Emerging Role of Flavonoids in Inhibition of NF-κB-Mediated Signaling Pathway: A Review

Kasi Pandima Devi* • Perumal Vijayaraman Kiruthiga • Shunmugiahthevar Karutha Pandian

Department of Biotechnology, Alagappa University, Karaikudi 630 003, Tamil Nadu, India

Corresponding author: * devikasi@yahoo.com

ABSTRACT

Nuclear factor kappa B (NF-κB) proteins comprise of a family of structurally-related eukaryotic transcription factors. They were originally discovered in lymphocytes, but later found to be ubiquitously expressed in almost all animal cell types. In mammals the NF-κB family (also known as the Rel family) consists of five members: p50 (product of the NF-κB1 gene), p52 (product of the NF-κB2 gene), p65 (also known as RelA), c-Rel and RelB. NF-κB dimers exist in a latent form in the cytoplasm bound by IκB inhibitory proteins. NF-κB-inducing stimuli (stress, cytokine, free radicals, UV radiation, oxidised LDL, bacterial or viral antigens) activate IκB kinase complex that phosphorylates IκB, leading to its ubiquitination and subsequent degradation. IκB degradation expose the DNA-binding domain and nuclear localization sequence of NF-κB and permit its stable translocation to the nucleus and the regulation of target genes. The NF-κB signaling pathway plays a key role in inflammation, immune response, cell growth control and protection against apoptosis. Downregulation/inhibiton of NF-κB is regarded as a potential drug targets for therapeutic intervention in many diseases like cancer, inflammatory and autoimmune diseases. Many natural plant products have been found to downregulate NF-κB production, including curcumin, quercetin, green tea, and resveratrol. In this review we describe flavonoids as NF-κB inhibitors and their role in preventing NF-κB signaling pathway mediated disorders.

CONTENTS

INTRODUCTION

The transcription factor nuclear factor-kappa B (NF-κB) was first identified by Sen and Baltimore in 1986 as a regulator of the expression of the kappa light-chain gene in murine B-lymphocytes, but was subsequently been found in many different cells. Several years following its discovery NF-κB endures as one of the most studied transcription factor in most cell types. It represents a group of structurally related and evolutionarily conserved proteins that belong to the Rel family and are regulated via shuttling from the cytoplasm to the nucleus in response to cell stimulation (Ghosh *et al.* 1998; Zhang and Ghosh 2001; Ghosh and Karin 2002). Rel/NF-κB is a collective name for inducible, ubiquitous dimeric transcription factors made up of members of the Rel family of DNA binding proteins that recognize a common sequence motif conserved from droso-

phila to humans (Chen and Ghosh 1999). Emerging evidence subserve to explain the signal transduction pathways that lead to the activation of Rel/NF-κB factors and the subsequent induction of gene expression (Pahl 1999). In most cell types, NF-κB is retained in the cytoplasm in an inactive form through association with any of several IκB inhibitor proteins (Ghosh *et al.* 1998). Rel/NF-κB can be activated within minutes by a wide array of stimuli, including inflammatory cytokines such as TNF-α and interleukin-1, T-cell activation signals, growth factors and stress inducers (Barnes and Karin 1997; Chen *et al.* 1999; Baldwin 2001). In response to those stimulus, IκB rapidly gets phosphorylated, ubiquitinated and degraded (Chen and Ghosh 1999). The liberated transcription complex then translocates to the nucleus where it can induce and control a broad spectrum of genes. Expression of targeted diverse gene products act as key regulators of many critical physiological pro-

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cesses including developmental processes, inflammation and immune responses, cell growth, cancer, expression of certain viral genes, cell adhesion, differentiation, redox metabolism and apoptosis (Pahl 1999; Shishodia and Aggarwal 2004). Furthermore, several studies have focused on other diverse functions of NF-κB, which clearly illustrate its 'good and evil' aspects, whereby NF-κB is mandatory for immunological functions (Pahl 1999) but is detrimental when it is dysregulated. Owing to its wide range of cellular roles, NF-κB has attracted wide spread interest to gain stature as treatments for certain cancers, neurodegenerative and inflammatory diseases. It has been blocked at various steps using a variety of natural and designed molecules, including antioxidants, proteosome inhibitors, peptides, small molecules and dominant negative or constitutively active polypeptides in the pathway (Epinat and Gilmore 1999). Because of increased awareness regarding the use of plant derived products, flavonoids have gained wide spread attention for management of several diseases including cancers and inflammation. Flavonoids are natural polyphenolic compounds whose main sources are fruits and vegetables and comprise of several classes (Ross and Kasum 2002). Presently, there is a growing evidence supporting that flavonoids has been used as NF-κB inhibitors because of its well-tolerated and non-toxic effects at large doses. Several flavonoid molecules act as general inhibitors of Rel/NF-κB induction, whereas some other flavonoids inhibit specific pathways of induction and profoundly decrease risk of some diseases. This review aims to highlight the role of flavonoids as NF-κB inhibitors.

TRANSCRIPTION REGULATOR NF-κB AND INHIBITORY IκB PROTEINS

The Rel/NF-κB transcription factor family is comprised of several structurally-related proteins that exist in organisms from insects to humans (Chen and Ghosh 1999). Mammals express 5 Rel (NF-κB) proteins, namely NF-κB1 (p50), NF-κB2 (p52), RelA (p65), RelB, c-Rel that belong to two classes shown in **Table 1** (Baldwin 1996; Ghosh *et al.* 1998; Hayden and Ghosh 2008). Vertebrate NF-κB transcription complexes can be any of a variety of homo and heterodimers formed by the subunits p50, p52, RelA (p65), Rel B and c-Rel. These proteins are structurally related through an approximately 300 aminoacids N-terminal domain called the Rel Homology (RH) domain (Rayet and Gelinas 1999) which contains sequences important for DNA binding, dimerization and inhibitor (IκB) binding and to generally activate specific target gene expression. The target gene specificity is thought to arise primarily from the specific Rel/NF-κB complexes that are in different cell types and the distinct κB target site binding specificities of different Rel/ NF-κB complexes. NF-κB works only when two members form a dimer (Zhong *et al.* 2002). The most abundant activated form consists of a p50 or p52 subunit and a p65 subunit. NF-κB dimers containing Rel A or c-Rel are held in the cytoplasm through interaction with specific inhibitors, the IκBs (Ghosh *et al.* 1998; Jacobs and Harrison

1998; Karin 1999; Hayden and Ghosh 2008). IκBs are a small family of related proteins with a core consisting of six or more ankyrin repeats, an N-terminal regulatory domain and a C-terminal domain that contains a PEST (sequences rich in Pro, Glu, Asp, Ser and Thr) motif. The IκBs are also members of a gene family that contains seven known proteins, IκBα, IκBβ, IκBε, IκBγ, Bcl-3 and precursor Rel proteins p100 and p105 (Gilmore and Morin 1993; Karin 1999). The IκBs are characterized by the presence of multiple ankyrin repeats and interact with NF-κB via Rel homology domain (RHD). The RHD serves several functions: it is the dimerization and DNA-binding domain for this class of proteins, it contains the nuclear localization sequence (NLS), and most important, it is site for binding of NF-κB inhibitors (Tripathi and Aggarwal 2006). IκBs undergo rapid ubiquitin dependent degradation after exposure to a variety of agonists, which activate IκB (IKK) complex (Baeuerle 1998).

SIGNALING PATHWAYS OF NF-κB

NF-κB dimers containing Rel A or c-Rel interact with specific inhibitors, IκBs which sterically block the function of their NLSs, thereby causing their cytoplasmic retention (Baldwin 1996; Pahl 1999; Ghosh and Karin 2002). In unstimulated cells, NF-κB resides in the cytoplasm in a latent form, and must be translocated to the nucleus to function. The cytoplasmic retention of NF-κB is provided by its interaction with inhibitory proteins known as IκB. Stimulation leads to a phosphorylation-targeted proteasomal degradation of IκB, allowing the 'active' NF-κB to enter the nucleus and initiate transcription (Makarov 2001). IκBs undergo rapid ubiquitin-dependent degradation after exposure to a variety of agonists, which activate IκB (IKK) complex. IKK is composed of three subunits, IKKα (IKK1), IKKβ (IKK2), and IKKγ (also known as NF-κB essential modulator, NEMO) (Jacobs and Harrison 1998; Yamaoka *et al.* 1998; Sun and Ley 2008). Stimulation by a diverse array of pathogens and other inducers, including viruses, cytokines, and stress-inducing agents led to activation of signalling cascades that culminate with the activation of the IKK complex and phosphorylation of IκB inhibitor (Pahl 1999). IκBα in their C-terminal portions subunit is phosphorylated by an upstream IKKα at serine residues 32 and 36, triggering ubiquitination and proteasomal degradation of IκBα, thereby facilitating the translocation of p50-p65 heterodimer into the nucleus (Chen *et al.* 1996; Lee *et al.* 1997). Phosphorylation of p65 facilitates its binding to a specific sequence in DNA, which in turn results in gene transcription (Yamaoka *et al.* 1998). Target genes are selectively regulated by the distinct transcriptional activation potential of different NF-κB subunit combinations. The consensus pathway for NF-κB activation in response to pro-inflammatory stimuli such as TNF-α and IL-1β has been extensively characterized. These cytokines act through distinct signalling pathways that converge on the activation of an IKK; the subsequent phosphorylation of IκB molecules targets them for degradation by the proteosomes (Chen *et al* 1999; Tripathi and Aggarwal 2006; Hayden and Ghosh 2008).

There are two signaling pathways leading to the activation of NF-κB known as the canonical pathway (or classical) and the non-canonical pathway (or alternative pathway) (Ghosh and Karin 2002; Bonizzi and Karin 2004) (**Fig. 1**). In the canonical signaling pathway, binding of ligand to a cell surface receptor such as a member of the Toll-like receptor superfamily leads to the recruitment of adaptors (such as TRAF) to the cytoplasmic domain of the receptor. These adaptors in turn recruit IKK complex which leads to phosphorylation and degradation of the IκB inhibitor. The canonical pathway activates NF-κB dimers comprising of RelA, c-Rel, RelB and p50.The non-canonical pathway is responsible for the activation of p100/RelB complexes and occurs during the development of lymphoid organs responsible for the generation of B and T lymphocytes (Karin and

Table 1 Members of the NF-κB/rel and IκB families of proteins.

NF-κB/Rel Proteins	Class 1	p50/p105
	(synthesized as mature products and	p52/p100
	do not require proteolytic processing)	Relish
	Class 2	RelA
	(synthesized as large precursors which	RelB
	require proteolytic processing to	c-Rel
	produce the mature proteins)	Dorsal
		Dif
IκB proteins		IκBα
		IκBβ
		IκBε
		IκBγ
		Bcl-3
		Cactus

Canonical pathway
TNF-α, IL-1, LPS, CD40L

Non-canonical pathway
LTα, LTβ, CD40L, BAFF, RANKL

MEKK3, TAK1

NIK

NEMO

IKK-1 IKK-2

IKK-1 IKK-2

p50 IκB-α Rel A

p100 RelB

p50 Rel A
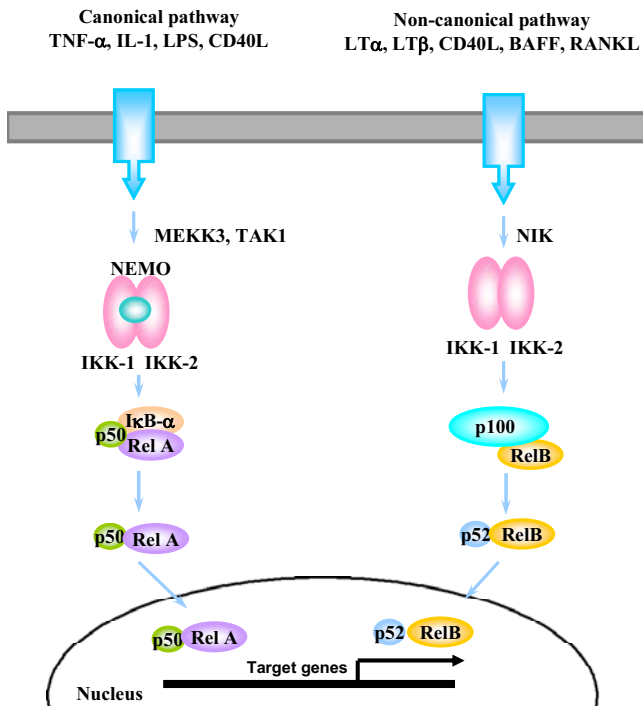
p52 RelB

p50 Rel A

p52 RelB

Target genes

Nucleus

Fig. 1 NF-κB signaling pathways. In the canonical pathway (left), the binding of a specific ligand (TNF-α, IL-1, LPS, CD40L) to a receptor leads to the phosphorylation of IκB through IKK complex (IKKα, IKKβ and NEMO) and ubiquitin-dependent proteosomal degradation of IκB. This releases active NF-κB dimer that translocates into the nucleus to transcribe target genes. In the non-canonical pathway (right), the binding of ligand (LTα, LTβ, CD40L, BAFF, RANKL) to receptor leads to processing of p100/RelB via NIK mediated activation of IKK complex comprising of two units of IKK. The processed p52/RelB translocates into the nucleus to transcribe target genes.

Lin 2002; Gilmore 2003; Beinke and Ley 2004; Tergaonkar 2006).

BIOLOGICAL FUNCTIONS OF NF-κB

More than a decade after its discovery, transcription factor NF-κB, remains an exciting and active area of study because of its diverse range of regulation and expression of signaling molecules involved in inflammation, cancer and oxidative stress related diseases. Recently, significant advances have been made in elucidating the details of the pathways through which signals are transmitted to the NF-κB:IκB complex in the cytosol. Because of involvement of NF-κB in wide range of function it has been used as drug target for variety of diseases. NF-κB regulates the transcription of an exceptionally large number of genes, particularly those involved in immune and inflammatory responses. Some of the functions of NF-κB are summarized here.

NF-κB: Effects in immune system

NF-κB is required for the development and function of many other cells, including T cells, thymocytes, dendritic cells, macrophages and fibroblasts. The targeted disruption of the p65 component of NF-κB is lethal because of the associated developmental abnormalities, whereas the lack of p50 components results in immune deficiencies and increased susceptibility to infection (Sha *et al.* 1995). Signalling through IKKβ is essential to protect T cells from TNF-α induced apoptosis during development. RelA is required for the development of T and B cells. Further, NF-κB regulates signaling pathway involved in T cell development. Macrophages from Rel B-/- mice have the inability to produce TNFα and also overproduce IL-1β (Weih *et al.* 1997), which explains the importance of NF-κB in macrophages.

NF-κB plays an important role in the development of dendritic cells also, which has been illustrated by the lack of CD8α and thymic dendritic cells in RelB-/- mice (Arron *et al.* 2001).

NF-κB: Regulation of immune cell functions

The classical NF-κB pathway, based on IKKβ-dependent IκB degradation, is essential for innate immunity. The activation and nuclear translocation of NF-κB is associated with increased transcription of a number of different genes, including those coding for chemokines (IL-8), adhesion molecules (endothelial leukocyte adhesion molecule, vascular cell adhesion molecule, and intercellular adhesion molecule), and cytokines (IL-1, IL-2, TNF-*a*, and IL-12). These molecules are important components of the innate immune response to invading microorganisms and are required for migration of inflammatory and phagocytic cells to tissues (Hawiger 2001). Bacterial products also activate NF-κB through activation of Toll like receptors (TLRs) as specific pattern recognition molecules. NF-κB controls the many bacterial and parasitic infections through production of ROS intermediates by regulation of induction of inducible nitric oxide synthase. Non immune cells such as fibroblasts, endothelial and epithelial cells are also capable of responding to pathogens by activating NF-κB. It is also involved in the ability of mast cells to make the T-cell and mast-cell growth factor IL-9 and their expression of TLR4 (Tripathi and Aggarwal 2006). Many of the events involved in triggering innate immune response to infection are essential for the development of protective T-cell responses. Thus, the abilities of accessory cells to present antigen provide costimulation, and produce cytokines in response to infection are critical to the subsequent adaptive immune response (Baeulle and Henkel 1994). RelA is required to express major histocompatibility complex class-I and CD40 molecules, which help in the development of CD8^{+} T-cell responses. Further, NF-κB is required for efficient CD4^{+} T-cell responses. NF-κB also plays a vital role in the regulation of accessory cell functions which affect adaptive responses. NF-κB play a necessary role in maintenance of long-term memory cells is the hallmark of adaptive immunity. Other studies also coupled the NF-κB to the commitment of the cell to DNA synthesis. NF-κB controls B cell functions like immunoglobulin class switching, regulate splenic architecture, B-cell proliferation and differentiation, controls B cells survival and mediates their effector functions (Tripathi and Aggarwal 2006).

NF-κB in apoptosis - Positive and negative aspects

Among their diverse role in cellular physiology, other pivotal role of NF-κB is apoptosis; it is strongly linked to inhibition of apoptosis. Apoptosis is a physiological process critical for organ development, tissue homeostasis, and elimination of defective or potentially dangerous cells in complex organisms. Apoptosis can be initiated by a wide variety of stimuli, which activate a cell suicide program that is constitutively present in most vertebrate cells. It was believed that NF-κB activates cell death pathways because of involvement in pathological responses. Apparently it can also protect cells from death and it actually serves as a survival factor. The paradoxical "survival" action of NF-κB is due to an induction of antiapoptotic factors. The NF-κB transactivation leads to expression of inhibitor of apoptosis protein-1 (IAP-1) and X-linked inhibitor of apoptosis protein (XIAP), which contains NF-κB elements in its promoters. Expression of those (IAP-1 and XIA) protein products inhibits several of the caspase enzymes involved in the cell-death program. Activation of the caspase cascade during apoptosis therefore downregulates NF-κB-dependent antiapoptotic pathways. Additionally, NF-κB may upregulate the mitochondrial antiapoptotic factor Bcl-2, following which Bcl-2 downregulates IκBα, thus increasing NF-κB activation.

Barkett and Gilmore (1999) and Valen *et al.* (2001) insinuated that NF-κB can induce both good survival signals as well as evil detrimental molecules. The exegesis behind this event may be that the inflammatory program mediated through NF-κB activation generates toxic molecules that can kill invading microorganisms without damaging the host cells. The induction by NF-κB of a survival program in parallel with potentially dangerous enzymes such as matrix metalloproteinase-9 and NO synthase might therefore provide protection for the cytokine-responding cell. In diverse cell types, Rel/NF-κB transcription factors have been shown to have a role in regulating the apoptotic program, either as essential for the induction of apoptosis or, perhaps more commonly, as blockers of apoptosis. In most cells, NF-κB activation protects the cell from apoptosis, through induction of survival genes such as TRAF1, TRAF2, c-IAP1, c-IAP2, IEX-IL, Bcl-x_L and BfI-1/A1. Whether Rel/NF-κB promotes or inhibits apoptosis appears to depend on the specific cell type and the type of inducer (Pah 1999; Tripathi and Aggarwal 2006).

Role of NF-κB in human diseases

The transcription factor NF-κB has attracted widespread recognition based on its atypical regulation. NF-κB can be activated by multifarious stimuli and it can also control diverse genes and biological responses. Some of the diseases associated to dysregulation of NF-κB are AIDS, atherosclerosis, asthma, arthritis, cancer, diabetes, inflammatory bowel disease, muscular dystrophy, stroke, and viral infections. In this section we discuss some of the evidences related to human diseases associated with abnormal NF-κB regulation.

1. Role in inflammatory disorders

Inflammatory processes are a hallmark of many diseases. NF-κB is focal key regulator of inflammatory responses which plays a crucial role in the initiation and amplification of inflammation (Senftleben *et al.* 2001; Kumar *et al.* 2003). Transcription of proinflammatory and anti apoptotic target genes can be triggered by stimulation of NF-κB which respond to IL-1β or TNF-α. Prolonged or imbalanced activation of NF-κB generates chronic inflammation and might favor tumorigenesis (Senftleben *et al.* 2002). More evidence has been published regarding the role of NF-κB in inflammatory responses. Moreover, NF-κB was shown to be involved in the transcriptional regulation of more than 150 genes with a significant portion demonstrating proinflammatory properties (Chen *et al.* 1996).Thus, it can be suggested that NF-κB deficiency or its inhibition *in vivo* leads to reduced inflammatory responses. The role of NF-κB in inflammatory disorders is discussed below.

2. Role in asthma

Asthma is defined as variable airway obstruction usually accompanied by airway hyperreactivity (AHR). Defining features of asthma include bronchoconstriction due to contraction or hypertrophy of airway smooth muscle (ASM), and inflammation within the airway. Symptoms often include dyspnoea, wheeze and tightening of the chest. The pathogenesis of asthma involves persistent expression of a broad array of genes, such as those encoding proinflammatory cytokines, chemokines, adhesion molecules, and inflammatory enzymes. Many of these genes contain the κB site for NF-κB within their promoters, suggesting that NF-κB plays a vital role in the initiation and perpetuation of allergic inflammation (Christman *et al.* 2000; Yamamoto *et al.* 2001). Several lines of evidence indicate enhanced NF-κB pathway activation in asthmatic tissues. Peripheral blood mononuclear cells (PBMCs) of adult uncontrolled, severe and moderate asthmatics have higher levels of NF-κB p65 protein expression, IκB phosphorylation and IKK-β protein levels than normal individuals (Gagliardo *et al.*

2003; LaGrutta *et al.* 2003). Furthermore, when compared to non-asthmatic individuals, nuclear extracts from bronchial biopsies, sputum cells (Hart *et al.* 1998), and cultured bronchial epithelial cells (Zhao *et al.* 2001) from stable, untreated asthmatics have greater levels of NF-κB p65 and p50 activation. These evidences support the role of NF-κB in the pathogenesis of asthma and other pulmonary diseases.

3. Role in rheumatoid arthritis

NF-κB has been shown to play diverse roles in the initiation and perpetuation of rheumatoid arthritis (RA) (Makarov *et al.* 2001). Activated NF-κB is a common feature in human rheumatoid arthritis synovium (Marok *et al.* 1996; Gilston *et al.* 1997; Miyazawa *et al*. 1998) and in various animal models of rheumatoid arthritis such as adjuvant arthritis in rats, collagen-induced arthritis in mice, and streptococcal cell wall induced arthritis in rats (Makarov *et al.* 2001; Mor *et al.* 2005). NF-κB is also suggested to be involved in the regulation of apoptosis in the synovium. In animal models of RA which were used to examine the relationship between inflammation, activation of NF-κB, and apoptosis in the synovium, it was demonstrated that in primary synovial fibroblasts, NF-κB is required for induction of multiple inflammatory molecules, including IL-1β and TNFα (Miagkov *et al.* 1998). Cartilage-pannus junction (CPJ) is the region of the synovial membrane that invades bone and cartilage resulting in erosions. The specific pathophysisological roles of Rel/NF-κB in chronic arthritis are supported by the predominant expression of NF-κB1 at tissues adjacent to the CPJ (Benito *et al.* 2004). Parallel findings were observed for IKKα and IKKβ level in RA patients which are constitutively expressed at the mRNA level, indicated that immunoreactive IKK level was also abundant in the primary fibroblast-like synoviocytes (Aupperle *et al.* 2001). Animal experiments also confirm that IKK activation is a crucial event in the initiation of synovitis (Tak *et al.* 2001).

4. Role in atherosclerosis

Atherosclerosis is an inflammatory disease, characterized by the accumulation of macrophage-derived foam cells in the vessel wall and accompanied by the production of a wide range of chemokines, cytokines, and growth factors. A wide range of molecules have been identified in atherosclerotic environments that are able to activate NF-κB *in vitro* which includes leukocyte adhesion molecules, such as vascular cell adhesion molecule 1, intercellular adhesion molecule 1, and E-selectin, as well as the chemokines IL-8 and monocyte chemoattractant protein 1 (Cybulsky *et al.* 1991; Boring *et al.* 1998; Iiyama *et al.* 1999). Numerous genes have been increasingly expressed during earlier stage of atherosclerotic lesion formation which is known to be regulated by NF-κB (Brand *et al.* 1997). Activated NF-κB was detected in human atherosclerotic lesions within smooth muscle cells, macrophages, and endothelial cells (Brand *et al.* 1996). Activation and increased levels of components of the NF-κB system is indicative for a role of NF-κB in atherosclerosis.

5. Role in septicemia

Septicemia is a life-threatening condition that may lead to sepsis and even septic shock. This cascade is usually accompanied by a pronounced inflammatory response, leading to high body temperature and elevated levels of laboratory markers of inflammation. The inflammatory response was significantly diminished in children with inherited disorders of nuclear factor (NF)-kappa B-mediated immunity (Von Bernuth *et al.* 2005). Moreover, NF-κB is involved in the development of sepsis-induced organ failure and also occupies a central role in signaling pathways important in sepsis (Abraham 2003). Hence modulation of NF-κB activity may be an appropriate therapeutic target in patients with sepsis.

6. Role in AIDS

Human immunodeficiency virus (HIV) infection leads to the progressive loss of CD4+ T cells and the near complete devastation of the immune system in the majority of infected individuals. Although NF-κB activation during viral infection has been interpreted as a protective response of the host to viral infection, some viruses including HIV have evolved strategies to interfere with NF-κB activation to evade the immune response. Interestingly, it has been reported that HIV infection induced NF-κB activation, which may suppress HIV induced apoptosis in infected myeloid cells. High levels of viral gene expression and replication results in part from the activation of NF-κB, which in addition to orchestrating the host inflammatory response also activates the HIV-1 long terminal repeat (Nabel and Baltimore 1987; Hiscott *et al.* 2001; Surabhi and Gaynor 2002)

7. Role in diabetes

Type I diabetes or insulin-dependent diabetes mellitus is a multifactorial autoimmune disease characterized by profound destruction of insulin-producing β cells. Accumulating evidence implicates free radicals and NF-κB in the destruction of β cells and disease progression (Ho and Bray 1999). It has been suggested that pancreas-specific reactive oxygen intermediates production plays a critical role in signaling the autoimmune/inflammatory response by activating NF-κB. Involvement of NF-κB activation in diabetes progression (Kwon *et al* 1995; Lamhamedi-Cherradi *et al.* 2003), signals that it can be targeted for the treatment of diabetes in animals (Yuan *et al.* 2001).

8. Role in carcinogenesis

NF-κB participates in many aspects of oncogenesis which includes suppression of apoptotis and induction of expression of proto-oncogenes such as c-myc and cyclin D1 (Guttridge *et al.* 1999; Pahl 1999). NF-κB also regulates the expression of various molecules which promote tumor cell invasion and angiogenesis (Bharti and Aggarwal 2002). Indeed, constitutive NF-κB activity has been observed in a number of human cancers, including breast cancer, non-small- cell lung carcinoma, thyroid cancer, T- or B-lymphocyte leukemia, melanoma, colon cancer, bladder cancer, and several virally induced tumors, and the inhibition of NF-κB abrogates tumor cell proliferation (Giri and Aggarwal 1998; Chen *et al.* 2001; Mukhopadhyay *et al.* 2001; Rath and Aggarwal 2001; Bharti *et al.* 2003; Younes *et al.* 2003). Chromosomal alterations of NF-κB family genes provided additional evidence for the role of NF-κB in oncogenesis. Although it is widely accepted that inhibition of NF-κB triggers apoptosis in many tumor cell types (Yamamoto and Gaynor 2001), there are a few exceptions in which NF-κB activation blocks malignant growth. These findings thus suggest that NF-κB plays a different role in the regulation of cell growth in a tissue context dependent manner.

9. Role in euthyroid sick syndrome

Euthyroid sick syndrome (ESS) also called low-T3 syndrome or nonthyroidal illness, is characterized by low serum T3 levels (De Groot 1999) and is caused mainly by a decrease in liver deiodinase type 1 (D1) mRNA. The disease is associated with a wide variety of disorders including sepsis, malignancy, and AIDS. Activation of NF-κB has been demonstrated to be the potential molecular factor of ESS (Nagaya *et al.* 2000). Hence the inhibition of NF-κB may be a therapeutic target for treatment of this syndrome.

10. Role in muscular dystrophy

Muscular dystrophy is an inherited group of muscle disorders that causes a slow but progressive degeneration of muscles, leading to life-long pain, disability, and eventual

Table 2 Diseases associated with NF-κB activation.

AIDS
Arthritis
Asthma
Atherosclerosis
Carcinogenesis
Diabetes
Euthyroid sick syndrome
Inflammation
Muscular dystrophy
Rheumatoid arthritis
Septicemia

Table 3 Genes targeted by NF-κB.

Genes coding for	References
Cytokines	
IL-1, IL-2, IL-6	Tak and Firestein 2001; Gilmore 2004; Karin and Greten 2005
Mediators of inflammation	
iNOS,	Gilmore 2004
TNF-α,	Gilmore 2004
COX	Marrogi *et al.* 2000; Heiss *et al.* 2001
Adhesion molecules	
ICAM-1, VCAM-1	Gilmore 2004
Immune receptors	
Class I	Israel *et al.*1989
Class II	Johnson and Pober 1994
Cell proliferation	
Cyclin D1, c-Myc, IAP-1	Gilmore 2004
Cell growth	
cyclin D1	Gilmore 2004
Cell differentiation	
p38, COX-2	Yamamoto *et al.* 1995
Apoptosis	
IAP-1, XIAP, Bcl-2 family proteins, TRAF-1, TRAF-2	Shishodia and Aggarwal 2002

death. Recent studies have shown that the onset of muscular dystrophy is associated with DNA-binding activity of NF-κB and the expression of NF-κB-regulated inflammatory cytokines such as TNF-α and IL-1β (Kumar *et al.* 2003). An elevated activity of NF-κB signaling pathway was also observed in the skeletal muscle fibers of patients with Duchenne muscular dystrophy (Monici *et al.* 2003), which suggests that the perturbation of the NF-κB signaling pathway is a common phenomenon in muscular dystrophies, and that aberrant regulation of NF-κB could be a potential cause of the onset of muscular dystrophy in animals. The regulation and control of NF-κB activity, which can be achieved by gene modification or pharmacological strategies, would provide a potential approach for the management of NF-κB related human diseases (**Tables 2, 3**).

TARGETING OF NF-κB: GLIMPSE OF THERAPEUTIC TARGETING

NF-κB is a key regulator in modulating the expression of different cytokines, which supports its role as a coordinating element in the body's response to stress, infection or inflammation (Shishodia and Aggarwal 2004). Consistent with the pivotal role of NF-κB in regulation and expression of cytokines, immune cell function and maintenance, it is an attractive target for array of diseases (Pahl 1999; Baldwin *et al.* 2002). It can be blocked at various steps, including its activation through different pathways, its translocation to the nucleus and its binding to DNA (**Table 4**). The list of therapeutics that inhibit NF-κB includes numerous natural and synthetic antioxidants, immunosuppressants, and natural plant compounds (Epinat and Gilmore 1999) suggesting that the ability to suppress NF-κB activation at least partially accounts for their therapeutic effects.

Among these agents, flavonoids also have the ability to inhibit NF-κB at multiple steps, including induction of

Table 4 Therapeutic targeting of NF-κB.

Direct therapeutic targeting of NF-κB
- Block NFκB activation (Staal *et al.*1993 Cho *et al.* 1998; Gilad *et al.* 1998; Islam *et al.* 1998; Manna *et al.* 1999b)
- Inhibits IKKβ (Murata *et al.* 2003; Liu *et al.* 2008; Syed *et al.* 2008)
- Phosphorylation of IκBα (Dhanalakshmi *et al.* 2002; Syed *et al.* 2008)
- Inhibits IKKα kinase activity (Dhanalakshmi *et al.* 2002; Syed *et al.* 2008)
- Inhibit proteosome degradation of IκB (Palombella *et al.* 1994; Jobin *et al.* 1998; Grisham *et al.* 1999)
- Interfering with any step in the NF-κB activation pathway by blocking a specific member of the cascade (Choi *et al.* 2004; Channavajhala *et al.* 2005; Gobert *et al.* 2007)
- Inhibiting its NF-κB translocation to the nucleus (Dhanalakshmi *et al.* 2002; Momose *et al.* 2007)
- Inhibits NF-κB binding to DNA (Qiu *et al.* 2007; Rao *et al.* 2007; Yang *et al.* 2008)
- Inhibits transactivation of NF-κB (Itoh *et al.* 2007; Samant *et al.* 2007; Kashima *et al.* 2009)

Indirect therapeutic targeting of NF-κB
- Transcriptional repression of several cytokines (Kim *et al.* 2008) and adhesion molecules (Jagielska *et al.* 2009)
- Inhibits TNF-α-induced NF-κB activation (Gohda *et al.* 2003; Lentsch *et al.* 2007)
- Blocking the incoming stimulating signal at an early stage and thus blocking its general effects (Epinat and Gilmore 1999)
- H_2O_2 induced NFκB activation (Musonada and Chipman 1998; Sen *et al.* 1998)
- Scavenging ROS (Sen *et al.* 1996b; Yamagishi *et al.* 2004)
- Inhibits mitochondrial electron transport (Schulze-Osthoff *et al.* 1993)
- Overexpression of enzymes that are involved in regulation of the redox state of the cell can block TNFα-induced activation of NF-κB (Schulze-Ostho *et al.* 1993; Manna *et al.* 1998; Manna *et al.* 1999b)
- Proteosome inhibitors (26S proteosome) (Grisham *et al.* 1999)
- Inhibits immune response by immunosuppressants (Frantz *et al.* 1994)

IκBα leading to enhanced binding to NF-κB and retention in the cytoplasm (Bosscher *et al.* 2000). Other ways to block NF-κB is to inhibit proteosome degradation of IκB, anti-cytokine therapy to prevent cytokine-mediated activation and introduction of IκBα super-repressor. There are concerns with the use of these inhibitors of NF-κB, which may have effects independent of the NF-κB pathway. However, use of inhibitors allows modulation of NF-κB at specific stages of the inflammatory response. It is important to note that such inhibitors may prevent the proper resolution of inflammation *in vivo*. Thus, the identification of NF-κB as a key player in the pathogenesis of inflammation suggests that NF-κB-targeted therapeutics might be effective in diseases like rheumatoid arthritis, inflammatory bowel disease, and various other animal models of inflammatory disease (Sun and Ballard 1999; Yamamoto and Gaynor 2001; Tripathi and Aggarwal 2006). The ultimate benefit of such targeted therapy will depend on the delicate balance between suppression of inflammation and interference with normal cellular functions. It is to be hoped that the development of therapeutic use of flavonoids against NF-κB will help to define the role of dietary flavonoids for treating human diseases.

FLAVONOIDS AS NF-κB INHIBITORS

Flavonoids are naturally occurring polyphenolic compounds containing two benzene rings linked together with a heterocyclic pyran or pyrone ring (Ross and Kasum 2002). Flavonoids are normal constituents of the human diet and are known for a variety of biological activities. Ample research with last few years has shown that flavonoids in certain fruits, vegetables, herbs, and plants exhibit innumerable properties including antioxidants, anticarcinogenic, anti-inflammatory, antiangiogenic, cytotoxic, antimicrobial, cytostatic, enzyme inhibitors and immunomodulators (Havsteen 1983; Harborne and Williams 2000; Havsteen 2002; Gazák *et al.* 2007; Sung *et al.* 2007). Because of its multitudinous properties and myriad range of application, flavonoid has been used for targeting prime molecules involved in diseased conditions (Moon *et al.* 2006; Fu *et al.* 2007; Mojzis *et al.* 2008; Akhlaghi and Bandy 2009). It has been already proposed that inhibition of NF-κB is one such target of flavonoids to prevent the inflammatory diseases and cancer (Orlowski and Baldwin 2002; Kim *et al.* 2004; Monks *et al.* 2004; Ahmed *et al.* 2006; Qin *et al.* 2007). Here we discuss some unprecedented targets and mechanism concerning flavonoids as NF-κB inhibitors which will provide new insight into the prevention of NF-κB mediated diseases

(Figs. 2, 3).

Fisetin

Fisetin (3,7,3',4'-tetrahydroxyflavone) is a flavonoid found in the smoke tree (*Cotinus coggygria*) and is also widely distributed in strawberry, apple, persimmon, grape, onion, and cucumber at concentrations of 2 to 160 μg/g (Arai *et al.* 2000). Fisetin is hydrophobic and readily passes through cell membranes and accumulates intracellularly, resulting in a good antioxidant activity and also has high Trolox equivalent antioxidative capacity (TEAC) values (Ishige *et al.* 2001). It suppresses the NF-κB activation, both constitutively and by induction with carcinogens, growth factors and inflammatory agents. It also down-regulates NF-κB dependent gene products involved in cell proliferation, in anti-apoptosis, and in invasion. The mechanism behind the suppression of NF-κB activation by fisetin was found to be due to inhibition of RIP, TAK1, and IKK activation, which led to inhibition of IκBα phosphorylation and degradation, suppression of p65 phosphorylation and translocation to the nucleus, and inhibition of NF-κB dependent reporter gene expression. It is also implied that, suppressive activity of fisetin may be due to the position and number of phenyl hydroxyl groups in the flavone. Fisetin also down-regulates the expression of NF-κB mediated gene products – MMP-9, ICAM-1, and VEGF. On the whole, work on fisetin by Sung *et al.* (2007) indicated that the anti-proliferative, pro-apoptotic, anti-angiogenic, and anti-metastatic effects of fisetin may be mediated through suppression of NF-κB regulated gene products.

Treatment of SRA01/04 cells with fisetin inhibited UVB-induced cell death and the generation of ROS. Fisetin inhibited UVB-induced activation and translocation of NF-κB/p65, which was mediated through an inhibition of the degradation and activation of IκB. Fisetin also inhibited UVB-induced phosphorylation of the p38 and c-Jun N-terminal kinase (JNK) proteins of the MAPK family at various time points studied. The results showed that fisetin could be useful in attenuation of UV radiation-induced oxidative stress through activation of NF-κB and MAPK signaling in human lens epithelial cells, which suggested that fisetin has a potential protective effect against cataractogenesis (Yao *et al.* 2008).

Park *et al.* (2007) suggested that fisetin modulates inflammatory reaction in stimulated human mast cells (HMC-1) by suppressing the induction of NF-κB promoter-mediated luciferase activity. In addition, the role of fisetin has been evaluated in apoptotic process, wherein the flavonoid
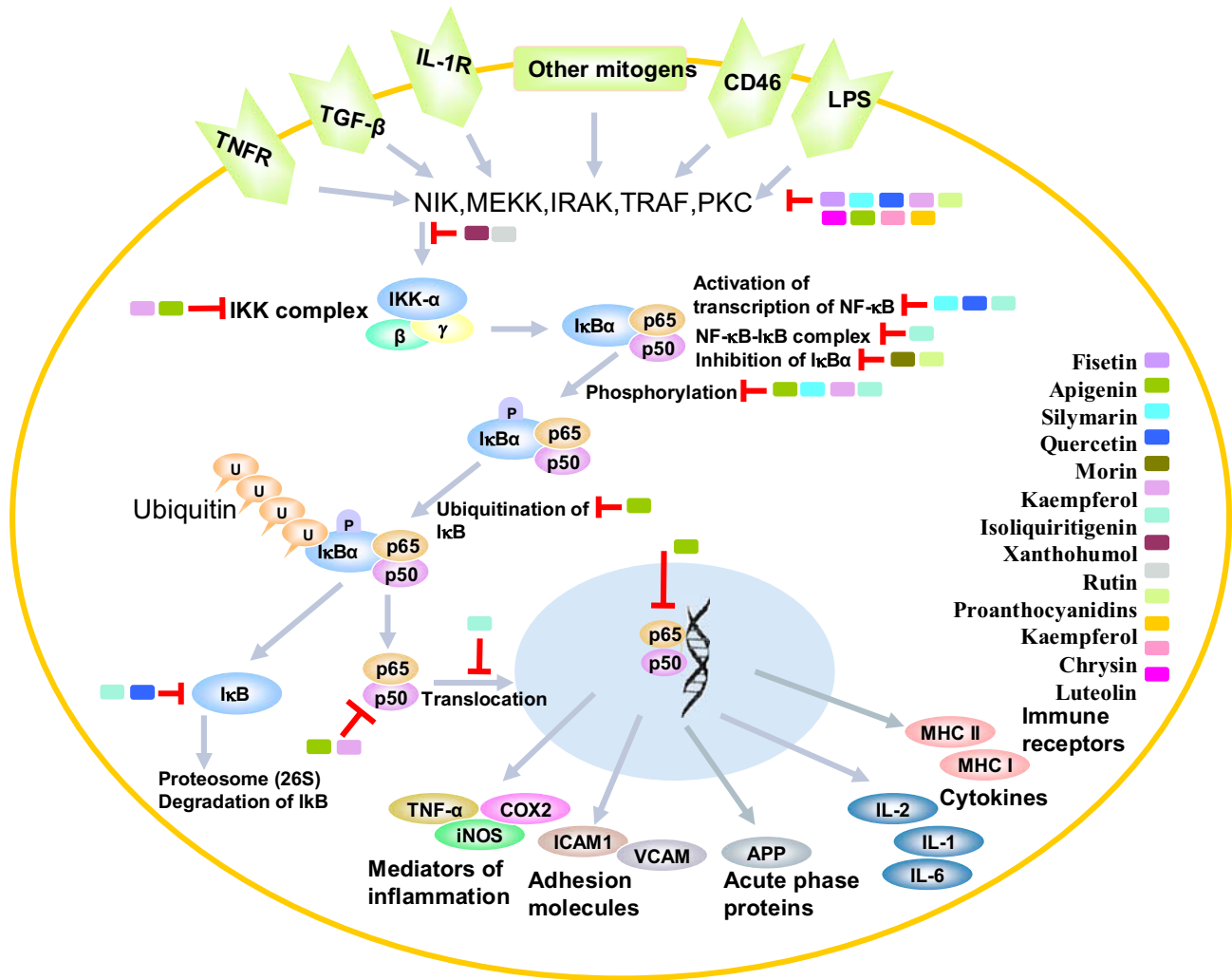
Fig. 2 Inhibitory effect of different flavonoids in NF-κB pathway. Under most circumstances, NF-κB dimers are located in the cytoplasm of cells bound to inhibitory IκB proteins. Upon receiving an inducing signals or conditions such as oxidative stress and inflammation, the proinflammatory cytokines stimulates the genes (NIK, MEKK, IRAK, TRAF, PKC) results in the activation of an IκB kinase (IKK). IκB is phosphorylated by active IKK and ubiquinatated by the ubiquitine ligase system (ULS). IκB is further degradated by the 26S proteasome (26S). Activated NF-κB can translocate into the nucleus to initiate NF-κB dependent gene transcription (e.g., TNF-α, IL-6, IL-1β, IL-2, iNOS, COX-2, MHC I, MHC II, APP, ICAM, VCAM). Activated NFκB can pass the nuclear membrane and interact with κB binding sequences in enhancers of NF-κB regulated genes. The system is eventually returned to its resting state through an auto-regulatory system, in that NF-κB turns on expression of the gene encoding IκB, and newly synthesized IκB then resequesters NF-κB in the cytoplasm. **NIK**, NF-κB inducing kinase; **MEKK**, mitogen activated protein kinase kinase; **IRAK**, interleukin-1-receptor-associated kinase; **TRAF**, tumour necrosis factor receptor associated factor; **PKC**, protein kinase C; **VCAM**, vascular cell adhesion molecule.

is reported to activate the expression of mitogen-activated protein kinases (MAPK, p38), protein phosphatases and NF-κB in human promyelocytic leukemia cells (HL-60 cells).

Apigenin

Apigenin (4',5,7,-trihydroxyflavone), a non mutagenic dietary flavonoid abundantly present in fruits and vegetables, may prove useful in the prevention and therapy of prostate cancer. It is identified that apigenin is a potent inhibitor of NF-κB, which may perform a pivotal function in the regulation of cell growth, apoptosis, and the regulation of the cell cycle (Yoon *et al.* 2006).

Inhibitory effect of apigenin on NF-κB expression was assessed in androgen-insensitive human prostate carcinoma cells which exhibited high constitutive levels of NF-κB. Treatment of cells with 10–40 μM doses of apigenin inhibited DNA binding and reduced nuclear levels of the p65 and p50 subunits of NF-κB. Apigenin inhibited IκBα degradation and IκBα phosphorylation and significantly decreased IKKα kinase activity. Apigenin also inhibited TNFα induced activation of NF-κB via IκBα pathway, thereby sensitizing the cells to TNFα induced apoptosis. The inhibition of NF-κB activation correlated with a decreased expression

of NF-κB dependent reporter gene and suppressed expression of NF-κB regulated genes [specifically Bcl-2, cyclin D1, cyclooxygenase-2, matrix metalloproteinase-9, nitric oxide synthase-2 (NOS-2), and vascular endothelial growth factor]. The results indicated that inhibition of NF-κB by apigenin may lead to prostate cancer suppression by transcriptional repression of NF-κB responsive genes as well as selective sensitization of prostate carcinoma cells to TNFα induced apoptosis (Shukla and Gupta 2004).

The influence of apigenin on immunostimulatory effect was assessed in dendritic cells (DC), which are the antigen presenting cells believed to be capable of initiating T-cell responses to both microbial pathogens and tumours. Apigenin induced the phenotypical and functional maturation of DC and suppressed the LPS-induced activation of ERK1/2, JNK, and p38 MAPK as well as the nuclear translocation of the NF-κB p65 subunit in DC. These findings provide new insight into the immunopharmacological functions of apigenin and its effects on dendritic cells, and they may also prove useful in the development of adjuvant therapies for individuals suffering from acute or chronic DC-associated diseases (Yoon *et al.* 2006).

The effect of apigenin to suppress the activation of NF-κB was assessed in LPS stimulated macrophages, since NF-
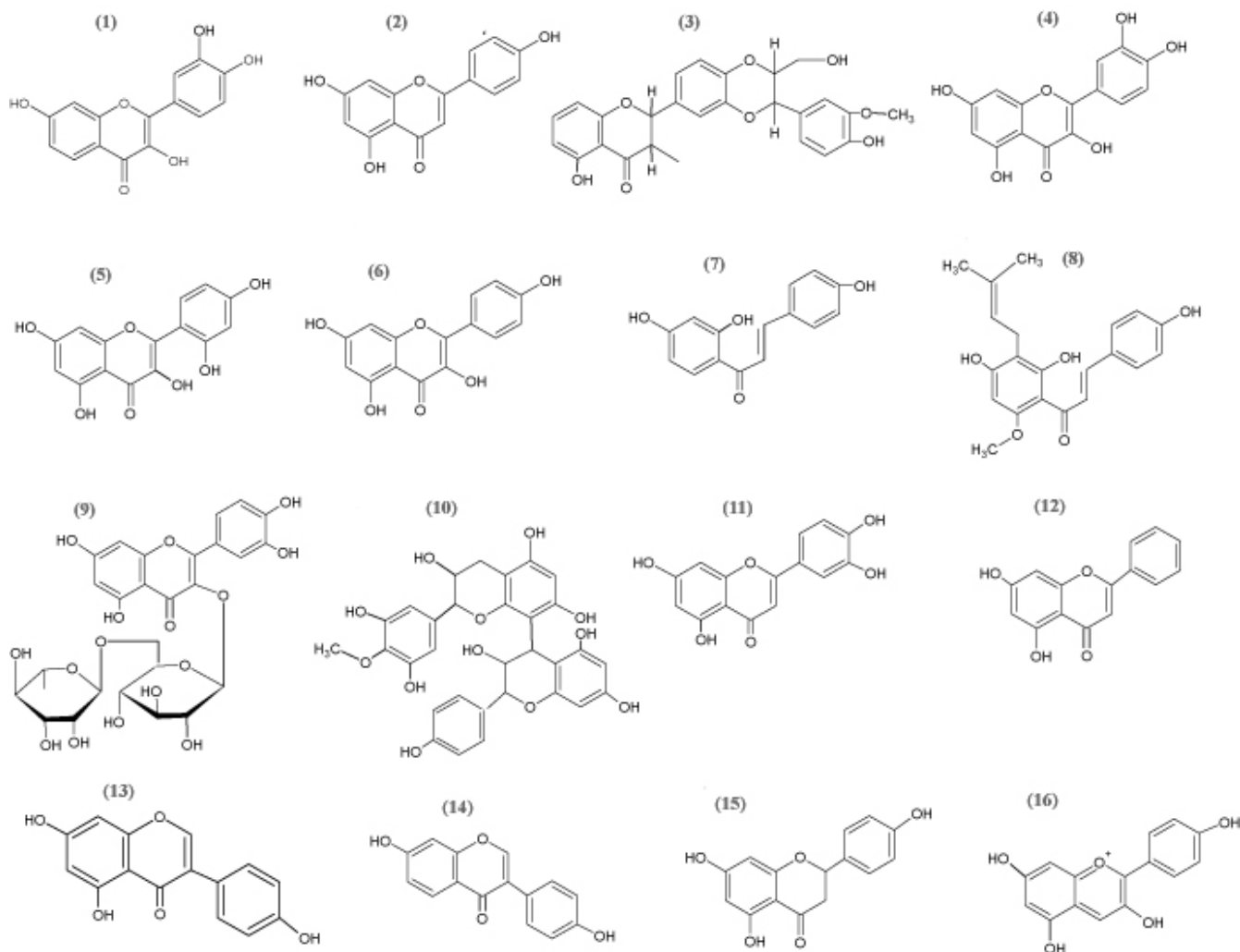
Fig. 3 Structure of flavonoids. (1) Fisetin, (2) apigenin, (3) silymarin, (4) quercetin, (5) morin, (6) kaempferol, (7) isoliquiritigenin, (8) xanthohumol, (9) rutin, (10) proanthocyanidins, (11) luteolin, (12) chrysin, (13) genistein, (14) diadzein, (15) naringenin, (16) pelargonidin.

κB is involved in the induction of expression of proteins involved in inflammatory processes. Incubation of RAW 264.7 cells with 100 ng/ml of LPS for 3 h increased NF-κB binding activity whereas induction of NF-κB binding activity by LPS was markedly inhibited by apigenin in a dose-dependent manner. Since activation of NF-κB is correlated with rapid proteolytic degradation of IκB, prevention of IκB degradation was also studied as an indication of NF-κB activation by apigenin. Apigenin was found to prevent the degradation of IκB and inhibited the LPS induced NF-κB transcriptional activity in a dose-dependent manner. Apigenin also significantly inhibited IKK activity induced by LPS of INF-γ. Hence these results suggest that anti-inflammatory activity of apigenin occurs via suppression of IKK activity resulting in the prevention of NF-κB activation (Liang *et al.* 1999).

Apigenin also protects C57BL/6J mice from LPS-induced lethal toxicity *in vivo*. This effect was accompanied by the decreased of LPS-stimulated production of TNF. These results provide novel insights into the molecular mechanisms by which apigenin regulates inflammation and show that apigenin functions by regulating NF-κB activity through the suppression of LPS-induced phosphorylation of p65 (Nicholas *et al.* 2007).

Silymarin

Silymarin is a flavonoid isolated from the fruits and seeds of the milk thistle, *Silybum marianum*. Silymarin has a wide variety of biological effects, including anti-carcinogenic (Bhatia *et al*. 1999), anti-inflammatory and anti-hepatotoxic

effects, attributed to its stabilizing effect on the plasma membrane and its inhibition of lipid peroxidation (Letteron *et al* 1990; Muriel and Mourelle 1990).

The inhibitory effect of silymarin on nitric oxide production and inducible nitric-oxide synthase (iNOS) gene expression was assessed in macrophages. Silymarin dose dependently suppressed the LPS induced production of nitric oxide in isolated mouse peritoneal macrophages and RAW 264.7, a murine macrophage-like cell line. In RAW 264.7 cells, the LPS-induced DNA binding activity of NF-κB/Rel was significantly inhibited by silymarin, and this effect was mediated through the inhibition of the degradation of inhibitory factor-κB. Silymarin also inhibited TNFα-induced NF-κB/Rel activation, whereas okadaic acid-induced NF-κB/Rel activation was not affected. NF-κB/Rel-dependent reporter gene expression was also suppressed by silymarin in LPS stimulated RAW 264.7 cells. Further study showed that silymarin suppressed the production of reactive oxygen species generated by H_2O_2 in RAW 264.7 cells. Collectively, these results suggest that silymarin inhibits nitric oxide production and iNOS gene expression by inhibiting NF-κB/Rel activation. Furthermore, the radical-scavenging activity of silymarin may explain its inhibitory effect on NF-κB/Rel activation (Kang *et al.* 2002).

The effect of silymarin on NF-κB activation induced by various inflammatory agents was investigated in human histiocytic lymphoma U-937 cells. Silymarin blocked TNF-induced activation of NF-κB in a dose- and time-dependent manner. This effect was mediated through inhibition of phosphorylation and degradation of IκBα, an inhibitor of NF-κB. Silymarin blocked the translocation of p65 to the

nucleus without affecting its ability to bind to the DNA. NF-κB-dependent reporter gene transcription was also suppressed by silymarin. Silymarin also blocked NF-κB activation induced by phorbol ester, LPS, okadaic acid, and ceramide, whereas H_2O_2- induced NF-κB activation was not significantly affected. The effects of silymarin on NF-κB activation were specific, as AP-1 activation was unaffected. Silymarin also inhibited the TNF-α induced activation of mitogen-activated protein kinase kinase and c-Jun N-terminal kinase Overall, the inhibition of activation of NF-κB and the kinases provide in part the molecular basis for the anti-carcinogenic and anti-inflammatory effects of silymarin Manna *et al.* (1999).

Dhanalakshmi *et al.* (2002) provided convincing evidence that pre- and post-treatment of DU145cells with silibinin results in significant inhibition of NF-κB activation, and that this effect was mediated via IκBα pathway. This observation raises the possibility that treatment of DU145 cells with silibinin and TNFα combination could make them more sensitive to apoptosis since the anti- apoptotic signaling elicited by TNFα-induced NF-κB activation is effectively blocked by silibinin. These results indicated that silibinin could be used to enhance the effectiveness of TNFα-based chemotherapy in advanced prostate cancer (PCA). The bioflavonoid silymarin is found to potently suppress both NF-κB-DNA binding activity and its dependent gene expression induced by okadaic acid in the hepatoma cell line HepG2. Surprisingly, TNFα-induced NF-κB activation was not affected by silymarin, thus demonstrating a pathway-dependent inhibition by silymarin. Many genes encoding the proteins of the hepatic acute phase response are under the control of the transcription factor NF-κB, a key regulator in the inflammatory and immune reactions. Thus, the inhibitory effect of silymarin on NF-κB activation could be involved in its hepatoprotective property (Saliou *et al.* 1998).

Quercetin

Quercetin is reported to have antioxidant properties associated with anti-thrombic, anti-hypertensive and anti-carcinogenic properties. Quercetin has also been shown to have anti-inflammatory properties and is reported to play a role in anti-inflammatory procedure. Intercellular adhesion molecule-1 (ICAM-1) is one of the important pro-inflammatory factors, especially in early stage of inflammation. The mechanisms regulating ICAM-1 expression by quercetin in human A549 cells were studied by Ying *et al.* (2008). Quercetin attenuated IL-1 beta-induced expression of ICAM-1 mRNA and protein in a dose-dependent manner, which suggested that quercetin exerts anti-inflammatory activity by inhibiting IκB degradation, and NF-κB activity.

Min *et al.* (2007) observed that quercetin may be suitable for the treatment of mast cell-derived allergic inflammatory diseases, since it attenuated calcium ionophore A23187 (PMACI)-induced activation of NF-κB and p38 mitogen-activated protein kinase. Administration of quercetin markedly alleviated histological abnormalities and inhibited oxidative stress and NF-κB activation in experimental model of portal hypertensive gastropathy induced by partial portal vein ligation (PPVL). Decreased IκB and induced iNOS protein were partially prevented by quercetin. Thus quercetin treatment may block the production of noxious mediators involved in the pathogenesis of portal hypertensive gastropathy by abolishing the NF-κB signal transduction pathway (Moreira *et al.* 2004). It has been reported that quercetin has a protective effect on homocysteine-injured vascular endothelial cells by antioxidant and anti-inflammatory mechanisms including the decreased expression of NF-κB (Lin *et al.* 2007). Ruiz *et al.* (2008) characterized the molecular mechanisms by which quercetin and its enteric bacterial metabolites, taxifolin, alphitonin, and 3,4-dihydroxy-phenylacetic acid, inhibit TNFα-induced proinflammatory gene expression in the murine small intestinal epithelial cell (IEC) line Mode-K as well as in

heterozygous TNFDARE/WT mice, a murine model of experimental ileitis. It was shown that quercetin inhibits TNF-induced expression of the proinflammatory cytokines IP-10 (interferon-γ-inducible protein) and MIP-2 (macrophage inflammatory protein) in primary IEC from TNF-DARE/WT mice as well as the epithelial cell line Mode-K. Consistent with its inhibitory function on various protein kinases, quercetin inhibited Akt phosphorylation in Mode-K cells but did not inhibit TNF-induced NF-κB/IκB phosphorylation/degradation or NF-κB reporter gene activity. Interestingly, and most important for understanding the mechanism involved, quercetin inhibited the recruitment of the NF-κB cofactor CBP/p300 to the IP-10 and MIP-2 gene promoters, suggesting that quercetin may target the TNF-induced transcriptional regulation at the chromatin.

Quercetin also modulates iNOS, COX-2 and C-reactive protein in Chang Liver cells by blocking NF-κB pathway mediated by upregulation of members of the IKK complex (García-Mediavilla *et al.* 2007). Other report also indicates that anti inflammatory activity of quercetin both *in vivo* and *in vitro* is mediated through inhibition of NF-κB pathway (Comalada *et al.* 2005).

Morin

Morin (3,5,7,2',4'-pentahydroxyflavone) is a flavone originally isolated from members of the Moraceae family, such as mulberry figs and others chinese herbs. It exhibits anti-proliferative, anti-tumor, and anti-inflammatory effects. The effect of morin was investigated by Manna *et al.* (2007) on NF-κB pathway which was activated by inflammatory agents, carcinogens, and tumor promoters. The effect of morin on expression of NF-κB regulated gene products involved in cell survival, proliferation, and invasion was also examined. The DNA-binding assay revealed that NF-κB activation induced by TNF, phorbol 12-myristate 13-acetate, lipopolysaccharide, ceramide, interleukin-1, and H_2O_2 was suppressed by morin. Further, the suppression of NF-κB by morin was mediated through inhibition of IκBα (inhibitory subunit of NF-κB) kinase, leading to suppression of phosphorylation and degradation of IκBα and consequent p65 nuclear translocation. Morin also inhibited the NF-κB dependent reporter gene expression activated by TNF, TNF receptor (TNFR) 1,TNFR1-associated death domain, TNFR-associated factor 2, NFκB inducing kinase, IκB kinase, and the p65 subunit of NF-κB. NF-κB regulated gene products involved in cell survival [inhibitor of apoptosis (IAP) 1, IAP2, X chromosome-linked IAP, Bcl-xL, and survivin], proliferation (cyclin D1 and cyclooxygenase-2), and invasion (matrixmetalloproteinase-9) were down-regulated by morin. These effects correlated with enhancement of apoptosis induced by TNF and chemotherapeutic agents. Overall, the results indicated that morin suppresses the activation of NF-κB and NF-κB regulated gene expression, leading to enhancement of apoptosis. This provides the molecular basis for the ability of morin to act as an anti-cancer and anti-inflammatory agent.

Kaempferol

Kaempferol is a flavonoid glycoside which is particularly abundant in fruits, vegetables, and beverages such as tea. The ability and mechanism of action of kaempferol, was investigated with regard to the inhibition of iNOS, TNF-α expression and NFκB activation (which includes IκB degradation in the cytoplasm and the nuclear translocation of NF-κB) in aged rat gingival tissues. The quantity of cytosolic p65 and IκBα were increased and reduced, respectively, in aged rat gingival tissues as the result of kaempferol treatment. Nuclear p65 levels were reduced, especially in the gingival tissues treated with 10 mg/kg/day of kaempferol. Moreover, the binding activity of NF-κB to cognate nucleotide sequences was reduced by kaempferol treatment. Kaempferol treatment reduced the quantities of p-NIK, one of the up-stream regulatory kinases of IKK and p-IKK

levels, in the aged rat gingival tissues. These results show that kaempferol effects a blockage of IKK activation followed by IκBα phosphorylation and degradation, thereby indicating that the effects of kaempferol on NF-κB may be attributable to the inhibition of phosphorylation, as well as the proteolysis of IκBα. The results of this study indicate that the anti-inflammatory effects of kaempferol are mediated via the modulation of NF-κB levels (Kim *et al.* 2007).

Kaempferol also modulates iNOS, COX-2 and CRP in Chang Liver cells by blocking NF-κB pathway through inhibiting upregulation of members of the IKK complex (García-Mediavilla *et al.* 2007).

Isoliquiritigenin

Isoliquiritigenin is a flavonoid with a chalcone structure, present in licorice root extract (derived from the plant *Glycyrrhiza glabra* or *G. radix*), is regarded as a promising chemopreventive agent. Kwon *et al.* (2007) assessed a novel anti-inflammatory property of licorice root components (which includes isoliquiritigenin), with respect to the monocyte trafficking on the activated endothelium. Isoliquiritigenin appeared to inhibit the endothelial CAM expression by blunting the degradation of IκB and translocation of NF-κB stimulated by TNF-α. The result shows that isoliquiritigenin-responsive mechanism(s) appear to be dependent of NF-κB-sensitive transcriptional regulatory mechanism(s). Kim *et al.* (2008) evaluated the anti-inflammatory effect of isoliquiritigenin in lipopolysaccharide (LPS)-treated RAW 264.7 macrophages. Isoliquiritigenin attenuated the LPS-induced DNA binding activity and the transcription activity of nuclear factor-kappa B (NF-κB), which was associated with a decrease in (IκBα) phosphorylation and subsequent blocking of p65 and p50 protein translocations to the nucleus. This result indicated that the anti-inflammatory properties of isoliquiritigen are caused by iNOS, COX-2, TNF-α, and IL-6 down-regulation due to NF-κB inhibition via the suppression of IKK, ERK1/2 and p38 phosphorylation in RAW 264.7 cells.

The status of NF-κB activation during isoliquiritigenin treatment in human primary endothelial cells demonstrated that isoliquiritigenin inhibits the translocation and activation of nuclear factor kappa B (NF-κB) by blocking the phosphorylation and subsequent degradation of IκBα (Kumar *et al*. 2007). These results have important implications for using isoliquiritigenin or its derivatives towards the development of effective anti-inflammatory molecules.

Xanthohumol

Xanthohumol (XN), the principal flavonoid of the hop plant (*Humulus lupulus* L.) and a constituent of beer, has been suggested to have potential cancer chemopreventive activities. Mechanisms of the anti-angiogenic activity were assessed by Albini *et al.* (2005) in endothelial cells. It was found that XN repressed both the NF-κB and Akt pathways in endothelial cells, indicating that components of these pathways are major targets in the molecular mechanism of XN. It hints that XN can be added to the expanding list of antiangiogenic chemopreventive drugs.

Rutin

Rutin, a glycoside of flavonol which is present in buckwheat, citrus fruits and many vegetables has been found to possess antioxidant, antitumor and anti-inflammatory activities (Guruvayoorappan and Kuttan 2007). To explore the role of rutin in inflammation, the effect of rutin was examined on the activation of NF-κB in PMACI-stimulated HMC-1 (phorbol 12-myristate 13-acetate; and calcium ionophore stimulated human mast cells) (Park *et al*. 2008). Normally NF-κB activation is involved in the coordinated expression of many genes that encode proteins such as cytokines, chemokines, and adhesion molecules involved in mediator synthesis and the further amplification and perpe-

tuation of the inflammatory reaction; and suppression of NF-κB activation has been linked with anti-inflammation. Rutin reduced the expression of proinflammatory cytokines but did not inhibit NF-κB, suggesting that rutin reduces the expression of proinflammatory cytokines without affecting NF-κB transcription. It implies that rutin may suppress the different signal transduction steps such as other transcription factors or repressor proteins in mast cell-mediated allergic inflammations (Park *et al.* 2008). Rutin also inhibits the formation of osteoclast through lowering of NF-κB activation. Osteoclast is a type of bone cell that removes bone tissue and the formation of osteoclasts requires the presence of RANK ligand (receptor activator of NF-κB). Rutin lowers the NF-κB activation in response to RANKL and also reduces reactive oxygen species produced by RANKL (Kyung *et al.* 2008). Rutin, which is one of the components of tartary buckwheat flavonoid (TBF) induced HL-60 leukemic cell apoptosis, partly through the inactivation of NF-κB (Ren *et al.* 2003).

Proanthocyanidins

Grapes (*Vitis vinifera*) are one of the most widely consumed fruits in the world. Grape seed proanthocyanidins (GSP) are a mixture of several polyphenols/flavanols and mainly contain proanthocyanidins (89%). *In vitro* treatment of normal human epidermal keratinocytes with GSPs inhibits UV-induced oxidative stress and oxidative stress–mediated activation of MAPK and NF-κB cellular signaling pathways (Mantena *et al.* 2006). *In vivo* study was designed by Sharma *et al.* (2007) to define the chemopreventive mechanism of action of GSPs against photocarcinogenesis in SKH-1 hairless mouse model, a recognized model in the field of analysis of cutaneous photodamage and photocarcinogenesis. It was observed that NF-κB/p65 was activated after UVB exposure to the mouse model and subsequently translocated to the nucleus; however, its activation and translocation to the nucleus was effectively inhibited by dietary GSPs. UVB irradiation also resulted in an increased degradation of IκBα protein. Whereas, GSPs block IκBα degradation in UVB-exposed skin, which suggests that the inhibitory effect of GSPs on UV-induced NF-κB/p65 activation may be mediated through the inhibition of proteolysis of the IκBα protein. This *in vivo* study provides conclusive evidence that dietary GSPs have the potential to attenuate UVB-induced oxidative stress and to inhibit the activation of the cellular signaling cascades involving the MAPK and NF-κB pathways that are associated with high risk of photocarcinogenesis.

Luteolin and chrysin

Chen *et al*. (2004) screened a number of flavonoids for the anti- inflammatory activity by investigating their effects on the TNF-α-stimulated ICAM-1 expression, in A 549 alveolar epithelial cells. In these cells, IKK/NF-κB pathway is required for TNF-α induced ICAM-1 expression. Therefore, the inhibitory mechanisms of chrysin and luteolin were first examined for their effects on IKKα/NF-κB pathway, like the IKK activity, IκBα degradation, NF-κB-specific DNA-protein complex formation, and NF-κB-luc activity. The results showed that luteolin had more significant inhibitions than chrysin on these parameters; however, their extent of inhibitions was not paralleled with that on TNF-α induced ICAM-1 expression. Therefore, it was proposed that other factors like AP-1 (a transcription factor), in addition to IKKα/NF-κB, might play a role in the flavonoid-induced inhibitions on ICAM-1 expression. It was found that chrysin and luteolin also inhibited TNF-α mediated AP-1 activation. The inhibitory effects of luteolin is mediated by the sequential attenuation of the ERK1/2, p38, and JNK activities, the c-fos and c-jun mRNA expressions, and the AP-1 transcriptional activity; however chrysin primarily mediate the attenuation of the JNK activity, the c-jun mRNA expression, and the AP-1 activity. Therefore, the transcription factor

AP-1 seems to play a more significant role than NF-κB in these inhibitions. The presence of a double bond at position C_2-C_3 of the C ring with OXO function at position 4, along with the presence of OH groups at positions 3' and 4' of the B ring, are required for the optimal inhibition of TNF-α-induced ICAM-1 expression by luteolin.

Genistein, daidzein, naringenin and pelargonidin

Hämäläinen *et al.* (2007) systematically investigated the effects of 36 naturally occurring flavonoids and related compounds on NO production in macrophages exposed to an inflammatory stimulus (lipopolysaccharide, LPS), and evaluated the mechanisms of action of the effective compounds. The isoflavones (daidzein, genistein), flavonols (isorhamnetin, kaempferol and quercetin), flavanone (naringenin), and the anthocyanin pelargonidin inhibited iNOS protein and mRNA expression and also NO production in a dose-dependent manner, by inhibiting the activation of NF-κB, which is a significant transcription factor for iNOS. Genistein, kaempferol, quercetin, and daidzein also inhibited the activation of the signal transducer and activator of transcription 1 (STAT-1), another important transcription factor for iNOS.

Structure activity relationship

Naturally occurring flavonoids have been proposed to exert biological effects on cells through the inhibitions of different enzymes and transcription factors involved in the signaling pathways. We have discussed several flavonoids so far, but the compelling evidence subserve that the effects of these flavonoids display characteristics inhibitory pattern towards NF-κB signal transduction pathways. The ability of inhibitory effects of these flavonoids towards NF κB targeting not only due to distinct signaling pathways it also depends on the specific cell types and origin of targeted cells. Chai *et al.* (2004) provided a hint regarding the effect of these flavonoids, which depends on the structure and functional group present in the basic structure of flavonoids. Flavonoids have remarkable antioxidant ability stem from their structure.

The capacities of 4 subclasses of flavonoids (flavanols, flavonols, flavanones, and flavones) for the inhibition of TNF-α-induced CAM expression were compared in human umbilical vein endothelial cells. The flavones (apigenin and luteolin) at doses of ≥ 25 μmol/L almost completely abolished the increased CAM protein and mRNA regardless of their anti-oxidative activity. They proved that this inhibition mediated by their interference with the NF-κB-dependent transcription pathway. Among different subgroups of flavonoids, the flavones were the most potent flavonoids and the potential to prevent pathological events involved in the atherosclerosis differs among individual flavonoid subclasses. They also epitomized that inhibitory mechanisms of the flavones appear to be independent of antioxidant sensitive transcriptional regulatory mechanism. Typically the flavonoids have the diphenylpropane (C6C3C6) skeleton, include monomeric flavanols, flavanones, flavones, and flavonols (**Fig. 4**). The variation in number and arrangement of the hydroxyl groups as well as from the nature and the extent of alkylation and/or glycosylation of these groups also explicit the individual differences in flavonoids.

Different subclasses of flavonoids have been used to study the inhibitory effect of TNF-α-induced ICAM-1 expression in A549 epithelial cells. Three flavonols (kaempferol, quercetin, and myricetin) and six flavones (flavone, chrysin, apigenin, luteolin, baicalein, and baicalin) have been used. The contributions of 4'-hydroxy structure in the B ring and 5,7-metadihydroxy arrangement in the A ring, which might help the design of analogs displaying anti-inflammatory effect, and provided evidence for the correlation between the anti-inflammatory properties of flavonoids and their efficiency in inhibiting signaling molecules. Presence of a double bond at position C2-C3 of the C ring with
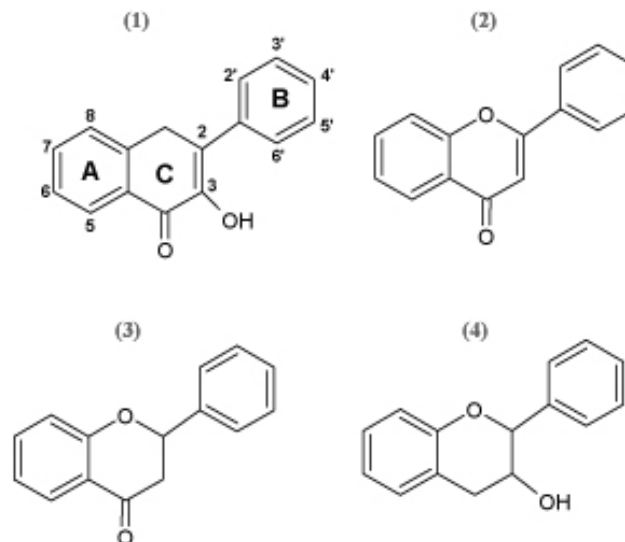

Fig. 4 Structure of (1) flavonol (2) flavone (3) flavonone (4) flavanol.

OXO function at position 4, along with the presence of OH groups at positions 3' and 4' of the B ring, is required for the optimal inhibition of TNF-α induced ICAM-1 expression by luteolin. The structure-activity relationships of flavonoids are also explored and found the significance of –OH groups at positions 5 and 7 of the A ring and at position 4' of the B ring (Chen *et al.* 2004).

Liang *et al.* (1999) also gives clues to the structural relation of flavonoids on the inhibitory effect and the flavonoids inability to inhibit the COX2 and iNOS activity through NF-κB might be due to the inclusion of more than two OH groups on the B ring. For EGCG and myricetin, presence of poly hydroxylated B ring might play a major role in the inhibitory effects. Regarding the structural requirements of flavonoids for the inhibition of NO production, Hämäläinen *et al.* (2007) discussed three main features could be essential. They are (a) a C-2,3 double bond is a common feature in the six most effective compounds, (b) a bulky group (e.g., glycoside, rhamnoside, rutinoside, or neohesperidoside) as a substituent lowered or abolished the compound's inhibitory effect (e.g., quercetin was highly effective whereas its rhamnoside-substituted derivative quercetin was ineffective), (c) 7 and 4' OH-groups were found in all effective compounds but this alone did not differentiate active from ineffective compounds. They compared the effects of 36 naturally occurring flavonoids and related polyphenolic compounds on LPS-induced NO production and iNOS expression in activated macrophages. The flavonoid classes containing the most effective com-pounds were isoflavones and flavonols. Four compounds (genistein, kaempferol, quercetin, and daidzein) inhibited activation of both of the important transcription factors for iNOS, that is, STAT-1 and NF-κB, whereas four compounds (flavone, isorhamnetin, naringenin, and pelargonidin) inhibited only NF-κB. The results partly explain the anti-inflammatory effects of flavonoids. Morin is a 3,5,7,2',4'-penta-hydroxyflavone, whereas apigenin is 5,7,4'-trihydroxyfla-vone and luteolin is 5,7,3',4'-tetrahydroxyflavone. However, 3,5,7,4'-tetrahydroxyflavone (kaempferol) and quercetin (3,5,7,3,4'-pentahydroxyflavone) have been found to be less active in blocking NF-κB activation (Manna *et al.* 2007).

CONCLUSION

Beginning with its discovery in 1986 and continuing through the present, the transcription factor NF-κB has attracted widespread interest based on its unusual regulation, the variety of stimuli that activate it, the diverse genes and biological responses that it controls, the striking evolutionary conservation of structure and function among family mem-

bers, and its apparent involvement in a variety of human diseases. The role of NF-κB in the transcriptional control of many inflammatory genes, such as cytokines, chemokines, growth factors, and leukocyte adhesion molecules and the involvement of ROS in its activation, made NF-κB a preferred target for inhibition by various agents like flavonoids. The experimental data outlined in this review lists and categorizes the flavonoids or their effect of NF-κB pathway.

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