Fungal metabolites modulating NF-κB activity: An approach to cancer therapy and chemoprevention (Review)

ROUMYANA D. PETROVA[1,2,3], ABRAHAM Z. REZNICK[3], SOLOMON P. WASSER[2], CVETOMIR M. DENCHEV[4], EVIATAR NEVO[2] and JAMAL MAHAJNA[1]

[1]Migal-Galilee Technology Center, Cancer Drug Discovery Program, P.O. Box 831, Kiryat Shmona 11016; [2]International Center for Cryptogamic Plants and Fungi, Institute of Evolution, University of Haifa, Mt. Carmel, Haifa 31905; [3]Department of Anatomy and Cell Biology, Faculty of Medicine, Technion, Haifa 31906, Israel; [4]Institute of Botany, Bulgarian Academy of Sciences, 23 Acad. G. Bonchev St., 1113 Sofia, Bulgaria

Received August 22, 2007; Accepted October 24, 2007

Abstract. Fighting cancer is considered one of the most important areas of research in medicine and immunology. Due to the ability of cancer cells to mutate and become resistant to available drugs, new scientific approaches, focused on molecular mechanisms of carcinogenesis, are needed. A new direction in cancer treatment has arisen, devoted to the adjuvant use of natural bioactive compounds in conventional chemotherapy. This kind of research is gaining more attention. In particular, fungi can be used not only as strong immunoceuticals but also as a source of potent metabolites, capable of penetrating cell membranes and interfering with particular signal transduction pathways linked to processes such as inflammation, cell differentiation and survival, carcinogenesis, and metastasis. One such a crucial pathway involved in the above-mentioned processes, is the activation of the nuclear transcription factor κB (NF-κB). This review compiles the available data on fungal metabolites, known to modulate the activity of NF-κB, thus demonstrating their potential use as novel anti-cancer agents in the rapidly advancing field of molecular therapy.

Correspondence to: Dr Jamal Mahajna, Migal-Galilee Technology Center, Cancer Drug Discovery Program, P.O. Box 831, Kiryat Shmona 11016, Israel
E-mail: jamalm@migal.org.il

Abbreviations: Akt, protein kinase B, serine/therionine kinase; AP-1, activator protein-1; CAPE, caffeic acid phenethyl ester; COX-2, cyclooxigenase-2; ERK, extracellular signal-regulated kinase; FB, fruit body; IκBα, inhibitory proteins κB; IKK, IκB kinase complex; IL-1, interleukin-1; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κB; NO, nitric oxide; STAT, signal transducer and activator of transcription; TNF-α, tumor necrosis factor α

Key words: fungal metabolites, nuclear transcription factor κB activity, chemoprevention

Contents

1. Introduction

Cancer is the second most predominant cause of death in the modern world, after cardiovascular diseases, taking many human lives every year. Depending on the stage of cancer progression, treatments include surgical operation, radiotherapy and chemotherapy. However, their side effects cause serious damage and suffering to patients. As an alternative to these treatment methods, immunotherapy is now gaining more attention than ever. Immunotherapy substantially reduces the side effects and the inherent pain of cancer sufferred by patients and helps to overcome cancer growth, even in the last stages of the disease.

Despite the observed success of most of the chemotherapeutic regimes, cellular adaptations have enabled tumor cells to evade many of these drugs. The nuclear transcription factor κB (NF-κB) is one of the factors responsible for cellular chemoresistance. It is a dimeric transcription factor belonging to the Rel/NF-κB family of transcription factors (1-4). The NF-κB complex is maintained in the cytoplasm in an inactive form by the inhibitory protein κB (IκBα), belonging to a family of inhibitory proteins, IκBs. The major activator of NF-κB is the IκB kinase complex (IKK). This complex is composed of two catalytic subunits, IKK-α and -ß and a regulatory subunit, IKK-γ (also known as NEMO) (2). The NF-κB pathway is triggered by bacterial and viral infections as well as pro-inflammatory cytokines and chemokines [e.g., tumor necrosis factor α (TNF-α), lipopolysaccharide (LPS), interleukins (IL-1, IL-6)], all of which activate the IKK complex. After activation, the IKK complex phosphorylates NF-κB-bound IκBs, thereby targeting them for proteasomal degradation. Simultaneously, the NF-κB dimers are released and free to enter the nucleus where transcriptional activation of target genes begins (Fig. 1). These target genes are mainly immunosuppresive, inflammatory and anti-apoptotic genes

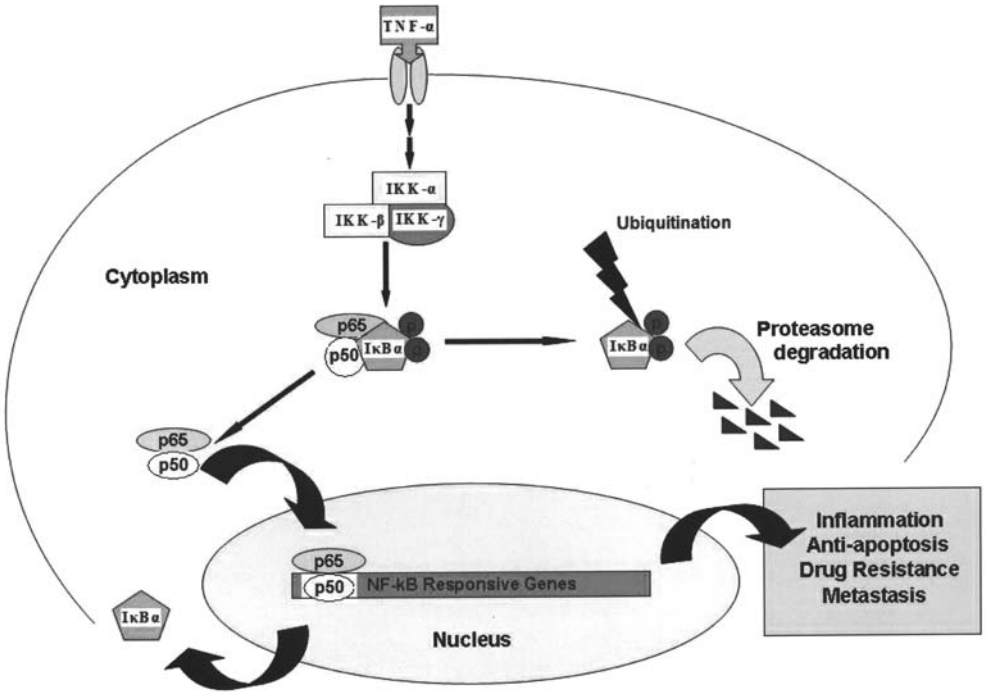

Figure 1. A model of the classical NF-κB activation pathway. TNF-α binds to its specific receptor on the cell membrane (TNFR) and induces activation of the IKK kinase complex. Subsequently, the IKKß subunit phosphorylates the IκBα inhibitory protein releasing the NF-κB dimers (p65 and p50). IκBα is ubiquitinated and degraded in the proteasome, while NF-κB is transferred into the nucleus, where the expression of target genes begins, including the genes related to inflammation, anti-apoptosis, drug resistance and metastasis. A new IκBα is synthesized and inhibits NF-κB by translocating it back to the cytoplasm. In the presence of appropriate stimuli a new cycle of NF-κB activation will start again.

(1,4). For instance, NF-κB induces the expression of genes coding for antigen receptors on immune cells, adhesion molecules, pro-inflammatory cytokines or chemoattractants for inflammatory cells (5). Moreover, the active NF-κB induces the transcription of a set of gene coding for anti-apoptotic proteins such as c-IAP-1 and -2 that directly block caspase functions or indirectly induce their ubiquitination and proteasome-dependent degradation. Another anti-apoptotic protein whose gene is under NF-κB control is c-FLIP that has a high homology with procaspase-8 but is without catalytic activity. Upon induction, c-FLIP associates with the TNF-receptor to compete with and block caspase-8 activation. In spite of its anti-apoptotic function, NF-κB is also known to promote cell death under certain conditions. Most of the pro-apoptotic effects of NF-κB are due to the induction of gene coding for the death receptor Fas or its ligand FasL. For example, NF-κB can interfere with the p53 pathway for the pro-apoptotic protein Bax. Generally, NF-κB and p53 express opposite functions although p53 can be induced by NF-κB (5).

In addition, NF-κB is associated with most processes that are believed to lead to neoplastic development, including self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of apoptosis, limitless replicative potential, tissue invasion and metastasis and sustained angiogenesis (6).

NF-κB is also known to be involved in mediating inflammation. Chronic inflammation has long been suggested as constituting a risk factor for a variety of epithelial cancers such as malignancies of the prostate, cervix, esophagus, stomach, liver, colon, pancreas and bladder. Indeed, more than 20% of all human cancers are caused by chronic infection or inflammatory states. Recent studies have highlighted the importance of the connection between NF-κB, inflammation and cancer and have underscored the value of therapies that regulate the activity of NF-κB for cancer therapy and chemoprevention in cases where chronic inflammation is the driven force for cancer initiation (7).

It is clear now that the aberrant gene expression is a fundamental cause of many diseases, including cancer. The pharmacological modulation of the intracellular signal transduction pathways and the activation of transcription factors regulating gene expression represent an attractive therapeutic approach to such disorders. Therefore, a significant part of the our research is focused on the identification of new natural compounds interfering with signal transduction pathways, the elucidation of their specific mechanism of action at the molecular level, as well as the application and evaluation of new targets for a rational development of novel therapeutics to cancer.

It is well documented that fungi produce a large and varied number of biologically-active compounds that not only stimulate the immune system but also modulate specific cellular responses by interfering with particular signal transduction pathway (8). For example, caffeic acid phenethyl ester (CAPE), which specifically inhibits DNA binding of the NF-κB and has shown some promising results in human breast cancer MCF-7 cells, was found to be produced by *Agaricus bisporus*, *Lentinus edodes* and *Phellinus linteus* (9,10). Moreover, a methanol extract of *Fomes fomentarius* was reported to inhibit the inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX) expression due to the down-regulation of the NF-κB binding activity to DNA (11).

Panepoxydone, a compound isolated from *Panus* spp. but also found in *Lentinus crinitus*, interferes with the NF-κB-mediated signal transduction by inhibiting the phosphorylation of IκBα (12). These reports demonstrate the fact that such substances can be used as molecular targets in malignant cells in order to combat certain cancers. Some of these substances are low-molecular-weight compounds that can penetrate the cell membrane. Among them there are lectins, lactones, terpenoids, alkaloids, antibiotics and metal chelating agents (13). Fungi also contain a number of enzymes such as laccase, superoxide dismutase, glucose oxidase and peroxidases. It has been shown that such an enzyme therapy can also play an important role in cancer treatment preventing oxidative stress and inhibiting cell growth (14).

Herein is the first attempt at summarizing the known data on fungal metabolites modulating the NF-κB activation pathway, which includes data on 26 fungal species known to produce bioactive substances interfering with the NF-κB function and their specific mechanisms of action and on fungal low-molecular-weight compounds that can penetrate the cell membrane and modulate NF-κB activation. Fungi also contain high-molecular-weight polysaccharides with significant biological activities. However, it is considered unlikely that such polysaccharides affect this mechanism. Furthermore, this review summarizes several known characteristics of additional fungal compounds and elaborates on their activities. This information may provide new insight into cancer therapy, demonstrating that fungi may serve as a promising natural source of anticancer therapeutics.

2. Fungal metabolites modulating NF-κB activity

Natural bio-products became popular in medicinal practice mainly because of their biological activity, which may be related to the nature of tumor-specificity and due to their novel chemical complexity. While there are examples where mushroom polysaccharides have demonstrated efficacy against specific types of cancer, such as in mono-therapy, the overwhelming successes have occurred when they were tested together with proven and accepted chemotherapeutic agents. The recent progress in fermentation, isolation and structure elucidation technologies has made research into the fungal secondary metabolism seem worthwhile. Based on these findings, an increasing amount of research on low-molecular-weight fungal substances modulating intracellular transduction pathways and, in particular, the NF-κB activation pathway has already been undertaken.

For example, the medicinal mushroom *Agrocybe aegerita* exhibits anti-tumor and immunostimulating activities due to the presence of bioactive glucans (15,16). Further studies demonstrated that a lectin, isolated from the same mushroom, exerts anti-tumor effects via apoptosis induction and the expression of DNAse activity (17). Also, COX-enzyme inhibitory and anti-oxidant activities of a fatty acid fraction, obtained from the same species, were reported (18). Data on the screening of 28 crude mushroom extracts with an NF-κB modulating activity were also published (19). The latter study described 15 mushroom extracts, which exhibited different effects on the IKBα level and these extracts were divided into four basic categories: i) extracts that inhibit the NF-κB function with a minor influence on IκBα phosphorylation and/or degradation, ii) extracts affecting IκBα phosphorylation and iii) degradation and iv) affecting both IκBα phosphorylation and degradation. Among these extracts, a crude ethyl acetate extract of *A. aegerita* culture liquid possessed a moderate inhibitory activity on both IκBα degradation and phosphorylation (Table I).

The most potent mushroom and the strongest inhibitor of IκBα phosphorylation was *Marasmius oreades*. These results were the first reported data on *M. oreades*, demonstrating its anti-NF-κB effect (19). It should be noted that previous studies on this fungus revealed the presence of agrocybin and drimane sesquiterpenes reported to induce anti-microbial and phytotoxic properties (20). In a separate study (21), it was demonstrated that an isolated lectin caused renal thrombotic microangiopathic lesions (Table I). Although *M. oreades* was distinguished as the strongest inhibitor of IκBα phosphorylation, several other mushrooms, including *Ganoderma* sp., *Pleurotus ostreatus* and *Schizophyllum commune* exhibited similar activities (19). All of them exerted considerable inhibitory effects on the phosphorylation of IκBα. Despite its effect on IκBα phosphorylation, *Ganoderma* sp. also slightly affected the degradation of IκBα.

Ganoderma is the most popular and well investigated genus among the medically active mushrooms. Many species of this genus possess anti-viral, anti-bacterial, anti-fungal, anti-cancer and immunostimulating activities and have been used in traditional Chinese medicine for thousands of years. These activities are due to the production of various metabolites such as terpenes, sterols, etc. Herein, only data on the *in vitro* and NF-κB-modulating effects of the most common species, *G. lucidum*, are included. Specifically, it was reported that an alcohol extract of this species induced G1 cell cycle arrest (22). Further investigations established that a spore extract inhibited breast and prostate cancer cell proliferation *in vitro* (23). However, only a few studies on the effect of *Ganoderma* spp. on the NF-κB activation pathway were conducted. It was reported that *G. lucidum* spores and the fruiting body (FB) extracts inhibited activator protein-1 (AP-1) and NF-κB (24,25). Recently, a *G. lucidum* organic extract was shown to interfere with the androgen receptor function, a molecular target implicated in prostate cancer development, inducing G1 arrest in androgen-dependent but not in androgen-independent prostate cancer cell lines (26).

Schizophyllum commune is another common edible and medicinal mushroom, which appears to modulate the NF-κB activity through the inhibition of IκBα phosphorylation (19). The main medicinal properties of this species are anti-tumor and immunomodulating, based on the production of the bioactive ß-glucan schizophyllan (27).

Pleurotus ostreatus, which also showed an inhibitory effect on IκBα phosphorylation, is an edible mushroom possessing biological activity related to immunoenhancement and *in vitro* anti-cancer effects (19). The first report showed anti-tumor activities of a *P. ostreatus* polysaccharide fraction (28). It was noted that the anti-tumor properties of this species are related to the production of heteroglucans (27). Another study showed that the *P. ostreatus* fruiting body extracts exhibited anti-tumor, hypocholesterol effects and hypotensive activity (29). Similarly, it was recorded that the fruiting body

Table I. Fungal species affecting the NF-κB function, including additional fungal compounds and their activities.

No.	Species	IC_{50}s[a] (μg/ml)	Effects on IκBα and phospho-IκBα[b]	Known activities	Bioactive compounds	References
1	*Agrocybe aegerita* (edible)	>125	Moderately inhibits IκBα degradation and IκBα phosphorylation	Anti-tumor	α-(1-3)-ß-glucan	15
				Anti-tumor and immunostimulating	Glucan	16
				Anti-tumor (apoptosis induction)	Lectin	17
				Inhibitor of COX and anti-oxidant activities	Fatty acid fraction, palmitic acid ergosterol, manitol, threhalose, etc.	18
2	*Cordyceps sinensis* (non-edible)	110	A strong inhibitor of IκBα degradation and a moderate inhibitor of IκBα phosphorylation	Anti-tumor	Polysaccharides, sterols, lipids, nucleosides, and deoxy-nucleosides	53
				Immunomodulating	Polysaccharides	54
				Inhibition of proliferation and differentiation of human leukemic U937 cells	Polysaccharide fraction from ethanol-precipitable aqueous extraction	55
				Anti-tumor (against various tumor cell lines, such as the K562 (CML), Jurkat (T-lymphoblastic), HL-60 (promyelocytic leukemia), WM1341 (malignant melanoma) and RPMI (multiple myeloma)	Sterols	56
				Anti-oxidant and anti-tumor activities: inhibit cell proliferation, induce apoptosis in colorectal and hepatocellular cancer	Polysaccharides (ethanolic extract), cordycepin	57
3	*Cyathus striatus* (non-edible)	<2	Dose-dependently inhibits both IκBα phosphorylation and degradation	Anti-bacterial	Striatins (antibiotics - diterpenoids)	35
				Inhibition of protein, RNA and DNA synthesis		
				Anti-bacterial and fungicidal	Striatins	20
				Anti-microbal and cytotoxic properties	Cyathins (antibiotics - diterpenes)	20, 34
4	*Fomes fomentarius* (non-edible)	>125	Strongly inhibits IκBα degradation and moderately inhibits IκBα phosphorylation	Anti-tumor	A polysaccharide fraction from culture filtrate	40
				Anti-microbal	-	41
				Anti-tumor	ß-glucan	27
				Inhibition of iNOS and COX expression due to down-regulation of NF-κB binding activity to DNA	Methanol extract	11
5	*Ganoderma* spp. (non-edible)	>125	Moderately inhibits IκBα degradation. A strong inhibitor of IκBα phosphorylation	G1 cell cycle arrest	Alcohol extract	22[c]
				Inhibits breast and prostate cancer cell proliferation *in vitro*	Spore extract	23[c]
				Inhibition of AP-1 and NF-κB activity	Spores and FB extracts	24, 25[c]
				G1 arrest in androgen-dependent prostate cancer cell lines	Organic extract	26[c]
6	*Marasmius oreades* (edible)	>125	A strong inhibitor of IκBα phosphorylation	Anti-microbal and phytotoxic properties	Agrocybin and drimane sesquiterpenes	20
				Renal thrombotic microangiopathic lesions	Lectin	21

Table I. Continued.

No.	Species	IC_{50}s[a] (μg/ml)	Effects on IκBα and phospho-IκBα[b]	Known activities	Bioactive compounds	References
7	*Phallus impudicus* (edible only immature)	>125	Strongly inhibits IκBα degradation	Anti-tumor	PI-2 glucomannan (FB and culture mycelium)	27
				Reducing the metastases in Lewis lung carcinoma	FB extract in the form of small particle spray	50
				Prevention of thromboembolic complications in cancer patients	Juice from fresh FB	51
8	*Pleurotus ostreatus* (edible)	>125	A strong inhibitor of IκBα phosphorylation	Anti-tumor	ß-glucan Heteroglucan	27
				Anti-tumor	Polysaccharide fractions	28
				Anti-tumor, hypocholesterol effects and hypotensive activity	FB extracts	29
				Cancer protective (cytostatic) and anti-inflammatory effects	FB extracts	30
				In vitro activity against rodent mammary adenocarcinoma 755	Fermentation products	31
				Suppression of aromatase activity	FB extract	32
				Inhibition of proliferation and differentiation of K562 human leukemia cells	Low-molecular-weight substances from mushroom crude extract	33
9	*Pleurotus pulmonarius* (edible)	>125	Strongly inhibits IκBα degradation and weakly inhibits IκBα phosphorylation	Anti-tumor	Protein-containing polysaccharides; p-anisaldehyde, (4-methoxyphenyl)-1.2-propandiol	37
				Hematological and cardiovascular effects	-	39
				Anti-fungal, nematocydal and cytotoxic properties	S-coriolic (linoleic) acid	20
				Anti-tumor	Xyloglucan(FB) Xylanprotein (FB)	27
				Anti-oxidant, anti-inflammatory and anti-tumor	Methanol extract of FB	38
10	*Sparassis crispa* (edible)	>125	Strongly inhibits IκBα degradation	Enhancing the hematopoietic response	ß-glucan fraction (CA1)	47
				Anti-tumor (improvement in lung, stomach, colon, breast, ovarian, uterine, prostate, pancreas and liver cancer)	ß-glucan	48
				Immunomodulating (complete response in breast cancer patients)	1.3-ß-D-glucan	49
11	*Schizophyllum commune* (edible)	40	A strong inhibitor of IκBα phosphorylation	Squalene synthetase inhibition	Schizostatin	20
				Anti-tumor and immunomodulating	ß-glucan (schizophyllan)	27
12	*Trametes gibbosa* (non-edible)	>125	A very strong inhibitor of both IκBα degradation and phosphorylation	Anti-tumor	ß-glucan (FB) Hot-water extracts (FB)	36
13	*Trametes zonata* (non-edible)	>125	Strongly inhibits IkBα degradation. Weakly inhibits IκBα phosphorylation	Inhibition of the growth, promotion of apoptosis and induction of erythroid differentiation of the K562 (CML), inhibition of the growth of the LNCaP (prostate cancer) cell line	Mycelial extract	52

Table I. Continued.

No.	Species	IC_{50}s[a] (μg/ml)	Effects on IκBα and phospho-IκBα[b]	Known activities	Bioactive compounds	References
14	*Agaricus bisporus* (edible)	-	Not included	Inhibits NF-κB binding to DNA Suppression of aromatase activity	CAPE -	9 32
15	*Agaricus brasiliensis* (edible)	-	Not included	Suppresses the activity of NF-κB and AP-1	Crude polysaccharides	42
16	*Chaetomium subspirale* (non-edible)	2.5	Not included	Inhibits the phosphorylation of ERK1/2 kinases and the activation of NF-κB	Oxaspirodion	46
17	*Cordyceps militaris* (non-edible)	-	Not included	Suppresses TNF-α gene expression, IκBα phosphorylation and nuclear translocation of p65. Decreases the expression of COX-2 and iNOS due to the down-regulation of NF-κB activation, Akt and p38 phosphorylation	Cordycepin	59
18	*Cordyceps pruinosa* (non-edible)	-	Not included	Inhibits IL-1ß, TNF-α, NO and prostaglandin E_2 in LPS-stimulated murine macrophages and primary macrophages by suppressing gene expression of IL-1ß, TNF-α, iNOS and COX-2 through NF-κB inhibition	Methanol extract	58
19	*Hericium erinaceum* (edible)	-	Not included	Enhances the activation of NF-κB by inducing the IL-1ß expression Induces macrophage activation through the activation of NF-κB	Water extract	43 44
20	*Inonotus obliquus* (non-edible)	-	Not included	Inhibits the DNA binding activity of NF-κB associated with the prevention of IκBα degradation and reduction of the nuclear p65 levels. Anti-inflammatory and anti-nociceptive activities related to the inhibition of iNOS and COX-2 expression through the down-regulation of the NF-κB binding activity	Methanol extract	60
21	*Lentinus edodes* (edible)	-	Not included	Inhibits NF-κB binding to DNA Suppression of aromatase activity	CAPE -	9 32
22	*Lentinus crinitus* (edible)	-	Not included	Interferes with the NF-κB-mediated signal by inhibiting the phosphorylation of IκBα	Panepoxydone	12
23	*Morchella esculenta* (non-edible)	-	Not included	Increases NF-kB luciferase expression in THP-1 human monocytes	Galactomannan	58
24	*Panus conchatus* (edible)	-	Not included	Panepoxydone inhibits the TNF-α- or TPA-induced phosphorylation and degradation of IκB. Cycloepoxydon also has a potent NF-κB inhibitory activity (shown in COS-1 cells)	Panepoxydone and cycloepoxydon	45
25	*Phellinus linteus* (edible)	-	Not included	Inhibits DNA binding of NF-κB Induces the maturation of dendritic cells via NF-κB, ERK and p38 MAPK signal pathways	CAPE	10 61

Table I. Continued.

No.	Species	IC_{50}s[a] (μg/ml)	Effects on IκBα and phospho-IκBα[b]	Known activities	Bioactive compounds	References
26	*Trametes versicolor* (non-edible)	-	Not included	Suppression of proliferation and increase in apoptosis in human U-937 and HL-60 leukemia cells. In HL-60, PSP decreased the levels of NF-κB, p65 and p50, associated with the inhibition of COX-2 expression. PSP increased the expression of STAT-1 but decreased the expression of ERK	Polysaccharo-peptide	62

[a]The IC_{50} values are given according to the data by Petrova *et al* (19). [b]Mushroom effects on IκBα phosphorylation and degradation are summarized and presented according to Petrova *et al* (19). [c]References presenting the activity of *Ganoderma lucidum*.

extracts of this mushroom possess cytostatic and anti-inflammatory effects (30). The *in vitro* studies on this species have a long history. Some *P. ostreatus* fermentation products were reported to exert *in vitro* activities against rodent mammary adenocarcinoma 755 (31), while a fruiting body extract suppressed aromatase activity (32). Most recently, it was noted that low-molecular-weight substances from the crude mushroom extract inhibited the proliferation and differentiation of the K562 human leukemia cells (Table I) (33).

Several bioactive metabolites with antibiotic effects were found in the fungal species *Cytahus striatus*. These are the diterpenoid derivate striatins and cyathins that exert anti-bacterial, fungicidal and cytotoxic properties (20,34,35). The inhibition effects of striatins on the protein and RNA and DNA synthesis were reported (35). Additionally, it was demonstrated that three out of four crude extracts of this species inhibited, dose-dependently and in very low concentrations, both IκBα phosphorylation and degradation (19). According to this study, *Trametes gibbosa* is a strong inhibitor of both IκBα degradation and phosphorylation. The anti-tumor effects of the fruiting body hot water extracts and a ß-glucan were previously recorded (36).

A very potent modulator of the NF-κB activity was detected in the liquid culture extraction of the edible mushroom *Pleurotus pulmonarius* which was found to significantly inhibit the degradation of IκBα (19). Although this was the first report on the NF-κB-modulating properties of the mushroom, there are many other studies showing its medicinal potential. For instance, it was shown to produce various biologically active substances such as proteins, glucans, and protein-containing polysaccharides, with anti-fungal, anti-tumor, anti-oxidant, anti-inflammatory and cytotoxic properties (20,27,37,38). Moreover, hematological and cardiovascular effects of this species were reported (39).

Another medicinal mushroom with NF-κB inactivating abilities is *Fomes fomentarius*. A methanol extract of this species inhibited iNOS and COX expression due to the down-regulation of the NF-κB binding activity to DNA (11). In an independent study, it was shown that an ethyl acetate extract of the mushroom biomass strongly inhibited the degradation of IκBα and moderately affected IκBα phosphorylation (19). However, these activities correlate with the previously reported anti-tumor and anti-microbial effects that are due mainly to the production of bioactive polysaccharides (27,40,41).

Several popular and traditionally-used medicinal mushrooms were also investigated and reported to exhibit NF-κB-modulating properties. Among them, *Agaricus bisporus*, *Lentinus edodes* and *Phellinus linteus* were shown to produce CAPE, which specifically inhibits the NF-κB binding to DNA (Fig. 2) (9,10). In addition, it was recorded that *Agaricus bisporus* and *Lentinus edodes* produce substances that suppress the aromatase activity (32). Another edible and medicinal mushroom, which was found to suppress the activity of NF-κB and AP-1, is *Agaricus brasiliensis* s. Heinem (42). A water extract of *Hericium erinaceum*, a well-known mushroom with medicinal activities, reportedly enhances the activation of NF-κB (through its pro-apoptotic function) by inducing interleukin-1ß (IL-1ß) expression (43). Son *et al* reported that the water extract also induced macrophage activation leading to the iNOS gene expression followed by nitric oxide (NO) production through the activation of NF-κB (44).

Panepoxydone and cycloepoxydon, biologically-active compounds that exert a potent NF-κB inhibitory activity, were isolated from the edible mushroom *Panus conhatus* (45). Panepoxydone inhibited the TNF-α- or TPA-induced phosphorylation and degradation of IκB. Cycloepoxydon was shown to exert a potent NF-κB inhibitory activity in COS-1 cells (Fig. 2). Panepoxydone was also found in *Lentinus crinitus* and was reported to interfere with the NF-κB-mediated signal by inhibiting the phosphorylation of IκBα (12). Another fungus, known to modulate the NF-κB activity is the ascomycete *Chaetomium subspirale*. Its metabolite oxaspirodion demonstrated an ability to inhibit the phosphorylation of ERK1/2 (extracellular signal-regulated kinases) and the activation of NF-κB (Fig. 2, Table I) (46).

Sparassis crispa was known for its anti-tumor and immunostimulating activities, based on the production of bioactive ß-glucans (47-49). Only recently has it been shown that a diethyl ether extract of this species inhibits the degradation of IκBα (19). Similarly, *Phallus impudicus* was distinguished as another inhibitor of IκBα degradation in the latter study. Although scarce data for this species were previously presented, its anti-cancer potential has been

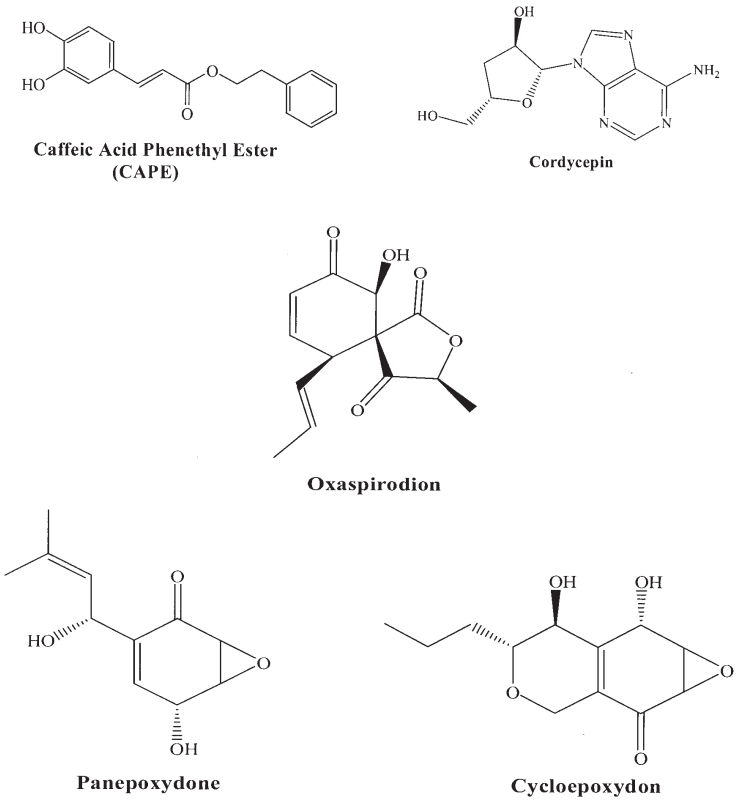

Figure 2. Fungal low-molecular-weight compounds exerting direct NF-κB inhibitory effects.

emphasized. More specifically, the anti-tumor effects include the prevention of thromboembolic complications in cancer patients and the reduction of metastases in Lewis lung carcinoma (27,50,51).

The same mode of action used in the screening study of Petrova *et al* was established for two other mushrooms, the basidiomycete *Trametes zonata* and the ascomycete *Cordyceps sinensis* (19). The crude ethyl acetate extracts of both species appeared to be strong inhibitors of IκBα degradation but were also moderate inhibitors of IκBα phosphorylation. Previously, a mycelial extract of *Trametes zonata* was shown to inhibit the growth, promote apoptosis and induce the erythroid differentiation of the K562 (erythroleukemia) cell line, as well as inhibit the growth of the LNCaP (prostate cancer) cell line (52). Regarding the activity of *C. sinensis*, it was reported to exert anti-tumor and immunomodulating properties, due to the production of polysaccharides, sterols, lipids, nucleosides and deoxynucleosides (53,54). It was also recorded that a polysaccharide fraction from an ethanol-precipitable aqueous extraction of that fungus inhibited the proliferation and differentiation of the human leukemic U937 cells (55). Further studies on this species resulted in the isolation of bioactive sterols, which exhibited anti-tumor activities against various tumor cell lines such as the K562, Jurkat (T-lymphoblastic), HL-60 (promyelocytic leukemia), WM1341 (malignant melanoma) and RPMI (multiple myeloma) (56). An ethanolic extract from this fungus as well as cordycepin, a newly-discovered bioactive compound, were reported to possess anti-oxidant and anti-tumor activities such as inhibiting cell proliferation, and inducing apoptosis in colorectal and hepatocellular cancer (57). Another study demonstrated that a methanol extract of *Cordyceps pruinosa* suppresses inflammation through the inhibition of the NF-κB-dependent inflammatory gene expression. The methanol extract inhibited IL-1ß, TNF-α, NO and prostaglandin E_2 *in vitro* and *in vivo*. The extract inhibited these inflammatory mediators in the LPS-stimulated murine macrophage cell line RAW264.7 and primary macrophages by suppressing the gene expression of IL-1ß, TNF-α, iNOS and cyclooxygenase-2 (COX-2) through NF-κB inhibition (58) (Table I). In another study (59), cordycepin was identified as a major component of a butanol fraction obtained from *Cordyceps militaris* (Fig. 2). The effect of cordycepin on the anti-inflammatory activities of the RAW264.7 macrophages was examined. Results revealed that cordycepin suppressed the TNF-α gene expression, IκBα phosphorylation and the nuclear translocation of p65. Moreover, the expression of COX-2 and iNOS were significantly decreased due to the down-regulation of the NF-κB activation, serine/therionine kinase (Akt) and p38 phosphorylation (59).

The enhancement of macrophage activation was also demonstrated by a galactomannan obtained from a polar extract of *Morchella esculenta* carpophores. The galactomannan polysaccharide increased the NF-κB luciferase expression in THP-1 human monocytes to levels of 50% of those achieved by the LPS activation (58).

A methanol extract obtained from *Inonotus obliquus* (MEIO) was studied for anti-inflammatory and anti-nociceptive properties *in vivo* and *in vitro* (60). MEIO

inhibited the DNA binding activity of NF-κB which was associated with the prevention of IκBa degradation and reduction of the nuclear p65 levels. This study indicated that the anti-inflammatory and anti-nociceptive activities of MEIO may be due to the inhibition of iNOS and the COX-2 expression through the down-regulation of the NF-κB binding activity (60).

A proteoglycan isolated from *Phellinus linteus* was investigated for its properties to interfere with the phenotypic and functional maturation of dendritic cells (61). This study revealed that the proteoglycan directly activated mitogen-activated protein kinases (MAPKs), such as ERK1/2 and p38 as well as NF-κB, thus inducing the maturation of dendritic cells via the NF-κB, ERK and p38 MAPK signal pathways (61).

A water extract of *Trametes versicolor* containing polysaccharo-peptide (PSP) was reported to induce cell cycle arrest at the G_1/S and G_2/M phases and alterations of anti-apoptotic and extracellular regulatory proteins in human the U-937 leukemia cells, resulting in the suppression of proliferation and increase in apoptosis (62). In HL-60 leukemia cells, PSP decreased the levels of the p65 and p50 forms of NF-κB associated with the inhibition of the COX-2 expression. PSP increased the expression of STAT-1 (signal transducer and activator of transcription) and decreased the expression of ERK (62).

3. Perspectives

This review describes the current knowledge on the low-molecular-weight fungal compounds, interfering with the NF-κB activation pathway. It is already clear that fungi, belonging to different ecological (parasites, saprotrophs, etc.) and taxonomic (Ascomycetes and Basidiomycetes) groups produce bioactive metabolites that not only stimulate the immune system but can also modulate specific cellular responses such as the NF-κB function. The Fungi kingdom covers about 82,000 species and many have yet to be discovered. There is no doubt that this group of organisms is one of the most diverse and holds numerous opportunities for science as well as for medical purposes.

Currently, only 26 fungal species have been found to affect the NF-κB function through different mechanisms. However, many of them still have unclear modes of action. The known data on their properties are based mainly on the activities of the crude fruit body, biomass or culture liquid extracts. Therefore, in order to investigate and establish the origin and structure of the active compounds on the one hand and their specific mechanisms of action on the other, further investigations are needed.

Fungi represent an immense source of bioactive substances with immunostimulating and anti-cancer properties that make them very potent natural supplements in cancer therapy. This fact continues to prompt molecular therapy, based on the application of low-molecular-weight compounds interfering with the intracellular signal transduction pathways and may provide a higher success in cancer treatment. Fungal metabolites can also affect such crucial cancer-related pathways as the NF-κB pathway, which seems to be a promising approach in the therapy of cancer. Thus, the activities of more and various fungal species are worth investigation in order to establish other, more potent fungal substances that can be reliable therapeutics for humans.

Acknowledgements

This research was partly supported by the Ministry of Science and Technology of Israel, Grant No. 3-998 to J.M.

References

1. Pahl H: Activators and target genes of Rel/NF-κB transcription factors. Oncogene 18: 6853-6866, 1999.
2. Zandi E and Karin M: Bridging the gap: composition, regulation and physiological function of the IκB kinase complex. Mol Cell Biol 19: 4547-4551, 1999.
3. Karin M and Ben-Neriah Y: Phosphorylation meets ubiquitination: The control of NF-[κ]B activity. Ann Rev Immunol 18: 621-663, 2000.
4. Karin M, Cao Y, Greten FR and Li ZW: NF-κB in cancer: from innocent bystander to major culprit. Nat Rev Cancer 2: 301-310, 2002.
5. Magné N, Toillon RA, Bottero V, Didelot C, Houtte PV and Gérard JP: NF-κB modulation and ionizing radiation: mechanisms and future directions for cancer treatment. Cancer Lett 231: 158-168, 2006.
6. Hanahan D and Weinberg RA: The hallmarks of cancer. Cell 100: 57-70, 2000.
7. Karin M: Nuclear factor kappaB in cancer development and progression. Nature 441: 431-436, 2006.
8. Petrova RD, Wasser SP, Mahajna JA, *et al*: Potential role of medicinal mushrooms in breast cancer treatment: current knowledge and future perspectives. Int J Med Mushr 7: 141-156, 2005.
9. Mattila P, Könkö K, Eurola M, *et al*: Contents of vitamins, mineral elements and some phenolic compounds in cultivated mushrooms. J Agric Food Chem 49: 2343-2348, 2001.
10. Nakamura T, Akiyama Y, Matsugo S, *et al*: Purification of caffeic acid as an anti-oxidant from submerged culture mycelia of *Phellinus linteus* (Berk. et Curt.) Teng (Aphyllophoromycetidae). Int J Med Mushr 5: 163-167, 2003.
11. Park YM, Kim IT, Park HJ, *et al*: Anti-inflammatory and anti-nociceptive effects of the methanol extract of *Fomes fomentarius*. Biol Pharm Bull 27: 1588-1593, 2004.
12. Erkel G, Anke T and Sterner O: Inhibition of NF-κB activation by panepoxydone. Biochem Biophys Res Commun 226: 214-221, 1996.
13. Wasser SP and Weis AL: Therapeutic effects of substances occurring in higher Basidiomycetes mushrooms: A modern perspective. Crit Rev Immunol 19: 65-96, 1999.
14. Ossowski L and Lopez MR: Proteolytic enzymes in cancer invasion. Introduction. Enzyme Prot 49: 5-6, 1996.
15. Mizuno T, Saito H, Nishitoba T and Kawagishi H: Anti-tumor-active substances from mushrooms. Food Rev Int 11: 23-61, 1995.
16. Yoshida I, Kiho T, Usui S, *et al*: Polysaccharides in fungi. XXXVII. Immunomodulating activities of carboxymethylated derivatives of linear (1-3)-alpha-d-glucans extracted from the fruiting bodies of *Agrocybe cylindracea* and *Amanita muscaria*. Biol Pharm Bull 19: 114-121, 1996.
17. Zhao CG, Sun H, Tong X and Qi YP: An anti-tumor lectin from edible mushroom *Agrocybe aegerita*. Biochem J 374: 321-327, 2003.
18. Zhang Y, Mills GL and Nair MG: Cyclooxygenase inhibitory and anti-oxidant compounds from the fruiting body of an edible mushroom, *Agrocybe aegerita*. Phytomedicine 10: 386-390, 2003.
19. Petrova RD, Mahajna J, Reznick AZ, *et al*: Fungal substances as modulators of NF-κB activation pathway. Mol Biol Rep 34: 145-154, 2007.
20. Lorenzen K and Anke T: Basidiomycetes as a source for new bioactive natural products. Curr Organic Chem 2: 329-364, 1998.
21. Warner RL, Winter HC, Speyer CL, *et al*: *Marasmius oreades* lectin induces renal thrombotic microangiopathic lesions. Exp Mol Pathol 77: 77-84, 2004.
22. Hu H, Ahn NS, Yang X, *et al*: *Ganoderma lucidum* extracts induce cell cycle arrest and apoptosis in MCF-7 human breast cancer cell. Int J Cancer 102: 250-253, 2002.

23. Lu Q-Y, Sartippour MR, Brooks MN, *et al*: *Ganoderma lucidum* spore extract inhibits endothelial and breast cancer cells *in vitro*. Oncol Rep 12: 659-662, 2004.
24. Sliva D, Labarrere C, Slivova V, *et al*: *Ganoderma lucidum* suppresses motility of highly invasive breast and prostate cancer cells. Biochem Biophys Res Commun 298: 603-612, 2002.
25. Sliva D, Sedlak M, Slivova V, *et al*: Biological activity of *Ganoderma lucidum* for the inhibition of highly invasive breast and prostate cancer cells. J Altern Compl Med 9: 491-497, 2003.
26. Zaidman BZ, Wasser SP, Nevo E and Mahajna J: *Coprinus comatus* and *Ganoderma lucidum* interfere with androgen receptor function in LNCaP prostate cancer cells. Mol Biol Rep 2007 Mar 13. DOI: 10.1007/s11033-007-9059-5.
27. Wasser SP and Weis AL: Medicinal properties of substances occurring in higher Basidiomycetes. Int J Med Mushr 1: 31-62, 1999.
28. Yoshioka Y, Ikekawa T, Noda M and Fukuoka F: Studies on anti-tumor activity of some fractions from Basidiomycetes. I. An anti-tumor acidic polysaccharide fraction of *P. ostreatus* (Fr.) Quél. Chem Pharm Bull 20: 1175-1180, 1972.
29. Buswell JA and Chang ST: Edible Mushrooms: Attributes and Applications. In: Genetics and Breeding of Edible Mushrooms. Chang ST, Buswell JA and Miles PG (eds.) Gordon and Breach, Amsterdam, pp297-324, 1993.
30. Lindequist U, Niedermeyer THJ and Julich WD: The pharmacological potential of mushrooms. eCAM 2: 285-299, 2005.
31. Jong SC and Donovick R: Anti-tumor and anti-viral substances from fungi. Adv Appl Microbiol 34: 183-261, 1989.
32. Grube BJ, Eng ET, Kao Y-C, *et al*: White button mushroom phytochemicals inhibit aromatase activity and breast cancer cell proliferation. J Nutr 131: 3288-3293, 2001.
33. Yassin M, Mahajna JA and Wasser SP: Submerged cultured mycelium extracts of higher Basidiomycetes mushrooms selectively inhibit proliferation and induce differentiation of K562 human chronic myelogenous leukemia cells. Int J Med Mushr 5: 261-276, 2003.
34. Allbutt AD, Ayer WA, Brodie HJ, *et al*: Cyathin, a new antibiotic complex produced by *Cyathus helenae*. Can J Microbiol 17: 1401-1407, 1970.
35. Anke T, Oberwinkler F, Steglich W and Höfle G: The striatins - new antibiotics from the basidiomycete *Cyathus striatus* (Huds. ex Pers.) Willd. J Antibiot 30: 221-225, 1976.
36. Ooi VEC and Liu F: Immunomodulation and anti-cancer activity of polysaccharide-protein complexes. Curr Med Chem 7: 715-729, 2000.
37. Zhuang C, Mizuno T, Shimada A, *et al*: Anti-tumor protein-containing polysaccharides from a Chinese mushroom Fengweigu or Houbitake, *Pleurotus sajor-caju* (Fr.) Sing. Biosci Biotech Biochem 57: 901-906, 1993.
38. Jose N, Aiith TA and Jananrdhan KK: Anti-oxidant, anti-inflammatory and anti-tumor activities of culinary-medicinal mushroom *Pleurotus pulmonarius* (Fr.) Quel. (Agaricomycetidae). Int J Med Mushr 4: 329-335, 2002.
39. Chang ST and Miles PG: Edible Mushrooms and their Cultivation. Boca Raton, CRC Press, FL, p345, 1989.
40. Ito H, Sugiura M and Miyazaki T: Anti-tumor polysaccharide fraction from the culture filtrate of *Fomes fomentarius*. Chem Pharm Bull 24: 2575, 1976.
41. Capasso L: 5300 years ago the Ice Man used natural laxatives and antibiotics. Lancet 352: 1864, 1998.
42. Hashimoto T, Nonaka Y, Minato K, *et al*: Suppressive effect of polysaccharides from the edible and medicinal mushrooms, *Lentinus edodes* and *Agaricus blazei*, on the expression of cytochrome P450s in mice. Biosci Biotechnol Biochem 66: 1610-1614, 2002.
43. Son C, Shin J, Cho J, *et al*: Induction of murine interleukin-1 beta expression by water-soluble components from *Hericium erinaceum*. Acta Pharmacol Sin 27: 1058-1064, 2006.
44. Son CG, Shin JW, Cho JH, Cho CK, Yun CH, Chung W and Han SH: Macrophage activation and nitric oxide production by water soluble components of *Hericium rerinaceum*. Int Immunopharmacol 6: 1363-1369, 2006.
45. Umezawa K: Inhibition of tumor growth by NF-κB inhibitors. Cancer Sci 97: 990-995, 2006.
46. Rether J, Erkel G, Anke T and Sterner O: Inhibition of inducible TNF-α expression by oxaspirodion, a novel spiro-compound from the ascomycete *Chaetomium subspirale*. Biol Chem 385: 829-834, 2004.
47. Ohno N, Harada T, Masuzawa S, *et al*: Anti-tumor activity and hematopoietic response of a ß-glucan extracted from and edible and medicinal mushroom *Sparassis crispa* Wulf. : Fr. (Aphyllophoromycetidae). Int J Med Mushr 4: 13-26, 2002.
48. Ohno N, Miura NN, Nakajima M and Yadomae T: Anti-tumor 1,3-ß-glucan from cultured fruit body of *Sparassis crispa*. Biol Pharm Bull 23: 866-872, 2000.
49. Ohno N, Nameda S, Harada T, *et al*: Immunomodulating activity of a ß-glucan preparation, SCG, extracted from a culinary-medicinal mushroom, *Sparassis crispa* Wulf. : Fr. (Aphyllophoromycetidae), and application to cancer patients. Int J Med Mushr 5: 359-368, 2003.
50. Kuznecovs S and Kuznecova G: *Phallus impudicus* in treatment of Lewis lung carcinoma. XVII International Congress of Allergology and Clinical Immunology - ICACI XVII, Sydney, p421, 2000.
51. Kuznecovs S, Jegina K, Kuznecovs I and Kuznecova G: *Phallus impudicus* in thromboembolic disease prevention in cancer. Abstracts of the 16th MASCC International Symposium Miami Beach, FL, p379, 2004.
52. Mahajna JA, Yassin M and Wasser SP: Mushroom extracts having anti-cancer activity. U.S. Patent Ser. No. 7,258,862 issued Aug 21, 2007.
53. Yoshida J, Takamura S, Yamaguchi N, *et al*: Anti-tumor activity of an extract of *Cordyceps sinensis* (Berk.) Sacc. against murine tumor cell lines. Jpn J Exp Med 59: 157-161, 1989.
54. Chen GZ, Chen GL, Sun T, *et al*: Effects of *Cordyceps sinensis* on murine T lymphocyte subsets. Chinese Med J 104: 4-8, 1991.
55. Chen YJ, Shiao MS, Lee SS and Wang SY: Effect of *Cordyceps sinensis* on the proliferation and differentiation of human leukemic U937 cells. Life Sci 60: 2349-2359, 1997.
56. Bok JW, Lermer L, Chilton J, *et al*: Anti-tumor sterols from the mycelium of *Cordyceps sinensis*. Phytochemistry 51: 891-898, 1999.
57. Wang BJ, Won SJ, Yu ZR and Su CL: Free radical scavenging and apoptotic effects of *Cordyceps sinensis* fractionated by supercritical carbon dioxide. Food Chem Toxicol 43: 543-552, 2005.
58. Lull C, Wichers HJ and Savelkoul HFG: Anti-inflammatory and immunomodulating properties of fungal metabolites. Mediators Inflamm 2: 63-80, 2005.
59. Kim HG, Shrestha B, Lim SY, Yoon DH, Chang WC, Shin DJ, Han SK, Park SM, Park JH, Park H, Sung JM, Jang Y, Chung N, Hwang KC and Kim TW: Cordycepin inhibits lipopolysaccharide-induced inflammation by the suppression of NF-κB through Akt and p38 inhibition in RAW 264.7 macrophage cells. Eur J Pharmacol 545: 192-199, 2006.
60. Park YM, Won JH, Kim YH, Choi JW, Park HJ and Lee KT: *In vivo* and *in vitro* anti-inflammatory and anti-nociceptive effects of the methanol extract of *Inonotus obliquus*. J Ethnopharmacol 101: 120-128, 2005.
61. Kim GY, Han MG, Song YS, Shin BC, Shin Y, Lee HJ, Moon DO, Lee CM, Kwak JY, Bae YS, Lee JD and Park YM: Proteoglycan isolated from *Phellinus linteus* induces toll-like receptors 2- and 4-mediated maturation of murine dendritic cells via activation of ERK, p38, and NF-κB. Biol Pharm Bull 27: 1656-1662, 2004.
62. Hsieh T, Wu P, Park S and Wu JM: Induction of cell cycle changes and modulation of apoptogenic/anti-apoptotic and extracellular signaling regulatory protein expression by water extracts of I'm-Yunity™ (PSP). BMC Compl Altern Med, 2006 Sep 11; 6:30. DOI: 10.1186/1472-6882-6-30.