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Over three decades of research on dietary flavonoid antioxidants and cancer prevention - What have we achieved?

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Abstract

Epidemiological studies keep confirming that the so-called Mediterranean Diet, which is characterised by a relatively high intake of fruit and vegetables, enhances health and provides protection against cancer. The first step in carcinogenesis, and possibly in a range of other degenerative diseases such as heart disease or degenerative dementia, is most likely damage to DNA and other macromolecules. Radical oxygen species - i.e. superoxide anion, hydrogen peroxide, and the hydroxyl radical - are generally considered a major cause of damage to macromolecules. It was long suggested that the antioxidant properties of food ingredients are essential to understanding the mechanism of action of what constitutes a healthy diet, i.e. a diet that prevents the onset of degenerative diseases.

However, since the levels of antioxidants in blood plasma required to see any health benefits is much higher than what we get through our diet, the role of dietary phytochemicals acting as anti-oxidants is now in doubt. Nevertheless, a correlation between presence of flavonoids in the diet and prevention of degenerative diseases has remained. Though there is a putative role for dietary flavonoids in the prevention of degenerative diseases, the exact mechanism of action of these phytonutrients is still a matter of debate.

The human body has its own defences against oxidative stress in the form of the enzymes superoxide dismutase (SOD) and catalase (CAT), and reduced glutathione (GSH). Rather than being antioxidants in their own right, plant constituents are more likely to act as triggers or inducers of expression of the human antioxidants SOD, CAT, and GSH.

Keywords

Polyphenols; antioxidants; flavonoids, kaempferol, quercetin; oxidative stress

Introduction

In 1983, Bruce Ames published a paper on dietary carcinogens and anticarcinogens, linking the risk of degenerative diseases to the occurrence of oxygen radicals in our diet (Ames, 1983). Using his eponymous bio-assay (Ames et al., 1973), Ames identified a range of phytochemicals that occur in the human diet but have mutagenic and possibly carcinogenic properties. Examples include phenylpropenes that are present in commonly used herbs like basil (*Ocimum basilicum* L.), fennel (*Foeniculum vulgare* Mill.), anise (*Pimpinella anisum* L.), and in sassafras (*Sassafras albidum* (Nutt.) Nees.); hydrazines that can be found in edible mushrooms, e.g. the false morel (*Gyromitra esculenta* (Pers. ex Pers.) Fr.), and the most common culinary species, the chestnut mushroom or portobello mushroom (*Agaricus bisporus* (J.E. Lange) Imbach). Other dietary mutagens are furanocoumarins - commonly found in vegetables from the family Apiaceae, e.g. celery (*Apium graveolens* L.), parsnips (*Pastinaca sativa* L.), and parsley (*Petroselinum crispum* (Mill.) Fuss), and allyl isothiocyanate - the pungent principle in various Brassicaceae including mustard (various *Sinapis* spec. or *Brassica* spec.), horseradish (*Armoracia rusticana* G.Gaertn., B.Mey. & Scherb.) and wasabi (*Eutrema japonicum* (Miq.) Koidz., syn. *Wasabia japonica*). Further compounds that were identified as mutagens are the xanthine alkaloid theobromine from cacao beans (*Theobroma cacao* L.) and kola nuts (*Cola acuminata* Schott & Endl.), and glycoalkaloids in potato tubers (*Solanum tuberosum* L.). Overall, the variety in toxic chemicals that can be found in the human diet is great and the various mechanisms by which these compounds exert their mutagenic and possible carcinogenic activities shows a similarly great diversity.

In addition to the identification of mutagenic compounds in the human diet, the Ames test assay led to the identification of a number of compounds derived from food that reduce the level of mutagenesis and can be considered anticarcinogens. The molecules that were

identified include vitamin E (tocopherol), β -carotene and related carotenoids, glutathione, vitamin C (ascorbic acid), and possibly phenolic compounds. Although there is a fair amount of structural variety in the molecules that were identified as beneficial, the common factor is that they are all small molecules with antioxidant properties. It was suggested that the antioxidant properties are essential to understanding the mechanism of action of what constitutes a healthy diet, i.e. a diet that prevents the onset of degenerative diseases.

The recommendation presented in the paper is modest and rather cautious: ‘a general increase in consumption of fibre-rich cereals, vegetables, and fruits and decrease in consumption of fat-rich products and excessive alcohol would be prudent’ (Ames, 1983). We may compare these recommendations with those of the World Cancer Research Fund/American Institute for Cancer Research in their Third Expert Report (WCRF/AICR, 2018): ‘Choose fibre-rich wholegrains for most grain servings. Suggested intake of 2 to 3 servings of fruit per day, 2 to 3 servings of vegetables per day, include sufficient consumption of dietary fibre, limit intake of saturated fatty acids’. The recommendations have changed very little over a period of 35 years, however the evidence-base for the recommendations has seen a considerable revision.

Figure 1:

Formation of reactive oxygen species (ROS) as part of normal oxidative metabolism

Radical oxygen species (ROS)

The first step in carcinogenesis, and possibly in a range of other degenerative diseases such as heart disease or degenerative dementia, is most likely damage to DNA and other macromolecules. Radical oxygen species - i.e. superoxide anion, hydrogen peroxide, and the hydroxyl radical - are generally considered a major cause of damage to macromolecules.

Sources of radical oxygen species include ionising radiation which can turn water into hydroxyl radical, inflammatory processes which convert oxygen into superoxide, cytochrome P450-mediated drug metabolism, and catabolic processes such as β -degradation of fatty acids. Unsurprisingly, the occurrence of degenerative diseases is positively correlated with exposure to ionising radiation or with chronic inflammation. Although the processes mentioned contribute to the level of ROS in the human body, the main source of ROS is our oxidative metabolism (Harman, 1981). The catabolic processes glycolysis, fatty acid oxidation, and citric acid cycle generate NADH and FADH₂ which are fed into the oxidative phosphorylation (electron transport) pathway in the mitochondria. In the latter process, electrons are transferred and used to reduce molecular oxygen to water in a step-wise fashion (Fig. 1), the free energy that is created in the process is used to generate ATP. The step-wise reduction of O₂ is mediated by a series of electron carriers, and the intermediates in this series of steps are ROS. The reduction is often portrayed as a conveyer belt series of reactions starting with O₂ and ending with H₂O, where the ROS intermediates are rapidly converted from one into the other. In reality, it is more a series of staggered steps and each of the ROS intermediates can accumulate and diffuse out of the mitochondria before it is converted further. Thus, the process of oxidative metabolism, which is essential for the maintenance of aerobic life forms, is the main source of superoxide anion, hydrogen peroxide, and hydroxyl radicals. It can be assumed that ROS generated during normal oxidative metabolism would produce changes in macromolecules. They are known to be involved in lipid peroxidation –

1. **Superoxide dismutase (SOD)**

2. **Catalase (CAT)**

3. **Glutathione (GSH) & Glutathione peroxidase (GPx)**

Figure 2: Antioxidant enzymes

and thus, in cell membrane damage, protein oxidation, and DNA damage. Mitochondrial DNA and membranes are possibly the first to be affected. These alterations may over time have an accumulative deleterious effect on the functioning of mitochondria and thus result in degenerative cell changes (Harman, 1981).

In order to limit the damage that can be caused by ROS that have leaked out of the mitochondria, a range of antioxidant enzymes are active: superoxide dismutase (SOD) converts the superoxide anion into the much less harmful hydrogen peroxide, catalase (CAT) catalyses the breakdown of hydrogen peroxide into water and oxygen, glutathione (GSH) and glutathione peroxidase (GPx) also help break down hydrogen peroxide into glutathione disulphide (GSSG) and water (Fig. 2). The GSH/GSSG ratio is often used as a measure to quantify the redox state of blood plasma (Jones, 2006).

It has been suggested that the expression of the human antioxidant enzymes and maintenance of a redox balance are triggered by ROS rather than directly by a pro-oxidant/antioxidant balance. A certain level of ROS may be essential, because at low levels ROS may function to trigger antioxidant response (Jones, 2006; Finley et al. 2011).

Figure 3: Delocalisation of electron from a radical species in the ascorbate radical.

Dietary antioxidants, ORAC values

Dietary antioxidants can remove radical species by accepting their unpaired electron and then stabilizing it through resonance and delocalization, as can be shown in the examples of vitamin C (Fig. 3). Naturally occurring antioxidants are characterised by the presence of

Figure 4: Structures of phytonutrients with anti-oxidative or radical scavenging activity

conjugated double bonds in their structure, which enables electrons to be delocalised and thus shared by many atoms (Fig. 4). Examples of some common dietary antioxidants are vitamin C, vitamin E, and carotenoids (Stahl & Sies, 2003), and various polyphenols including stilbenoids (Lorenz et al., 2003), chalcones (Rozmer & Perjési, 2016), flavonoids (Trembl & Šmejkal, 2016) and isoflavones (Sreerama et al. 2010). The idea that dietary antioxidants may ameliorate a poor redox status, and thus boost human health rapidly gained popularity. Linus Pauling, who had been awarded the Nobel Prize for Chemistry in 1954 and the Nobel Peace Prize in 1962, was one of the main advocates for taking large doses of vitamin C to ward off illnesses, including cancer. His books on the topic (Pauling 1970; Cameron & Pauling 1979/1993; Pauling 1986) were bestsellers in their time, though his theory of orthomolecular therapy never gained much traction outside complementary and alternative medicine. In the 1990s onwards, antioxidant status of fruit, vegetables, and dietary supplements and the link with health enhancement, were widely discussed, in popular media but also in peer-reviewed scientific papers.

The antioxidant capacity of plant extracts is relatively easy to measure using assay techniques that can be applied in most laboratories. The most popular assay is the DPPH [2,2-diphenyl-1-picrylhydrazyl] assay - where the stable free radical DPPH reacts with radical scavengers, resulting in a colour change which can be quantified by measuring absorbance 517 nm (Sharma & Bhat, 2009). DPPH in solution can also be used as a spray reagent in TLC analysis; the reagent loses colour when it reacts with spots that have radical scavenging activity.

The ABTS [2, 2'-azinobis (3-ethylbenzthiazoline-6-acid)] radical scavenging assay requires a bit more effort. First ABTS is converted to its radical form with the aid of hydrogen peroxide and horseradish peroxidase - then, if radical scavengers are present, the blue ABTS radical

cation is converted back to its colourless neutral form and this conversion can be monitored by measuring decrease in absorbance at 730 nm (Cano et al., 2000). This assay also known as the Trolox equivalent antioxidant capacity (TEAC) assay, because the reactivity of antioxidants tested are commonly compared to that of the known antioxidant Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid).

A third assay, regularly used to indicate antioxidant activity is the Folin-Ciocalteu assay, though strictly speaking this is an assay to determine the presence of polyphenols rather than an antioxidant assay per se (Agbor et al., 2014).

Further antioxidant tests include the total radical absorption potential (TRAP) assay (Evelson et al., 2001), ferric reducing/antioxidant power (FRAP) assay (Benzie et al., 1999), and oxygen radical absorption capacity (ORAC) assays (Cao & Prior, 1999). The latter was long considered industry standard for estimating antioxidant strength of whole foods, juices and food additives, mainly from the presence of polyphenols (Ou et al. 2002). In the ORAC assay, the fluorescent compounds β -phycoerythrin or fluorescein are used as an indicator, and AAPH (2,2'-azobis(2-amidinopropane) dihydrochloride) as a peroxy radical generator. Trolox is used as a control standard, and the degree of protection of the indicator molecules against oxidative degeneration is quantified using a fluorometer. Results are expressed as ORAC units, where 1 ORAC unit equals the net protection produced by 1 μ M Trolox.

The relative ease of antioxidant assays, and the wide availability of ORAC values in the scientific literature, on governmental databases, and through nutritional and health care information sources (both governmental and commercial), made that many observations on health-enhancing properties of dietary products were linked with their antioxidant properties. However, it would be prudent here to note the adage that correlation is not causation.

In addition, the effects of flavonoids with known antioxidant activity were often tested in *in vitro* assays, e.g. the inhibitory effect of flavonoids on growth of tumour cell lines, on

enzyme activities, or oxidation of low-density lipoproteins (LDL) were assessed (e.g. review by Harborne & Williams, 2000). Many in vitro assay methods showed inhibitory activity, but at concentrations that would not be achieved in blood plasma, i.e. in systemic circulation. For example, the quercetin IC₅₀ values for inhibition of LDL oxidation was 10 µM, hypolaetin (8-hydroxyluteolin) inhibits 5-lipoxygenase with an IC₅₀ of 10 µM, pedicin (3',6'-dihydroxy-2', 4', 5'-trimethoxychalcone), from leaves of *Fissistigma languinosum* (Annonaceae), was found to inhibit tubulin assembly into microtubules (IC₅₀ value 300 µM) (Alias et al., 1995). Polyphenols are found in concentrations ranging from nmol/L to lower µmol/L ranges in plasma (Manach et al., 2005) Polyphenol levels higher than 1 µmol/L are not maintained long in the plasma; maximum polyphenol concentrations in plasma are reached after 1-9h and their elimination half-lives vary from 1-28h (Zamora-Ros et al., 2014).

Antioxidants and cancer

The period since the publication of Ames' landmark paper (Ames, 1983) saw an exponential increase in papers on the topic 'antioxidants and cancer'. However, it has become apparent for a while that, as research continues, some paradoxical effects accumulate (Halliwell, 2000; Halliwell, 2013). Whereas there is general consensus that consumption of fruit and vegetables help maintain human health, supplements of ascorbate (Jacob et al., 2003), vitamin E (GISSI, 1999), or β-carotene (Vance et al., 2013; Khurana et al., 2018) do not seem to offer protection against oxidative damage, i.e. a protective effect of diet is not equivalent to a protective effect of antioxidants in diet.

The Agricultural Research Service (ARS), which is part of the United States Department of Agriculture (USDA), is a not-for-profit governmental organisation. Its mission is to develop authoritative food composition databases and state-of-the-art methods to acquire, evaluate, compile and disseminate composition data on foods. Between 2007 and 2012, USDA/ARS

had made available a database listing the ORAC values of selected foods. However, they withdrew the ORAC database from their website when it became increasingly apparent that the beneficial effects of polyphenol-rich foods cannot be attributed to the antioxidant properties of these foods (Finley et al, 2011). In its justification, the USDA/ARS stated that there are a number of bioactive compounds which are theorized to have a role in preventing or ameliorating various chronic diseases such as cancer, coronary vascular disease, Alzheimer's, and diabetes. However, the associated metabolic pathways are not completely understood and non-antioxidant mechanisms, still undefined, may be responsible. In addition, concern was expressed that ORAC values are routinely misused by food and dietary supplement manufacturing companies to promote their products and by consumers to guide their food and dietary supplement choices.

Diet and cancer

Epidemiological studies keep confirming that the so-called Mediterranean Diet, which is characterised by a relatively high intake of fruit and vegetables, enhances health and provides protection against cancer (Benetou et al., 2008; Bonaccio et al., 2018; WCRF/AICR, 2018).

Though the role of dietary phytochemicals acting as anti-oxidants is now in doubt, a correlation between presence of flavonoids in the diet and prevention of degenerative diseases has remained (Grosso et al., 2017; Lei et al., 2016; Zhang et al., 2016).

Though there is a putative role for dietary flavonoids in the prevention of degenerative diseases, the exact mechanism of action of these phytonutrients is still a matter of debate.

They should exert their effect at plasma concentration of less than 1 $\mu\text{mol/L}$, so in vitro models that show activities only at higher concentrations should be regarded critically.

The structural resemblance of some flavonoids with human steroid hormones has been noted before (Arroo et al. 2014). These so-called phytoestrogens can interfere with oestrogen

receptors (ER) and oestrogen metabolism. Oestrogens play a key role in human physiology and development - including positive effects on development of the brain, bone, heart, liver, and vagina, although prolonged oestrogen exposure has negative effects such as increased risk of breast and uterine cancers. Modulation of estrogens and ERs by dietary phytoestrogens can be accomplished by inhibition of ER binding, by downregulating ERs, or by decreasing oestrogen production (reviewed by Balunas et al. 2008). Of the flavonoids tested, flavones have been tested most often and have been the most active. Though the similarity of phytoestrogens with human oestrogen has received most attention, several reports have highlighted interference with non-aromatic androgens as well, suggesting a possible role in prevention of prostate cancer (Knowles et al., 2000; Chen et al., 2001; Fu et al., 2004; Khan et al., 2008).

In spite of the large amount of papers that have been written over the last three decades claiming anti-cancer properties for flavones, not a single flavone-based anticancer drug is in clinical use. The closest we have to a potentially therapeutically useful flavone is Flavopiridol (Alvocidib, L86-8275), which has been granted orphan drug designation by the FDA for the treatment of patients with acute myeloid leukemia. Ironically, Flavopiridol is not a derivative of any known dietary flavone, but rather of the chromone alkaloid rohitukine (Fig. 5) extracted from *Dysoxylum binectariferum* or *Amoora rohituka* (Meliaceae) (Mohana Kumara et al. 2014; Safia et al., 2015). Flavopiridol and rohitukine are both kinase inhibitors and their mechanism of action is through interference with cell signalling and modulation of the apoptosis pathways.

In the previous decade, modulation of cell signalling pathways by flavones has received increasing interest as a potential model for the preventative properties of phytonutrients. Depending in the signalling pathways, this can either trigger or mitigate expression specific cytochromes P450 that affect the levels of toxins that are involved in early carcinogenesis

Figure 5: Rohitukine and its semi-synthetic flavone derivative flavopiridol

(Dong et al. 2010; Priyadarsini & Nagini, 2012). Some natural compounds that exhibit antioxidant activity may not act as antioxidants in their own right but rather act to trigger cell signalling pathways that lead to changes in gene expression, which in turn result in the activation of enzymes that eliminate ROS (Finley et al., 2011; Priyadarsini & Nagini, 2012). Considering that the human body has its own defences against oxidative stress in the form of the enzymes superoxide dismutase (SOD) catalase (CAT), and glutathione peroxidase (GPx): plant constituents may act as triggers or inducers of expression of the human antioxidants SOD, CAT, and GSH (Taha et al., 2014; Sharifzadeh et al., 2017).

Antioxidants and cancer - revisited

Human aging is characterized by a chronic, low-grade inflammation which is positively correlated with the occurrence of degenerative diseases (Franceschi & Campisi, 2014). This chronic low-grade inflammation occurring in the absence of overt infection, referred to as

‘inflammaging’, is thought to trigger a range of age-related multi-factorial conditions - e.g. cancer, cardiovascular disease, Alzheimer’s disease, and type II diabetes (Sanada et al., 2018). Ageing and chronic inflammatory conditions are also associated with increased oxidative stress (Bauer & Fuente, 2016).

A string of recent papers (Table 1) reported on how polyhydroxy flavonoids attenuate the effects of inflammation and oxidative damage. The general methodology is that some kind of injury is caused to animals - e.g. streptozotocin-induced diabetes, or acetaminophen-induced liver injury - which triggers inflammation-associated cytokines including tumour necrosis factor- α (TNF- α), interleukin-6 (IL-6), and IL-1 β as well as proteins such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). This in turn results in an increase of pro-inflammatory mediators such as nitric oxide (NO), reactive oxygen species (ROS) and the lipid peroxidation indicator malondialdehyde (MDA). Oral application of polyhydroxy flavonoids up-regulates the activities of antioxidant enzymes (heme oxygenase 1 / HO-1, SOD, CAT and GSH-Px) which results in a decrease of ROS and MDA. In addition, the flavonoids prevent or reverse increase in inflammatory factors TNF- α and interleukins. Further, a range of flavonoids seem to decrease nuclear factor-kappa B (NF- κ B) and mitogen-activated protein kinases (MAPKs) activation via inhibition of the degradation of NF- κ B, inhibitory B-a (IkB-a), extracellular regulated kinase (ERK), c-Jun-N-terminal (JNK), and p38 active phosphorylation. Thus, the flavonoids alleviate the effects of injury through suppression of ROS- and/or NF- κ B-mediated inflammasome activation - specifically the nucleotide-binding domain, leucine-rich repeat family, pyrin domain containing 3 / NLRP3 (Li et al., 2018). Several papers report the activation of transcription factor Nrf2 which regulates the expression of antioxidant proteins that protect against oxidative damage triggered by injury and inflammation (see Table 1).

Flavanone treatment	Antioxidant and anti-inflammation markers	Model	Injury treated	Reference
Naringenin 25-100 mg/kg/day, 2 weeks	CAT, GSH, SOD, up MDA, NO down IL-1 β , IL-6, TNF- α and NF- κ B down	Mice	olfactory bulbectomy-induced neuroinflammation	Bansal et al., 2018
Naringenin 10-50 mg/kg/day, i.p., 2 weeks	CAT, GSH, SOD, up MDA, NO down IL-1 β , TNF- α down	Mice	Social defeat stress	Umukoro et al., 2018
Naringenin 100 mg/kg/day, 5 weeks	CAT, GSH, GSH-Px, HO-1, SOD, up MDA down IL-6, NF- κ B p65, COX-2 TNF- α down	Mice	Alcohol-Induced Hepatic Damage	Zhao et al., 2018
Eriodictyol 5-20 μ M, 24 hours	CAT, GSH-Px, HO-1, SOD, up ROS down Nrf-2 up IL-8, TNF- α down	Rat RGC-5 cells	high glucose-induced oxidative stress	lv et al., 2018
Eriodictyol 50-200 mg/kg, i.v., 24 hours	GR, GSH, GSH-Px, GST, SOD up MDA down CYP-2E1 and CYP-3A11 down	Mice	Acetaminophen (APAP)-induced liver injury	Wang et al., 2017
Hesperetin 10-20 mg/kg/day, 3 weeks	CAT, GSH-Px, GR, GSH, SOD up MDA down	Rats	Streptozotocin (STZ)-induced Alzheimer's Disease	Kheradmand et al., 2018
Hesperetin 100 mg/kg/day, 6 days	CAT, HO-1, SOD up iNOS, MDA down Nrf-2 up NF- κ B down	Mice	lipopolysaccharide/D-galactosamine (LPS/ D-GalN)-induced acute liver failure	He et al., 2019
Hesperetin 50 mg/kg/day, 46 days	GSH, GSH-Px, SOD up MDA, ROS down IL-17, TNF α down	Rats	STZ-induced diabetes	Samie et al., 2018

Dihydromyricetin 250 mg/kg/day, 2 weeks	SOD up MDA, ROS down SIRT-3 up	Mice	Transverse aortic constriction- induced myocardial hypertrophy	Chen et al.,2018
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Flavone treatment	Antioxidant and anti-inflammation markers	Model	Injury treated	Reference
Chrysin 25-50 mg/kg/day, 6 days	CAT, GSH, GSH-Px, SOD up MDA down IL-1 β , TNF- α down	Rats	paracetamol-induced liver injury	Eldutar et al.,2017
Chrysin 100 mg/kg/day, 6 days	CAT, HO-1, SOD up iNOS, MDA down Nrf-2 up NF- κ B down	Mice	LPS/ D-GalN-induced acute liver failure	He et al.,2019
Chrysin 25-50 mg/kg/day, 6 days	CAT, GSH-Px, SOD up MDA down IL-1 β , IL-33, TNF- α down	Rats	Paracetamol-induced oxidative stress	Kandemir et al., 2017
Apigenin 100 mg/kg/day, 6 days	CAT, HO-1, SOD up iNOS, MDA down Nrf-2 up NF- κ B down	Mice	LPS/ D-GalN-induced acute liver failure	He et al., 2019
Apigenin 15 mg/kg/alternate days, i.p., 30 days	CAT, GSH, GSH-Px, SOD up ROS down IL-6, TNF- α down pIKK β , JNK, NF- κ B-p65 down	Rats	High calorie diet-induced oxidative stress	Jagan et al., 2017
Apigenin 10 μ M, 2 hours	CAT, GSH-Px, SOD up iNOS, ROS down TNF- α , IL-6, NF- κ B, COX-2 down	H9C2 cells (Rats)	Isoproterenol-induced apoptosis	Thangaiyan et al., 2018

Apigenin 100 mg/kg/day, 5 weeks	CAT, GSH, GSH-Px, HO-1, SOD, up MDA down IL-6, NF- κ B p65, COX-2 TNF- α down	Mice	Alcohol-Induced Hepatic Damage	Zhao et al., 2018
Apigetrin 15-30 mg/kg/day, 10 days	HO-1 up ROS down Nrf2 up IL-1 β , IL-6, p-NF- κ B (p-p65), TNF- α down	Mice	LPS-induced acute otitis	Guo et al., 2019
Luteolin 100 mg/kg/day, 6 days	CAT, HO-1, SOD up iNOS, MDA down Nrf-2 up NF- κ B down	Mice	LPS/D-GalN-induced acute liver failure	He et al., 2019
Luteolin 30 mg/kg/day, i.p., 30 days	SOD, GSH-Px up MDA down IL-1 β , IL-6, TNF- α down	Rats	Chronic cerebral hypoperfusion (CCH)-induced cognitive dysfunction	Yao et al., 2018
Isoorientin 50 mg/kg/12h, i.p., 24 hours	GSH, SOD up MDA down Nrf2 AMPK/Akt/GSK3 β up JNK down	Mice and HepG2 cells	APAP-induced liver injury	Fan et al., 2018
Orientin 10-40 mg/kg/day, i.p., 12 days	GSH, SOD up MDA down IL-1 β , IL-6, IL-10, TNF- α down TLR-4/NF- κ B down	Rats	Spinal nerve ligation	Guo et al., 2018
Diosmin 50-100 mg/kg/day, 10 days	CAT, GSH, GSH-Px, GR, GST, SOD up MDA, NO down IL-1B, IL-6 and TNF- α down	Mice	Methotrexate (MTX)-induced hepatic, renal, and cardiac injury	Abdel-Daim, et al., 2017

Flavonol treatment	Antioxidant and anti-inflammation markers	Model	Injury treated	Reference
Galangin 8 mg/kg/day, 45 days	GSH, GSH-Px, SOD up TBARS down	Rats	Streptozotocin (STZ)-induced diabetes	Aloud et al., 2018
Galangin 75-100 mg/kg/day, 3 days	CAT, GSH, GSH-Px, SOD up TNF- α , IL-1 β and IL-6 down ERK and NF- κ B signalling down	Mice	Cisplatin-induced renal injury	Huang et al., 2017
Galangin 27 mg/kg, single i.p., 2 hours	CAT, GSH, SOD up MDA down	Rats	Liver ischemia-reperfusion injury	Li et al., 2018
Kaempferol 20 nM, 24 hours	SOD up ROS down	ARPE-19 cells (Human)	hydrogen peroxide-induced oxidative cell damage	Du et al., 2018
Kaempferol 62-250 mg/kg, single dose, 18 hours	CAT, GSH, GSH-Px, HO-1, SOD up ROS, TBAR down CYP2E1 down Nrf2 up IL-6, TNF- α down	Mice	Propacetamol-induced acute liver injury	Tsai et al., 2018
Kaempferol 15 mg/kg/12h, 6 days (mice) 50 μ M, 24 hours (MH-S cells)	SOD up ROS, MDA, down IL-1b, IL-6, TNF- α down NF- κ B p65 down	Mice MH-S cells	influenza virus-induced acute lung injury	Zhang et al., 2017
Astragalin (Kaemp-3-OGlc) 3-30 mg/kg/day, 8 weeks	CAT, GSH-Px, SOD up NO, MDA down TNF- α , iNOS down	Mice	STZ-induced diabetes	Han et al., 2019
Quercetin 100 mg/kg/day, 4 weeks	CAT, GSH, GSH-Px, SOD up MDA down	Rats	Diazinon induced neurohepatic inflammation	Abdel-Daim et al., 2018

Quercetin 30-120 mg/kg/day, 7 days	CAT, GSH, SOD up MDA down NF- κ B pathway (TNF- α , IL-6 and IL-17) down	Mice	Imiquimod (IMQ)-induced psoriasis	Chen et al., 2017
Quercetin 50 mg/kg/day, i.p., 56 days	CAT, GSH, GSH-Px, SOD up TBARS down IL-6, iNOS, NF- κ B down	Rats	Ammonium chloride-induced hyperammonemia	Kanimozhi et al., 2016
Quercetin 20 μ M, 24 h	SOD up MDA down	RAW264.7 cells (mice)	Oxidized low density lipoprotein (ox-LDL)-induced lipid deposition	Li et al., 2018b
Quercetin 25 mg/kg/day, 18 weeks	CAT, SOD up ROS down CYP1A1 and 1B1 down NF κ B signalling down	Hamsters	7,12-dimethylbenz[a]anthracene (DMBA) induced buccal pouch carcinomas	Priyadarsini & Nagini, 2012
Quercetin 20 μ M, 72 hours	GSH up MDA and NO down	HUVEC cells (Human)	High glucose-induced oxidative stress	Rezabakhsh et al., 2019
Quercetin 50 mg/kg/day, 10 days	CAT, SOD up MDA, NO down COX-2, IL-6, IL-10, TNF- α down	Rats	Cyclophosphamide-induced bladder injury	Sherif et al., 2017
Quercetin 20 mg/kg/day, 10 days	IL-6, IL-17, TLR-4, NF- κ B down	Mice	Triptolide (TP)-induced liver injury	Wei et al., 2017
Quercetin 100 mg/kg/day, 5 weeks	CAT, GSH, GSH-Px, HO-1, SOD, up MDA down IL-6, NF- κ B p65, COX-2 TNF- α down	Mice	Alcohol-Induced Hepatic Damage	Zhao et al.,-2018
Quercetin 60 mg/kg/day, 3 weeks	SOD, GSH-Px up MDA down IL-1 β , IL-6, IL-10, iNOS down	Mice	Alcohol-induced liver injury	Zhu et al.,-2017
Rutin 150 mg/kg/day, 14 days	CAT, GSH, GSH-Px, SOD up MDA down	Rats	cisplatin-induced testicular injury	Aksu et al., 2016

Rutin 60 mg/kg/day, 7 days	SOD up MDA down IL-1 β down	Mice	Formalin-induced paw inflammation	Fikry et al., 2017
Rutin 50 mg/kg/day, 31 days	CAT, GSH-Px, SOD up MDA down IL-1 β , TNF- α down	Rats	Fluoride-induced neuro-inflammation	Nkpaa et al., 2018
Rutin 5-15 mg/kg/day, 10 days	SOD, GSH, GSH-Px up MDA down IL-1 β , TNF- α , pNFkB p65 down	Rats	Freund's adjuvant-induced arthritis	Sun et al., 2017
Rutin 10-100 mg/kg/day, 30 days	CAT, GSH, GSH-Px, GR, SOD up MDA, ROS down iNOS down Nrf2 up	Mice Erythrocytes (human)	Hydrogen peroxide-induced oxidative stress	Singh et al., 2018
Rutin 10-100 mg/kg/day, 7 days	CAT, GSH, GSH-Px, GR, SOD up MDA, ROS down iNOS down Nrf2 up	Mice Erythrocytes (Human)	<i>t</i> -butyl hydroperoxide-induced oxidative stress	Singh et al., 2019
Myricetin 50 mg/kg/day, 5 days	CAT, GSH-Px, SOD up MDA down	Rats	MTX-induced liver injury	Ekinci-Akdemir. , 2018
Myricetin 1-25 mg/kg/day, 1 week	GSH/GSSG ratio, SOD up, MDA down NF- κ B/p65, IL-1 β , IL-6, TNF- α down	Rats	Cerebral ischemia injury	Sun et al., 2017
Myricitrin 5-30 mg/kg/day, i.p., 5 days	CAT, GSH-Px, SOD up MDA down NF- κ B p65, IL-1 β , IL-6, TNF- α , COX-2 down	Rats	traumatic injury of the spinal cord	Lei et al., 2017

Table 1: Flavonoids ameliorate the effects of oxidative injury by activating antioxidant enzymes and downregulating pro-inflammatory signalling pathways.

Intervention studies should look at chronic effects of flavones rather than acute effects. The bioactivities of most flavones listed in Table 1 were assessed after regular oral application of relatively high amounts, typically over a one- or two-week period. This reflects the fact that flavonoids are part of any regular diet rich in fruits and vegetables, and their levels in blood plasma can build up after prolonged intake. For example, after 10 days of dietary supplementation of (–)-epicatechin, metabolites were detected in the brain of rodents at levels that could be of physiological relevance (Wang et al., 2012).

Ames already stated that among the most important defences may be those against oxygen radicals and lipid peroxidation. A variety of small molecules in our diet are required for antioxidative mechanisms (Ames, 1983). The statement is true, although the most effective small molecules do not act directly against oxygen radicals and lipid peroxidation as was initially thought. Instead, they are effective mediators of human cell signalling and, at sub-micromolar concentrations, trigger the expression of antioxidant proteins after every meal - provided the meal contains abundant fruit and vegetables. This is consistent with the advice that consumption of fibre-rich cereals, vegetables, and fruits and decrease in consumption of fat-rich products be prudent (Ames, 1983; WCRF/AICR 2018).

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