. The aglycone, however, may be set free from its conjugates in specific tissues and exert those effects found experimentally *in vitro*. Some *in vivo* studies have already demonstrated that strong effects were observed after intraperitoneal or after oral administration of non-toxic doses of luteolin.

New instruments were developed during the last decades which now allow to estimate dietary contents of total flavonoids and their most important subclasses: the USDA database lists flavones, flavonols, flavanones, flavan-3-ols, anthocyanidins, as well as isoflavones and proanthocyanidins, and a number of important single components. Investigations in different populations have revealed, that mean total flavonoid intake is usually in the order of 100–200 mg/d. Flavonols (with quercetin and kaempferol as main components) contribute 5–20 mg/d, flavones only about 1 mg, often less. Up to 90% of the flavone fraction may be apigenin, leaving about 0.1 mg/d for luteolin. Expectations to find health effects of flavone (or even luteolin) intake by epidemiological correlations should be moderate. It is surprising, after all, that there are indeed some promising new data for these substances. This concerns some species of cancer (by mechanisms which are not subject of this article), but also cardiovascular disease (CVD), which is nowadays regarded as the result of long-lasting inflammatory processes in combination with other factors like high levels of (oxidized) blood lipids.

Risk reduction for CVD by flavonoid intake has been suggested in a number of studies, e.g., by Knekt et al. [71] with 20 years follow-up of more than 5000 Finnish men and women. In a 28 years follow-up cohort study with 9000 Finnish people, a correlation of CVD risk with apple intake was reported, but not with the main flavonol component quercetin [72].

Marniemi et al. [73] assessed dietary and serum vitamins and minerals for prediction of acute myocardial infarction (AMI) and stroke in elderly subjects in Finland. In a population-based health survey with special emphasis on the diet, 361 men and 394 women aged between 65–99 years were followed up for up to 10 years. Low intakes of vitamin D emerged as the sole predictor for stroke, while high dietary intakes of luteolin and kaempferol were associated with lowered risk of AMI in a statistically significant way ($p < 0.01$).

Mink et al. [74] investigated dietary intake of total flavonoids and 7 subclasses to evaluate the association between flavonoid intake and CVD mortality in 34,489 postmenopausal women in the Iowa Women's Health Study. Mean total flavonoid intake was 13.9 mg/d, quercetin 9.7 mg/d, kaempferol 3.4 mg/d, myricetin 0.74 mg/d, luteolin 0.05 mg/d, and apigenin 0.01 mg/d. After 16 years of follow-up no significant inverse associations were observed for total flavonoid intake, but between anthocyanidins and CHD, CVD, and total mortality; between flavanones and CHD; and between flavones and total mortality (OR: 0.88 (0.82–0.96)). No association was found between flavonoid intake and stroke mortality. In an earlier evaluation of the same study population after 10 years follow-up, Yochum et al. [75] had analyzed single flavonols and flavones. Quercetin and kaempferol significantly reduced CHD death, while luteolin was at the border of significance [OR: 0.8 (0.63–1.02)].

It appears that nutritional uptake of luteolin or its glycosides in very low amounts contributes to protection from cardiovascular disease (but not from stroke); there are, however, also some studies which could not find such a correlation [76], [77].

Besides inflammatory and allergic reactions, cardiovascular disease and cancer, various indications like diabetes mellitus, multiple sclerosis, or leishmaniasis have been discussed as possible therapeutic fields for luteolin. Luteolin is already marketed as a nutrition supplement providing 100 times higher dosage than normal diet, with reference to anti-ageing and specific therapeutic activities. This is questionable in view of the scarce clinical and safety data for such high amounts. Recent publications indicate possible interactions of luteolin with the cytochrome P450 system and other detoxification systems [78], [79], so that high-dose oral uptake needs further scientific backing. As far as epidemiological studies on nutritional effects are concerned, the question of luteolin uptake in form of its glycosides or the aglycone needs also further elucidation before consequences for supplementation can be drawn. An interesting application of luteolin was presented by Ahlenstiel et al. [80] for the preservation of renal transplantation tissue.

Considering the biotransformation in which flavonoids and also luteolin are involved after oral uptake the topical application of luteolin for inflammatory skin diseases may be very interesting. Preliminary pharmacokinetic data suggest that luteolin is able to penetrate into deeper skin layers [63] and might be used for the treatment of inflammatory and allergic skin diseases as well as for skin protection from solar radiation. Skin damage from solar radiation is mediated via generation of reactive oxygen species and radicals, which induce inflammatory processes. Luteolin may counteract this process by its anti-oxidant as well as anti-

inflammatory mechanisms. Protection from solar induced erythema by topical application of luteolin and long-term anti-ageing effects on dermal structure should be an interesting field for further investigations.

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