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Regulation of inflammation and redox signaling by dietary polyphenols

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Abbreviations:
AP-1, activator protein 1
ARE, antioxidant response elements
ATF1, activating transcription factor
CAPE, caffeic acid phenethyl ester
COPD, chronic obstructive pulmonary disease
COX-2, cyclooxygenase-2
CRE, cyclic AMP response element
CREB, CRE-binding protein
EC, (2)-epicatechin
EGC, epicatechin gallate
EGCG, epigallocatechin gallate
EGFR, early growth response
ELAM-1, endothelial leukocyte adhesion molecule 1
EpRE, electrophilic response element

ABSTRACT

Reactive oxygen species (ROS) play a key role in enhancing the inflammation through the activation of NF-κB and AP-1 transcription factors, and nuclear histone acetylation and deacetylation in various inflammatory diseases. Such undesired effects of oxidative stress have been found to be controlled by the antioxidant and/or anti-inflammatory effects of dietary polyphenols such as curcumin (diferuloylmethane, a principal component of tumeric) and resveratrol (a flavanoid found in red wine). The phenolic compounds in fruits, vegetables, tea and wine are mostly derivatives, and/or isomers of flavones, isoflavones, flavonols, catechins, tocopherols, and phenolic acids. Polyphenols modulate important cellular signaling processes such as cellular growth, differentiation and host of other cellular features. In addition, they modulate NF-κB activation, chromatin structure, glutathione biosynthesis, nuclear redox factor (Nrf2) activation, scavenge effect of ROS directly or via glutathione peroxidase activity and as a consequence regulate inflammatory genes in macrophages and lung epithelial cells. However, recent data suggest that dietary polyphenols can work as modifiers of signal transduction pathways to elicit their beneficial effects. The effects of polyphenols however, have been reported to be more pronounced *in vitro* using high concentrations which are not physiological *in vivo*. This commentary discusses the recent data on dietary polyphenols in the control of signaling and inflammation particularly during oxidative stress, their metabolism and bioavailability.

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ERK, extracellular signal-regulated kinase
FEV_1, forced expiratory volume in 1 s
GCLC, glutamate-cysteine ligase catalytic subunit
G-CSF, colony-stimulating factor 3
GM-CSF, colony-stimulating factor 2
GSH, reduced glutathione
H_2O_2, hydrogen peroxide
HAT, histone acetyltransferase
HDAC, histone deacetylase
HDAC2, histone deacetylase-2
HO-1, heme oxygenase-1
ICAM-1, intercellular adhesion molecule-1
IFN-γ, gamma interferon
IK, I-kappa kinase
IL-1α, interleukin 1 α
IL-1β, interleukin 1 β
IL-6, interleukin 6
iNOS, inducible nitric oxide synthase
IRAK, IL-1β receptor-associated kinase
IRF-1, IFN regulatory factor
IκBα, inhibitory kappa B
JNK, c-Jun N-terminal kinases
LPS, lipopolysaccharide
MAPK, mitogen activated protein kinase
MAPKK, mitogen-activated protein kinase kinase
MCP-1, monocyte chemotactic peptide-1
M-CSF, colony-stimulating factor 1
MEK, mitogen-activated kinase
MMP-9, matrix metallo proteinases
MSK-1, mitogen and stress-activated protein kinase-1
NF-E2, nuclear factor erythroid derived 2
NF-κB, nuclear factor-kappa B
NIK, NF-κB inducing kinase
NO, nitric oxide
Nrf2, nuclear redox factor
$O_2^{\bullet -}$, superoxide anion
PUFA, polyunsaturated fatty acids
RANTES, regulated on activation normal T cell expressed and secreted
ROS, reactive oxygen species
SOD, superoxide dismutase
TGF-beta, transforming growth factor beta
TNF, tumor necrotic factor
TRAIL, tumor necrosis factor related apoptosis-inducing ligand
VCAM-1, vascular cell adhesion molecule-1
VEGF, vascular endothelial growth factor

1. Polyphenols: an overview

A wide variety of dietary plants including grains, legumes, fruits, vegetables, tea, wine, etc. contain polyphenols [1]. The disease preventive abilities of fruit and vegetables have been attributed to the antioxidants/polyphenols present in these dietary sources [2]. It is noteworthy that most reports on the beneficial effects of polyphenols have been obtained from *in vitro* studies and more detailed investigations are required to extrapolate these results to *in vivo* situations. This is particularly relevant in view of the fact that polyphenols are known to undergo various biochemical transformations which affects their bioavailability as well as bio-efficacy. In this review, we will discuss the various aspects of polyphenol metabolism, their role as antioxidants and modulators of cell signaling and inflammation, as well as the efficacy of these compounds.

2. Chemistry of polyphenols

Polyphenols, with over 8000 structural variants, are secondary metabolites of plants and denote a huge gamut of substances having aromatic ring(s) bearing one or more hydroxyl moieties. The structure of natural polyphenols varies from simple molecules, such as phenolic acids, to highly polymerized compounds, such as condensed tannins [3]. Polyphenols are effective free radical scavengers and metal chelators which are mediated by the presence of *para*-hydroxyl group. The most widely distributed group of plant phenolics are flavonoids. Their common structure is that of diphenylpropanes (C6–C3–C6) and consists of two aromatic rings linked through three carbons that usually form an oxygenated heterocycle [3]. Fig. 1 shows the basic structure and the system used for the carbon numbering of the flavonoid nucleus, the structures of important polyphenols such as resveratrol, curcumin and catechins and their structural relationship with steroids. The flavonoids subclasses include: flavonols, flavones, flavanols, isoflavones, antocyanidins and others. Glycosylated flavonoids are more water-soluble and less reactive toward free radicals (free radical scavenger).

Considering the large number of polyphenolic compounds present in dietary sources, we have therefore attempted to focus this review on the more well known and studied polyphenols such as resveratrol, curcumin and the catechins.

2.1. *Resveratrol*

Resveratrol (3,5,4′-trihydroxystilbene) was discovered as a phytoalexin in grapes in 1976 by Langcake and Pryce [4], and is composed of two phenolic rings connected by a double bond (Fig. 1). It exists in two isoforms; *trans*-resveratrol and *cis*-resveratrol where the *trans*-isomer is the more stable form [5]. While *trans* to *cis* isomerisation is facilitated by ultraviolet light and high pH, the *cis* to *trans* conversion is facilitated by visible light, high temperature, or low pH [5]. Resveratrol has been of

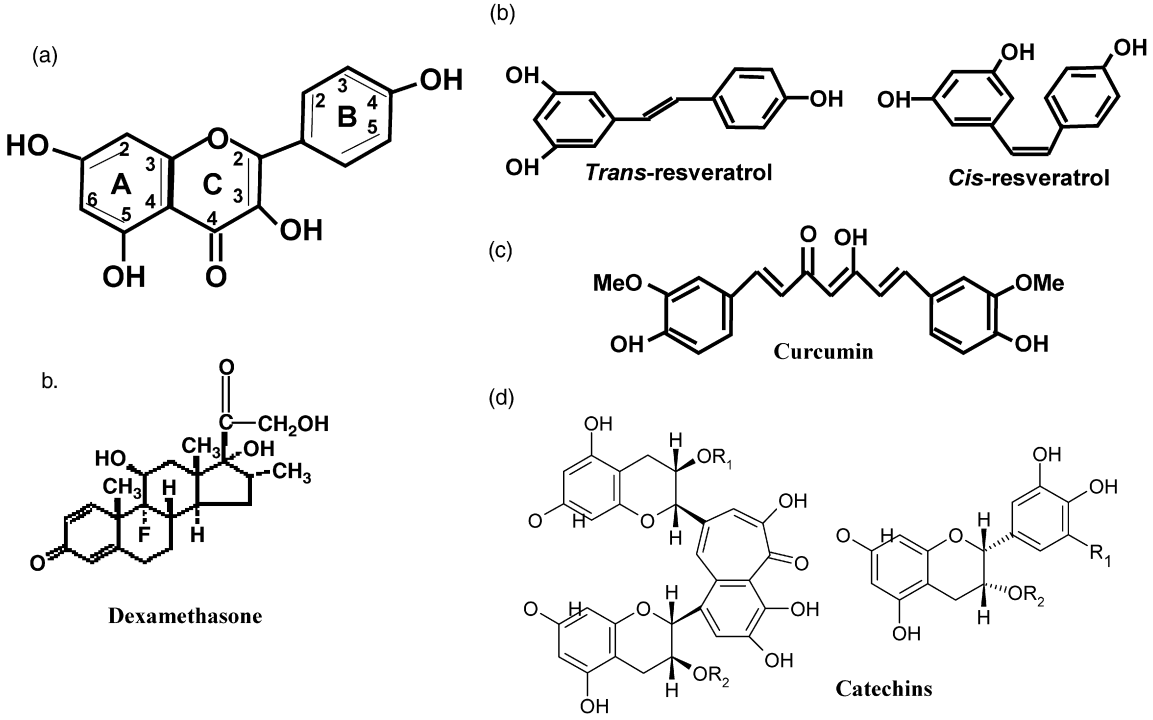

Fig. 1 – Flavonoids (C6–C3–C6), basic structure and system used for carbon numbering of the flavonoid nucleus (a), the structures of important polyphenols such as resveratrol (c), curcumin (d) and catechins (e) and their structural relationship with steroids (dexamethasone) (b). Me = methyl group.

great scientific interest over the years. This interest is mainly due to numerous reports, both *in vitro* and *in vivo*, suggesting its cancer preventive properties and the protective effects of wine against coronary heart disease, the so called ''French Paradox''.

2.2. Curcumin

Curcumin, a member of the curcuminoid family of compounds, is a yellow coloured phenolic pigment obtained from powdered rhizome of *C. longa* Linn. (Family Zingiberaceae). The antioxidant and anticarcinogenic activities of curcumin have been attributed to its hydroxyl and methoxy groups (Fig. 1). A typical crude extract of rhizomes of *C. longa* contain 70–76% curcumin, 16% demethoxycurcumin and 8% bisdemethoxycurcumin. Extensive investigations on curcumin have demonstrated its varied therapeutic effects as: anti-inflammatory, antibacterial, antiviral, antifungal, antitumor, antispasmodic and hepatoprotective [6].

2.3. Catechins

These are monomers of flavanols with a variety of similar compounds such as catechin, epicatechin, epigallocatechin, epicatechin gallate (EGC) and epigallocatechin gallate (EGCG) [7]. Green tea contains mainly catechins whereas black tea contains theaflavins and thearubigins. Oolong tea extracts fall in between green and black tea with respect to their flavanol content.

3. Absorption, pharmacokinetics, tissue distribution and metabolism of polyphenols

In view of polyphenols having complex absorption, biotransformation and bioavailability characteristics, it is important to probe these aspects before we embark on the investigation of the molecular mechanisms and therapeutic applications of these versatile compounds. A proper understanding of how polyphenols are absorbed and transformed pre- and post-absorption is helpful in understanding how *in vitro* observations can be translated into the *in vivo* context.

3.1. Resveratrol

Resveratrol is absorbed mainly in the duodenum with approximately 20% of the available resveratrol being absorbed as evidenced from studies in rat intestines. Resveratrol-glucuronide was the major form absorbed when compared to the very minute amounts of unconjugated resveratrol and resveratrol sulfate [8]. Studies with radio-labeled resveratrol in mice have revealed that resveratrol is distributed to all organs. After 1.5 h post-administration, it was detected in the duodenum as well as in the liver and kidney [9], and remained detectable at these sites for up to 6 h. By 3 h post-administration it could be detected in the lung, spleen, heart, brain, and testis.

Resveratrol is glucuronated in the human liver and sulfated in both the liver and the duodenum. The major derivatives of resveratrol glucuronidation are *trans*-resveratrol-3-*O*-glucuronide, *trans*-resveratrol-4′-*O*-glucuronide, and *trans*-resveratrol-3-*O*-sulfate [10]. Kinetic analysis of resveratrol transformation suggests that in the liver, glucuronidation is favored over sulfation with almost similar rates of reaction. The metabolic modifications of resveratrol can be inhibited by quercetin, a polyphenol also found in wine. Clinical and *in vivo* studies have indicated that free *trans*-resveratrol in plasma is very sparse and short lived.

3.2. Curcumin

Pharmacokinetic measurements have revealed that about 40–85% of ingested curcumin is unaltered in the gastrointestinal tract, most of the absorbed flavonoid being metabolized in the intestinal mucosa and liver [11]. Curcumin undergoes *O*-conjugation to curcumin glucuronide and curcumin sulfate. It is also reduced to tetrahydrocurcumin, hexahydrocurcumin, and hexahydrocurcuminol in rats and mice *in vivo* and in human hepatic cell suspensions [12]. Products of curcumin reduction are also subject to glucuronidation. Certain curcumin metabolites, such as tetrahydrocurcumin, possess anti-inflammatory and antioxidant activities similar to those of their metabolic progenitor. However, recent data indicate that the anti-inflammatory property is lost when curcumin is reduced to tetrahydrocurcumin, although its antioxidant property was still intact. It has been suggested that the intestinal tract plays an important role in the metabolic disposition of curcumin, a notion which is based predominantly on experiments with [^{3}H] labeled curcumin [13]. Metabolites of curcumin such as curcumin glucuronide, curcumin sulfate, tetrahydrocurcumin, and hexahydrocurcumin were identified in intestinal and hepatic microsomes and hepatic cell cytosol from humans and rats. Curcumin is unstable at neutral and basic pH values and is degraded to ferulic acid ([4-hydroxy-3-methoxycinnamic acid]) and feruloylmethane (4-hydroxy-3-methoxycinnamoyl-methane) [14]. Although the exact mechanism of degradation is still not fully obvious, the idea that an oxidative mechanism may be involved gains ground from observations that the presence of antioxidants such as ascorbic acid, *N*-acetyl-L-cysteine, or glutathione completely blocks the degradation of curcumin at pH 7.4. Curcumin should be stable in the stomach and small intestines because the pH is between 1 and 6, and degradation of curcumin is extremely slow under these conditions. Thus, although the pharmacokinetic properties of curcumin have been investigated many questions still remain as to its fate after ingestion.

3.3. Catechins

Tea polyphenols EGCG, EGC, and (2)-epicatechin (EC), are believed to be responsible for the beneficial effects of tea. In a study using decaffeinated green tea in rats [15], it was found that β-elimination half-lives was greatest for EGCG followed by EGC and then EC. An intravenous administration of decaffeinated green tea recorded the highest levels of EGCG in the intestinal samples, and levels declined with a half-life of 173 min. The highest levels of EGC and EC were observed in the kidney, and levels here declined rapidly with half-life of 29 and 28 min, respectively. The liver and lung levels of EGCG, EGC, and EC were generally lower than those in the intestine and

the kidney. Distribution studies indicate that EGCG is mainly excreted through bile, and EGC and EC are excreted through both the bile and urine.

4. Bioavailability of polyphenols

Although the knowledge of absorption, bioavailability, biodistribution and metabolism of polyphenols is not entirely known, in general it appears that some polyphenols are bioactive and are absorbed through the intestine in their native or modified form. The absorbed forms are then metabolized and the end products may be detected in plasma in nanomolar ranges. The plasma forms of polyphenols may retain at least part of their antioxidant capacity before being excreted [16]. In general, the bioavailability of flavonoids is limited due to low absorption and rapid elimination. Nevertheless, flavonoid aglycones and flavonoid glucosides are absorbed in the small intestine. However, they are rapidly transformed into methylated, glucuronidated or sulfated derivatives [16]. Colonic bacteria play an important role in flavonoid metabolism and absorption and the resulting derivatives do not necessarily possess the same biological activity as that of the parent flavonoids [17]. Therefore one has to be careful in extrapolating *in vitro* results obtained from flavonoid studies using purified compounds.

Pharmacologically, curcumin has been found to be safe and human clinical trials indicated no dose-limiting toxicity when administered at doses up to 10 g/day [18]. Curcumin has low oral toxicity in man but also has low oral bioavailability (500–1000 nM after 8 g/day oral dose). However, consumption of curcumin along with piperin (active ingredient in peppers), a known inhibitor of intestinal and hepatic β-glucuronidation of curcumin, may improve the oral bioavailability 20-fold but it still has a short half-life of only 40 min [19]. In the case of resveratrol, dose studies revealed that a 70 kg person could safely consume at least 14 mg resveratrol per day. It is also calculated that daily consumption of pure resveratrol and its analog piceatannol with a dose of 25–50 mg daily leads to nanomolar concentrations of resveratrol in the blood system. Interestingly, on a lighter note, it is estimated that 20 glasses of red wine can provide up to 25 mg of resveratrol, although the ensuing effects of the alcohol would no doubt limit ones uptake via this route.

Bioavailability differs markedly among catechins. EGCG is the only known polyphenol present in plasma with a large proportion (77–90%) in a free form [15]. The other catechins are highly conjugated with glucuronic acid and/or sulfate groups. In a study using pure catechins, van Amelsvoort et al. [20] demonstrated that galloylation of catechins reduces their absorption. They found that only epigallocatechin was methylated and that 4′-O-methyl-epigallocatechin accounted for 30–40% of the total metabolites of epigallocatechin. A study by Meng et al. [21] showed that EGCG was preferentially methylated at the 3′-position. Therefore, the mean bioavailability parameters calculated may be underestimated due to diverse derivatization of the catechins *in vivo* [22]. Several microbial metabolites of catechins mostly in conjugated forms, were also identified in plasma and urine of volunteers after ingestion of green tea [21]. Since polyphenols are poorly absorbed and undergo extensive biotransformation, clinical studies have recently demonstrated that it is safe to consume EGCG or polyphenol E (a defined, decaffeinated green tea polyphenol mixture) in amounts equivalent to the EGCG content in 8–16 cups of green tea once a day, or in divided doses twice a day for 4 weeks.

5. Polyphenols as antioxidants

In recent years there has been a remarkable increment in scientific knowledge dealing with the beneficial role of polyphenols during oxidative stress. This is due to the identification of flavonoids and other dietary polyphenol antioxidants present in plant foods as bioactive molecules. Data supports the idea that the health benefits associated with fruits, vegetables and red wine in the diet are probably linked to the polyphenol antioxidants they contain. Indeed, the high content of polyphenol antioxidants in fruits and vegetables appear to be important factors responsible for these effects.

The antioxidant activity of curcumin was reported as early as 1976 [23] and has been reported to be an effective oxygen free radical scavenger. Curcumin (in the micro to millimolar range) was shown to scavenge ROS, such as superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and nitric oxide (NO), both *in vitro* and *in vivo* [24]. Moreover, it has been shown to be highly protective against H_2O_2-induced damage in human keratinocytes, fibroblasts and in NG 108-15 cells (a mouse neuroblastoma-rat glioma hybrid cell line) [25]. Our recent findings indicated that curcumin, between 1 and 50 μM, could scavenge ROS in 1–4 h as determined by electron pulse resonance spectroscopy (unpublished observation). Furthermore, curcumin was much faster in terms of quenching ROS than other polyphenols tested (resveratrol and quercetin). The antioxidant properties of curcumin are based on its lipid peroxidation lowering effects through its ability to maintain the cellular status of antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase [26]. Indeed curcumin has been shown to increase reduced glutathione (GSH) levels, which leads to lowered ROS production [27]. Since ROS have been implicated in the pathogenesis of various chronic and inflammatory conditions, curcumin therefore has the potential to control these diseases through its potent antioxidant activity. Contradictory to the afore-mentioned antioxidant effects, a pro-oxidant nature of curcumin has also been demonstrated in view of its failure to prevent single-strand DNA breaks by H_2O_2, a damage that was prevented by Vitamin E [28]. The pro-oxidant property is believed to be due to the generation of phenoxyl radicals of curcumin by the peroxidase–H_2O_2 system, which co-oxidizes cellular glutathione or NADH, accompanied by O_2 uptake to form ROS [29]. Thus curcumin may not be a complete antioxidant under situations of oxidative stress. Nevertheless, the antioxidant properties of curcumin can be attributed to its unique conjugated structure, which includes two methoxylated phenols and an enol form of a β-diketone [30].

Catechins are effective scavengers of free radicals at least *in vitro* [31]. Catechins however, may not be effective as antioxidants *in vivo* since, even with very high intakes, plasma and intracellular flavonoid concentrations in humans are

likely to be 100–1000 times lower than concentrations of other antioxidants, such as Vitamin C or glutathione. Moreover, most circulating flavonoids are actually flavonoid metabolites, some of which have lower antioxidant activity than the parent flavonoid. For these reasons, the relative contribution of dietary flavonoids to plasma and tissue antioxidant function *in vivo* is likely to be relatively minor [17]. Therefore, it has been suggested that these compounds may play a major role in cell signaling rather than as an antioxidant [17].

The antioxidant properties of catechins have been attributed to the di- or trihydroxyl groups on the B-ring and the *meta*-5,7-dihydroxyl groups on the A ring. A trihydroxyl structure on the D-ring (gallate) in EGCG and ECG further enhances the antioxidant functions of these compounds [32]. Among tea catechins, EGCG is the most effective in scavenging ROS. Another feature which adds to the antioxidant nature of catechins is the metal ion chelating capacity through their vicinal dihydroxyl and trihydroxyl structures, which can prevent the generation of free radicals. In addition catechins have been reported to inhibit Cu^{2+} catalyzed oxidation of lipoproteins *in vitro* in macrophages [33]. Although effects of catechins on biomarkers of oxidative stress, such as DNA oxidative damage, have been demonstrated in animals, more such investigations are required in humans. Only transient and modest increases in plasma total antioxidant activity after tea ingestion have been reported in human subjects [34]. It should be remembered, however, that the bioavailability of tea polyphenols limits the biological activity *in vivo* [35].

Akin to other antioxidants, EGCG and other tea polyphenols have also been shown to act as pro-oxidants. The relatively short half-life of EGCG, 0.5–2 h *in vitro*, could be prolonged by the addition of superoxide dismutase (SOD) [36], suggesting that the superoxide radical may be responsible for the short half-life. Since the pO_2 in a cell culture system is much higher (160 mmHg) than that in the blood or tissues (<40 mmHg) [37], it is not yet clear whether the pro-oxidant properties of EGCG can also be envisaged at relatively lower pO_2 conditions *in vivo*. Under appropriate conditions EGCG and other catechins can be oxidized to form phenolic radicals, superoxide radicals, and hydrogen peroxide, thus contributing to cell apoptosis.

Resveratrol has potent antioxidant properties and has a global effect on oxidative stress conditions. There is evidence to support that resveratrol is a potent inhibitor of the oxidation of polyunsaturated fatty acids (PUFA) that play a major role in atherosclerosis [38]. Compared to flavonoids, resveratrol was shown to be more potent in protection against copper-catalyzed oxidation [39]. Although these reports provide evidence for anti-lipogenic and anti-atherogenic properties of resveratrol, *in vivo* data do not corroborate these findings. This was demonstrated in hyperlipidemic rabbits fed on resveratrol, which showed no decrease in serum cholesterol levels or atherosclerotic lesions [40]. Interestingly, the anti- and pro-oxidant activities of resveratrol, appear to be concentration and cell type dependent. For example, in human leukemia cells, resveratrol was found to induce formation of ROS, whereas in prostate cancer cells a dose-dependent decrease in intracellular ROS (in particular, O_2^-) was recorded. Similarly, the cardio-protective ability of resveratrol was demonstrated in studies wherein resveratrol was shown to modulate the production of NO from vascular endothelium [41]. In another study resveratrol was also shown to inhibit platelet aggregation, another major contributor in the process of atherosclerosis [42]. The above observations are further supported by human *in vivo* studies demonstrating increased antioxidant activity in the blood of moderate red wine consumers. However, more investigations are warranted to establish whether the beneficial effects of resveratrol on human cardiovascular, neurological, and hepatic systems are indeed a function of this polyphenol.

6. Cellular signaling, NF-κB and polyphenols

Much of the earlier studies on polyphenols have viewed these compounds from the perspective of antioxidants. The antioxidant property of these molecules was later explained on the basis of the availability of –OH and the system of conjugated double bonds present in these molecules. However, many other effects of polyphenols such as anti-inflammatory, anti-tumor, anti-atherogenic abilities could not be explained solely on the basis of their antioxidant properties. Investigations into the mechanism of action of these molecules have thrown light on the fact that polyphenols may not merely exert their effects as free radical scavengers, but may also modulate cellular signaling processes during inflammation or may themselves serve as signaling agents [43]. In the following section, an overview of the anti-inflammatory properties of various polyphenols with respect to nuclear factor-kappa B (NF-κB) and mitogen activated protein kinase (MAPK) signaling are outlined to obtain a general idea of the wide variety of cellular inflammatory processes such compounds can modulate.

6.1. *Resveratrol*

A direct impact of specific polyphenolic compounds on inflammation has been studied both *in vitro* and *in vivo*. In a recent study, Birrel et al. [44] have demonstrated that *in vivo*, resveratrol can inhibit inflammatory cytokine expression in response to lipopolysaccharide (LPS) challenge in rat lungs. Furthermore, in both monocytic U937 cells and alveolar epithelial A549 cells, resveratrol inhibits NF-κB and activator protein-1 (AP-1) activation [45,46]. Resveratrol had no effect on the binding of NF-κB proteins to the DNA, but it did block the tumor necrotic factor (TNF)-induced translocation of p65 subunit of NF-κB and reporter gene transcription. Similarly, the activation of c-Jun N-terminal kinases (JNK) and its upstream kinase mitogen-activated protein (MEK) are inhibited by resveratrol, which may explain the mechanism of suppression of AP-1 by resveratrol.

Previously, it has been shown that (PMA)-induced cyclooxygenase-2 (COX-2) is blocked by resveratrol [47]. This gene and inducible nitric oxide synthase (iNOS) gene are both known to be regulated by NF-κB activation. Inducible NO synthase (iNOS) gene is also regulated by NF-κB. Thus, it is possible that resveratrol suppresses COX-2 and iNOS expression by inhibiting NF-κB activation. Besides COX-2, various other genes, including those for matrix metalloproteinase-9 (MMP-9) and cell surface adhesion molecules (e.g., intercellular adhesion molecule-1 (ICAM-1), endothelial leukocyte adhesion

molecule-1 (ELAM-1), and vascular cell adhesion molecule-1 (VCAM-1)), are also regulated by NF-κB. It also appears now that the anti-carcinogenic properties assigned to resveratrol may be via suppression of NF-κB-mediated expression of such genes and their corresponding translated equivalents [48].

NF-κB activation can have an anti-apoptotic role and the suppression of apoptosis by resveratrol may at first seem paradoxical. However, NF-κB activation does not block apoptosis induced by all agents. Other studies using a *cis* isomer of resveratrol (c-resveratrol) in peritoneal macrophages stimulated with LPS and gamma interferon (IFN-γ), have shown that c-resveratrol significantly attenuated the expression of NF-κB family of genes, adhesion molecules and acute-phase proteins [49]. In addition, c-resveratrol also inhibited transcription of Scya2 (chemokine monocyte chemotactic peptide-1 (MCP-1)), the chemokine RANTES (regulated on activation, normal T cell expressed and secreted), pro-inflammatory cytokines that attract monocyte–granulocyte cells such as colony-stimulating factor 1 (M-CSF), colony-stimulating factor 2 (GM-CSF) and colony-stimulating factor 3 (G-CSF), the transforming growth factor beta (TGF-beta) and the extracellular ligand interleukin 1 (IL-1α). In contrast, c-resveratrol stimulated transcription of the pro-inflammatory cytokines interleukin 6 (IL-6) and TNF-α, the extracellular ligand IL-1β, and the IFN regulatory factor (IRF)-1. Therefore, it appears that c-resveratrol can significantly modulate a wide variety of pro-inflammatory pathways via inhibiting the activation of NF-κB [49]. Piceatannol, a stilbene and a structural homologue of resveratrol, has anti-inflammatory, immunomodulatory and anti-proliferative properties. It has also been shown to modulate cytokine signaling pathways and TNF-induced DNA binding activity of NF-κB [50]. In contrast, stilbene or rhaponticin (another analog of piceatannol) had no effect, suggesting a critical structure activity relationship of the hydroxyl groups within piceatannol.

Resveratrol has been known to modulate MAP kinase signaling. In undifferentiated and retinoic treated cells a small amount of resveratrol could induce phosphorylation of extracellular signal-regulated kinase (ERK1/2) [51], as well as JNKs and p38MAPK in mouse epidermal cells, with concomitant increase in p53-serine-15 phosphorylation [52,53]. In contrast the papillary and follicular thyroid carcinoma cell lines required higher doses of resveratrol to activate nuclear translocation of ERK1/2 [52]. However, at still higher concentrations (50–100 μM) resveratrol inhibited phosphorylation of MAPK, depressing MAPK activity and reducing phosphorylation at the active sites of ERK1/2, JNK1 and p38 MAPK at still higher doses (37 mM) [53]. Another related study showed that resveratrol activated JNKs at the same dose that inhibited tumor promoter-induced cell transformation [54]. Therefore, resveratrol appears to activate MAPK in some cells, while it inhibits in others. While the above reported effects are dose dependent, in general, resveratrol is stimulatory at lower concentrations (<20 μM) and inhibitory at higher. A recent study employing 10 μM resveratrol showed it enhanced phosphorylation of p38MAPK and ERK1/2 [55]. Moreover, it was found to induce CREB phosphorylation via adenosine A1 and A3 receptors through the activation of AKT survival pathway [56]. Therefore, resveratrol-mediated CREB activation may be routed through the phosphorylation of MSK1, since MSK1 inhibition in turn inhibited phosphorylation of CREB.

6.2. *Curcumin*

Curcumin has also been reported to inhibit NF-κB activation, with concomitant suppression of IL-8 release, COX-2 expression and neutrophil recruitment in the lungs [57]. The inhibition of cigarette smoke-induced NF-κB activation by curcumin has been reported to be via inhibition of Iκ-Bα kinase in human lung epithelial cells [58]. This finding corroborates an earlier study on the inhibitory effect of curcumin on NF-κB activation [59]. Curcumin has also been demonstrated to down regulate other NF-κB-regulated genes involved in inflammation and cellular proliferation such as leukotrienes, PLA2, COX-2 and 5-LOX, cyclin D1 and c-myc, antiapoptotic factors, e.g. IAP1, IAP2, XIAP, Bcl2, Bcl-xL, Bf1-1/A1, TRAF1, cFLIP and metastatic factors such as VEGF, matrix metallo proteinases (MMP-9) and ICAM-1. Curcumin mediated suppression of NF-κB transactivation was associated with inhibition of nuclear translocation of p65, which was further associated with the sequential suppression of IκB kinase activity and phosphorylation, IκBα degradation, p65 phosphorylation, p65 nuclear translocation, and p65 acetylation. Furthermore, curcumin was shown to inhibit TNF-induced NF-κB-dependent reporter gene expression and also suppressed NF-κB reporter activity induced by TNFR1, TNFR2, NIK, I-kappa kinase (IKK), and the p65 subunit of NF-κB. Cigarette smoke, which contains numerous oxidants and carcinogenic agents, such as superoxide, hydroxyl radicals, H_2O_2 and benz(a)pyrenes, activates NF-κB, blocks apoptosis and induces proliferation and carcinogenesis. Curcumin also abolishes the cigarette smoke-mediated induction of NF-κB binding to the DNA, blocks IKK activation, IκBα phosphorylation and degradation as well as NF-κB p65 translocation [59]. Inhibition of NF-κB by curcumin is certainly an interesting strategy in chronic inflammatory diseases where NF-κB is activated [60].

In addition, curcumin has been reported to down regulate expression of iNOS, MMP-9, TNF-α, chemokines, cell surface adhesion molecules and growth factor receptors (such as EGFR and HER2) [61]. Curcumin also modulates a number of other kinase signalling pathways such as JNK, p38, AKT, JAK, ERK and PKC in a variety of different cell types [62]. Interestingly, curcumin and tumor necrosis factor related apoptosis-inducing ligand (TRAIL) has been reported to promote cell death in a cooperative manner [63]. The pleiotropic nature of curcumin in targeting so many cell signalling pathways complicates the process of identifying which pathway is essential for the anti-inflammatory effects. On the other hand, it may be that the ability to prevent cross-talk between the myriad of signalling pathways is a pre-requisite for its anti-inflammatory properties.

6.3. *Catechins*

Among the numerous polyphenols isolated from green tea, EGCG predominates and is considered to be the major therapeutic agent. In many model studies of cancer, EGCG has been shown to induce apoptosis, cell-growth arrest, and deregulation of the cyclin kinase inhibitor p21WAF [64].

The ability of EGCG to inhibit cell cycle progression causing a G0/G1-phase arrest and a subsequent induction of apoptosis has been also reported in human epidermoid carcinoma (A431) cells [65], probably through a mechanism mediated by NF-κB inhibition [66]. More recently, using a cDNA microarray, it has been found that EGCG treatment of LNCaP cells resulted in induction of genes that functionally exhibit growth-inhibitory effects, and repression of genes that belong to the G-protein signaling network [64]. One study has also demonstrated that EGCG can induce specific temporal changes in gene expression in response to H_2O_2 [67]. This has lead to a proposal that EGCG may cause differential oxidative environments in tumor versus normal epithelial cells. However, the role that EGCG, H_2O_2 and intracellular catalase play in the epithelial system is largely unknown [68]. That EGCG can modulate NF-κB/AP-1 activity has been demonstrated by EGCG suppression of malignant transformation in PMA-stimulated mouse epidermal JB6 cells via inactivation of AP-1 [69] and/or NF-κB [70]. In another study, expression of genes related to angiogenesis such as VEGF and those related to metastasis, such as (MMP)-2 and MMP-9 were found to be inhibited by green tea or purified EGCG administered to mice in the drinking water [71].

Expression of interleukin-8 (IL-8), the major human neutrophil chemoattractant and inflammatory mediator, is dependent on IL-1β activation of NF-κB. EGCG markedly inhibited IL-1β-mediated IL-1β receptor-associated kinase (IRAK) degradation and the signaling events downstream from IRAK degradation: IKK activation, IκBα degradation, and NF-κB activation [72]. The functional consequence of this inhibition was evident by inhibition of IL-8 gene expression. Catechins, especially EGCG, have also been shown to down regulate CD11b expression on CD8+ T cells and thereby inhibit infiltration of these cells into sites of inflammation [73]. Green tea polyphenols are also able to stimulate MAPK pathways in HepG2 cells [74] and can increase mRNA levels of the immediate-early genes such as c-jun and c-fos. Chen et al. [75] has shown that not all polyphenols in green tea extracts have similar activity and their effects appear to be structurally related to the 3-gallate group. The degree of activation of MAPK by the five tea polyphenols was related to the structure, dose and time. Of the five predominant polyphenols, only EGCG showed potent activation of all three MAPKs (ERK, JNK and p38) in a dose- and time-dependent manner, whereas EGC activated ERK and p38. Furthermore, whilst at lower concentrations, EGCG activated MAPK, at higher concentrations it elicited sustained activation of JNK leading to apoptosis [75]. Similar to curcumin, green tea polyphenols thus appear to modulate a myriad of inflammatory signaling pathways and as such it is difficult to attribute the anti-inflammatory properties at present to one particular pathway. Clearly further investigations are warranted.

7. Polyphenols and cellular redox system

Since a variety of oxidants, free radicals and aldehydes are implicated in the pathogenesis of chronic inflammatory diseases, therapeutic intervention with a variety of polyphenolic antioxidants may therefore be an effective alternative for the treatment of chronic inflammatory diseases. An alternative mechanism may be that polyphenolic components of dietary plants may increase the endogenous antioxidant defense potential and thus modulate cellular redox state. It is therefore apt to consider how polyphenols may modulate the redox system and its components in a cell during normal and pathophysiological conditions. In the following section we have considered the effects of polyphenols on Nrf2 as this important cellular redox dependent transcription factor regulates the expression of several genes involved in the modulation of inflammatory processes.

7.1. *Nrf2*

Nrf2 is a member of the ''cap '*n*' collar'' family of transcription factors. These transcription factors bind to nuclear factor-erythroid derived 2 (NF-E2) binding sites (GCTGAGTCA) that are essential for the regulation of erythroid specific genes. The NF-E2 binding site is a subset of the antioxidant response elements (ARE) that have the sequence GCNNNGTCA. Nrf2 is expressed in a wide range of tissues, many of which are sites of expression for phase 2 detoxification genes. The AREs are regulatory sequences found on promoters of several phase 2 detoxification genes that are inducible by xenobiotics and antioxidants. ARE-mediated expression and coordinated induction of antioxidant enzymes is a critical mechanism of protection against chemically induced oxidative/electrophilic stress [76] (Fig. 3).

Selected Nrf2-Keap1-ARE activators, such as curcumin, caffeic acid phenethyl ester and 4′-bromoflavone, are potential chemopreventive agents [77]. Alteration of the Nrf2-Keap1 interaction enables Nrf2 to translocate into the nucleus, bind to the ARE and initiate the transcription of genes coding for detoxifying enzymes and cytoprotective proteins. This response is also triggered by a class of electrophilic compounds including polyphenols and plant-derived constituents. Natural antioxidants like curcumin and caffeic acid phenethyl ester (CAPE) have been identified as potent inducers of heme oxygenase-1 (HO-1), a redox-sensitive inducible protein that provides protection against various forms of stress [78]. Both curcumin and CAPE stimulate the expression of Nrf2 in a concentration- and time-dependent manner. From several lines of investigation it is also reported that curcumin (and, by inference, CAPE) stimulates HO-1 gene activity by promoting dissociation of the Nrf2-Keap1 complex, leading to increased Nrf2 binding to the resident HO-1 AREs. Recently, the role of Nrf2 in the transcriptional regulation of rat glutamate-cysteine ligase catalytic subunit (GCLC) has also been investigated [79]. Furthermore, Nrf2 was found to regulate the rat GCLC promoter by modulating the expression of a key AP-1 family of proteins. Interestingly, the authors have earlier reported that curcumin could increase GSH synthesis in A549 cells via increasing the expression of the GCL gene. Since curcumin is also known to stimulate Nrf2 expression [78,79], it appears that the antioxidant function of curcumin may be mediated via an Nrf2-ARE-GCLC axis. EGCG also induced transcriptional activation of phase II detoxifying enzymes through ARE/EpRE by activation of all three MAPK pathways (ERK, JNK and p38) [74]. Similarly, other polyphenols, such as resveratrol, have also been shown to stimulate Nrf2 in PC12 cells through MAP kinase signal transduction pathways [77,80]. However, caution should be

used in using polyphenols to activateNrf2 as changes in Nrf2 signaling in various chronic inflammatory diseases has not been fully addressed.

8. Polyphenols and glucocorticoid signaling

Although corticosteroids are highly effective in the control of asthma and other chronic inflammatory and immune diseases, a small proportion of patients with asthma fail to respond even to high doses of oral corticosteroids. Resistance to the therapeutic effects of corticosteroids is also recognized in other inflammatory and immune diseases, including rheumatoid arthritis and inflammatory bowel disease. Patients with corticosteroid-resistant asthma, although uncommon, present considerable management problems. Patients with chronic obstructive pulmonary disease (COPD) show a poor clinical response to corticosteroids and have a largely steroid-resistant pattern of inflammation [81]. New insights into the mechanisms whereby corticosteroids suppress chronic inflammation have shed light on the molecular basis for corticosteroid resistance in asthma and COPD [82].

It is generally accepted that oxidative stress plays a key role in the pathogenesis of COPD [83]. However, a more insidious aspect of oxidative stress is the role it is proposed to play in promoting the poor efficacy of corticosteroids in COPD and severe asthma (Fig. 2). Ito et al. [84] have shown a role for histone acetylation and deacetylation in IL-1β-induced TNF-α release in alveolar macrophages derived from cigarette smokers. Both *in vitro* and *in vivo* studies have shown that oxidants may play an important role in the modulation of histone deacetylase (HDAC) and inflammatory cytokine gene transcription [84,85]. Furthermore, we have shown that both cigarette smoke/H_2O_2 and TNF-α caused an increase in histone acetylation (HAT activity) leading to IL-8 expression in monocytes and alveolar epithelial cells *in vitro* [86]. Glucocorticoid suppression of inflammatory genes requires recruitment of histone deacetylase-2 (HDAC2) into the proinflammatory transcriptome complex by the glucocorticoid receptor [87]. This results in deacetylation of histones and a decrease in inflammatory gene transcription. A reduced level of HDAC2 was associated with increased proinflammatory response and reduced responsiveness to glucocorticoids in alveolar macrophages obtained from smokers [84]. Culpitt et al. [88] have shown that cigarette smoke solution stimulated release of IL-8 and GM-CSF, which was not inhibited by dexamethasone, in alveolar macrophages obtained from patients with COPD compared to that of smokers. They suggested that the lack of efficacy of corticosteroids in COPD might be due to steroid insensitivity of macrophages in the respiratory tract. Thus, the cigarette smoke/oxidant-mediated reductions in HDAC2 levels in alveolar epithelial cells and macrophages will not only increase inflammatory gene expression but will also cause a decrease in glucocorticoid function in patients with COPD. HDAC activity has also been measured in bronchial biopsies and alveolar macrophages from COPD patients and smoking controls, demonstrating a significant decrease in HDAC activity, the magnitude of which increased with severity of disease [89]. Moreover, protein expression of HDAC2 was also decreased in a similar manner in COPD patients. Consequently, a potential means by which to treat COPD would be to increase HDAC2 expression and activity such that steroids regain their anti-inflammatory activity. This has been demonstrated with theophylline in

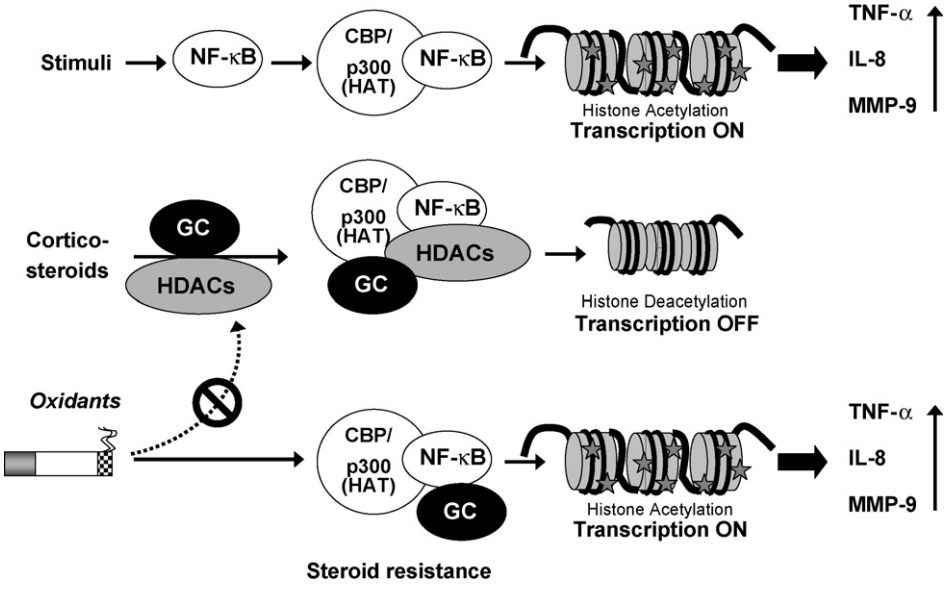

Fig. 2 – Impact of oxidative stress on the regulation of chromatin structure and pro-inflammatory gene expression. Pro-inflammatory cytokines activate transcription factors, such as NF-κB, recruiting transcriptional co-activator molecules CBP/p300 containing intrinsic HAT activity resulting in histone acetylation and DNA unwinding, allowing DNA polymerases access to the DNA and pro-inflammatory gene expression. Activated corticosteroid receptors recruit HDAC into the transcriptome complex promoting histone deactylation, chromatin condensation and expulsion of DNA polymerases, shutting off gene expression. Oxidative stress inhibits HDAC activity as well as activating NF-κB, facilitating histone acetylation by the transcriptome complex even in the presence of activated glucocorticoid receptor.

lung macrophage cells, increasing HDAC2 activity and expression and re-sensitizing the cells to steroids [90].

The antioxidant and/or anti-inflammatory effects of dietary polyphenols (curcumin-diferuloylmethane and resveratrol), the bronchodilator theophylline and glucocorticoids have all been shown to play a role in either controlling NF-κB activation or chromatin remodeling through modulation of HDAC activity and subsequently inflammatory gene expression in lung epithelial cells. Recent studies in the authors' laboratories show that curcumin can also restore glucocorticoid function in response to oxidative stress imposed by cigarette smoke or hydrogen peroxide. Furthermore, this appeared to be mediated through up regulation of HDAC2 activity independent of any anti-oxidant activity in the monocyte/macrophage (U937) cells. Interestingly, in Mono-Mac6 cells this was also associated with restoration of HDAC1 and HDAC3 levels [91]. Clearly the restoration of a ROS induced HAT-HDAC imbalance by dietary polyphenols could have a significant impact on inflammation. This would manifest itself by facilitating steroid-mediated HDAC recruitment in attenuating NF-κB mediated chromatin acetylation and subsequent pro-inflammatory gene expression. The concept that a HAT-HDAC imbalance regulating inflammatory gene expression could be modulated by dietary polyphenols is corroborated by other independent reports, where curcumin at very high concentration (100 μM) could inhibit HAT activity, preventing NF-κB mediated chromatin acetylation [92]. However, other possible mechanisms of polyphenol-mediated inhibition of inflammatory response should not be overlooked, such as quenching or reversing post-translational protein modifications induced by oxidants and damaging reactive aldehydes. This might be achieved through the induction of enzymes such as, tyrosine denitrase, carbonyl reductase or aldo–keto reductase. It is interesting to speculate that these dietary polyphenols and flavonols may not only act as antioxidant/anti-inflammatory agents, but it is also possible that they will increase the efficacy of glucocorticosteroids in COPD.

The beneficial anti-inflammatory effect of polyphenols was demonstrated by a Finnish study involving over 10,000 participants, wherein a significant inverse correlation was observed between polyphenol intake and the incidence of asthma [93]. Similar beneficial associations were also observed for COPD in a study encompassing over 13,000 adults. In this study Tabak et al. [94], reported that increased polyphenol intake correlated with improved symptoms, as assessed by cough, phlegm production and breathlessness, and improved lung function as measured by forced expiratory volume in 1 s (FEV_1) [95]. Two further studies appeared to corroborate these findings. The first study showed a beneficial protective effect against COPD symptoms for increased fruit intake, high in polyphenol and Vitamin E content [96]. In the second more recent study, a standardised polyphenol extract administered orally was shown to be effective in reducing oxidant stress and increasing PaO_2, as well as improvements in FEV_1 between enrolment and the end of the study [96]. More importantly, while single component intake, such as catechin was independently associated with FEV_1 and all three COPD symptoms, flavonol and flavone intake was independently associated with chronic cough only. The importance of this study was further substantiated by Walda et al. [95] who showed the protective effect of fruit containing polyphenols and Vitamin E intake against COPD symptoms in 20-year COPD mortality study from three European countries consisting of the Finnish, Italian and Dutch cohorts. These important studies certainly encourage carrying out further multi-national clinical studies to demonstrate the beneficial effects of a high intake of polyphenols/bioflavanoids against COPD symptoms.

9. Challenges for research on polyphenols

Hundreds of polyphenols with antioxidant activity are potential contributors to the antioxidant mechanisms present in humans and animals. Although these compounds are excellent candidates to explain the health benefits of diets rich in fruits and vegetables, there is still not enough information on food composition data, bioavailability, interaction with other food components and their biological effects. There is evidence that polyphenols are metabolized by intestinal flora and that they and their metabolites are also absorbed. In this respect, the known chelating capacity of polyphenols raises the question of their participation in aspects related to metal metabolism and pathology [97]. Considering the complexities of polyphenol absorption and metabolic transformations and the still less understood aspect of polyphenol bioavailability, it becomes more important to address the following questions: (a) What doses of polyphenols should be taken, (b) What post-absorption metabolic modifications would render the polyphenols bioactive? (c) Since most reports on the beneficial effects of polyphenols are based on *in vitro* studies, will identical doses yield similar effects in whole animal? It is perhaps surprising that, to our knowledge, there has been hardly any attempt to probe the link between target organ levels of the polyphenols, efficacy *in vivo*, and activity observed in cells *in vitro*.

Another aspect of polyphenol metabolism that warrants detailed investigation is their cross-reactivity with other biological antioxidants. For example, ascorbate and catechin have been shown to cross react with each other [98], leading to the hypothesis that polyphenol antioxidants are part of the antioxidant network of the organism. Although attempts have been made to estimate the relative contribution of polyphenols to the total antioxidant capacity in plasma, insufficient knowledge however, on the nature and concentration of circulating polyphenol species render these results as only speculative at present. Another rapidly developing aspect of free radical metabolism is its participation in the process of mediating and regulating cellular function. It is possible that dietary polyphenols continuously participate in the regulation of cellular function independent of its anti-oxidant properties.

10. Conclusions

Polyphenols and flavonoids seem to be important metabolic modulators by virtue of their ability to moderate and influence several cellular processes such as signaling, proliferation,

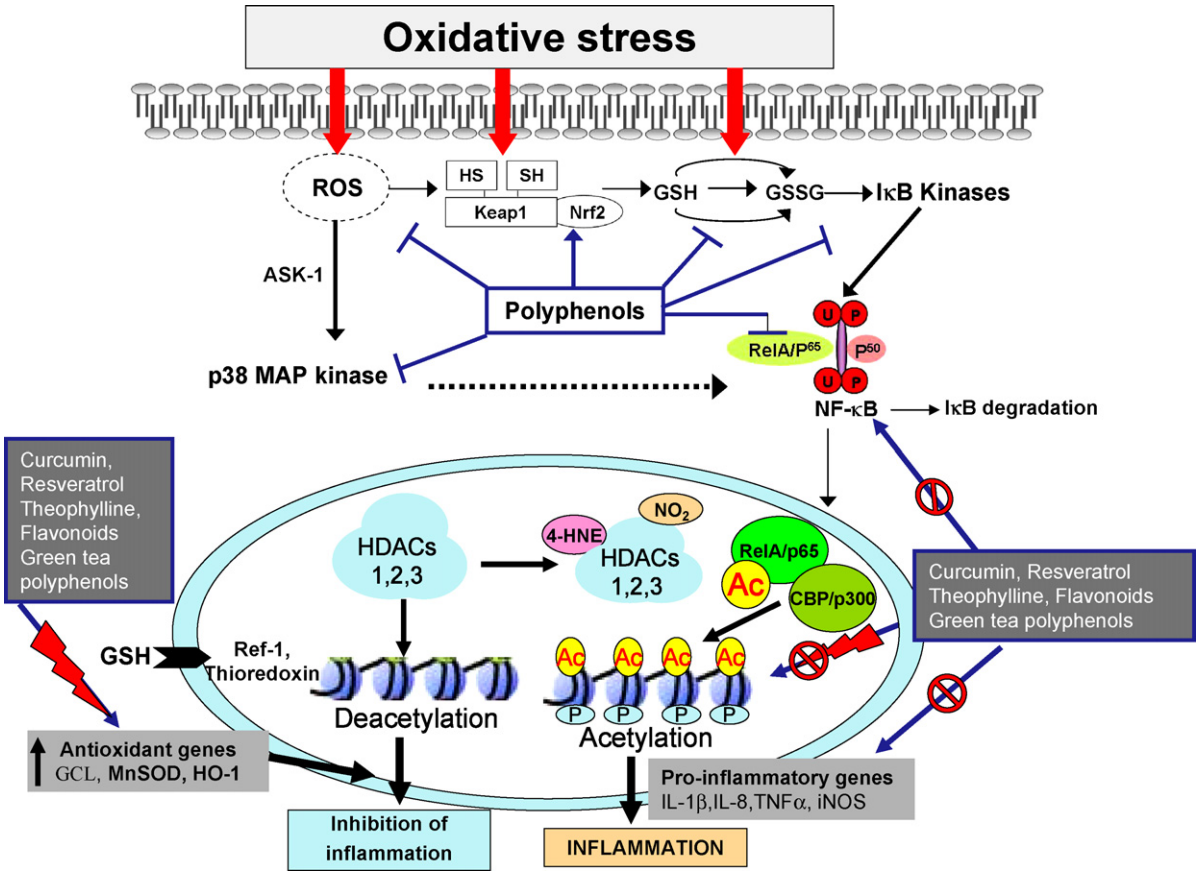

Fig. 3 – A schematic model for polyphenols and flavonoids mediated modulation of cell signaling: oxidative stress induced inflammation is mediated by NF-κB activation, MAP kinases and affect a wide variety of cellular signaling processes leading to generation of inflammatory mediators and chromatin remodeling. The later allows expression of pro-inflammatory genes such as IL-1β, IL-8, TNFα and iNOS. On the other hand to counter the effects of oxidative stress, the cells also concomitantly express protective antioxidant genes such as GCL, MnSOD, HO-1. Polyphenols and flavonoids inhibit pro-inflammatory gene expression via inhibition of IκB, thus inhibiting NF-κB transactivation, as well as restoring transrepressive pathways through the activation of histone deacetylases. In addition, expression of antioxidant genes such as GCL, MnSOD, HO-1 via modulation of MAPK-ARE-Nrf2 pathway are upregulated.

apoptosis, redox balance, differentiation, etc. (Fig. 3). Although abundant in most dietary sources such as fruits, vegetables, tea and wine, more detailed studies are still required to determine their true absorption and bioavailability. It would serve well to remember that most studies and results on the effects of polyphenols have been obtained from *in vitro*/cell culture studies. Given the fact that polyphenols undergo considerable degree of chemical modifications during digestion and absorption and that the modified forms may have altered biological properties and potencies, it is extremely important to practice caution before claiming any definite pharmacological applications for these compounds. Moreover, despite their beneficial health effects, polyphenols have also been shown to have adverse effects too. Future experimental designs should consider the above factors. In addition, most human exposure studies using polyphenols have been on a short-term basis and therefore more studies should be undertaken on an extended basis in order to determine the long-term effects of these diverse compounds. In view of their anti-inflammatory and antioxidant abilities and their capacity to modulate important inflammatory and anti-inflammatory signaling pathways, glucocorticoid efficacy, polyphenols and flavonoids hold great promise as potential therapeutic strategies for controlling lung inflammation and related diseases. In fact, polyphenols and flavonoids may be perceived as future pharmacological agents and may be used as antioxidant and anti-inflammatory enforcements to combat oxidative challenges.

Clearly, further studies are required to understand the effect of ROS on basic cellular functions and the differential responses seen in different cell types and how this in turn impacts on the pathology of different inflammatory disease states. At the same time, endeavors into identifying new and more efficacious antioxidants as a therapeutic strategy should continue. Indeed, elucidating the mechanism of action for some of the naturally occurring antioxidants, such as the potent enzyme mimetics and polyphenols, may lead to new therapeutic targets that can be modulated through more conventional pharmacological approaches.

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