Biological properties of andrographolide, an active ingredient of *Andrographis Paniculata*: a narrative review

Vaclav Vetvicka[1], Luca Vannucci[2]

[1]Department of Pathology, University of Louisville, Louisville, KY, USA; [2]Department of Immunology, Institute of Microbiology, Prague, Czech Republic

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Correspondence to: Vaclav Vetvicka. Department of Pathology, University of Louisville, Louisville, KY 40202, USA.
Email: vaclav.vetvicka@louisville.edu.

Abstract: Andrographolide is a labdane diterpenoid isolated from *Andrographis paniculata* and traditionally used in Chinese and Indian medicine. Reported effects include anti-bacterial, anti-inflammatory and anti-cancer functions. Most of the studies support the hypothesis that andrographolide supplementation stimulates immune system, so the observed effects migh in fact be secondary to the stimulation of defense reactions. As andrographolide is involved in regulation of inflammation, it is not surprising that it is also evaluated in inflammation-mediated diseases such as ulcerative colitis. Anticancer effects of the andrographolide have been tested on various cancer panels. Colon cancer, breast cancer, and head and neck carcinomas were the most investigated, followed by prostate cancer and glioblastoma. The results looked promising. However, problems with solubility and low level of active substance in natural extract leads to preparation of chemical analogs. Objective of this short review is to summarize current knowledge of the biological effects of andragrapholide. We conclude that despite documented effects and some partly characterized mechanisms of action, more research is clearly needed. At present, the doses, types of treatment and possible negative side effects are not yet established. In addition, various isolations and compound formulas have been used for treatment of various diseases, making final conclusions problematic.

Keywords: Andrographolide (AG); immunity; cancer; infection; signaling

Introduction

Andrographolide (AG) is a natural diterpenoid and represents the major biologically active ingredient of *Andrographis paniculata*. Among described effects are stimulation of the immune system (1), blocking the development of psoriasis and antibacterial and antiviral activities (2). The major biological effects are summarized in *Figure 1*. This results in its use in traditional Chinese and ayurvedic medicine. A pulmonary delivery of liposomal AG powder resulted in amelioration of bacterial pneumonia via downregulation of inflammation and inhibition of phosphorylation of the IκBα in the NF-κB pathway (3).

Molecular mechanisms of AG action are still unclear. Most of the studies reported suppression of NF-kB activation. However, others described changes in PI3K and MAPK pathways, and inhibition of NFAT activation (4).

Despite repeatedly demonstrated significant biological activity, further use of AG in pharmacology and medicine is hindered by its poor selectivity and low solubility, leading to attempts to modify its structure and formation of several analogs. Low levels of AG in various extracts has been lately improved by use of different inducers (5), but the results are not satisfactory thus far.

In this review, we focused on the most documented and most promising aspects of the use of AG in several health
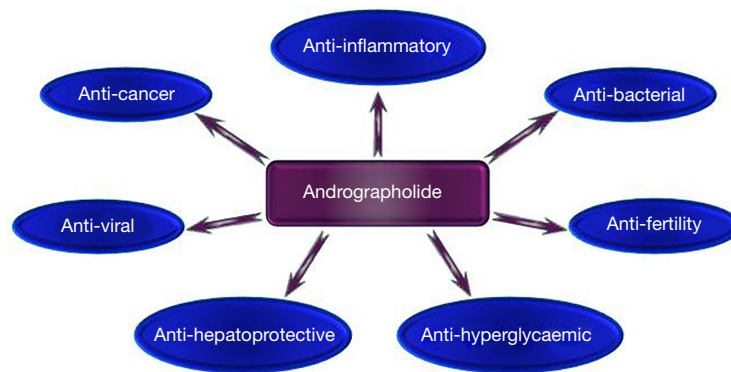
Figure 1 Major biological effects of andrographolides.

problems. We present the following article in accordance with the Narrative Review reporting checklist (available at https://dx.doi.org/10.21037/atm-20-7830).

Methods

An English language literature search utilizing the PubMed for years 2000 to 2020 was used. Collected data were based on keywords including AG, inflammation, cancer, immunity, infection and medicine.

Inflammation

AG and its derivatives have some anti-inflammatory activity. The study of possible mechanisms of AG action focused on the interaction with TLRs. The investigation confirmed that AG suppressed NF-κB activation and COX-2 expression caused by signaling via TLR3 and TLR4 by targeting TBK1 (6). The strength and distinction of these effects makes AG a promising candidate for anti-inflammatory treatment.

When tested on an experimentally induced murine colitis, this reduction of inflammation resulted in improvement of the disease and even reversed the pathology (7). The possible mechanisms of action include secretion reduction of several inflammatory cytokines including IL-6 and IFN-γ. Based on computational chemistry, AG has at least three different mechanisms of anti-inflammatory activity including inhibition of production of oxygen radicals, suppression of inducible iNOS expression and inhibition of NF-κB (8).

Most studies describing activities of *Andrographis paniculata* extracts focused on andragrapholide. However,

additional components such as dehydroxyandrographolide and neoandrographolide also exert some biological effects, most of all interactions with inflammatory processes [for review see Parichatikanond *et al.* (9)]. Direct evaluation of anti-inflammatory activities of all three types of *Andrographis paniculata* extracts revealed all three inhibit inflammation via interfering COX and inflammatory cytokines. AG has an additional effect in downregulating the expression of some genes participating in inflammatory processes (9).

As AG is involved in regulation of inflammation, it is not surprising that it is also evaluated in inflammation-mediated diseases such as ulcerative colitis. Continuous delivery of AG resulted in significant decrease of pro-inflammatory cytokines IL-1β, TNF-α, IL-6 and IL-17A. In addition, a reduction in numbers of Th17 cells in colon tissue was found. The authors concluded that AG supplementation decreased Th17 immune response, probably due to the inhibition of STAT3 signaling (10). Using a similar model of experimental colitis, Jing and co-workers confirmed palliative effects of AG, but found different mechanisms. Their *in vitro* and *in vivo* experiments showed that AG inhibited the expression of phosphorylated NF-κB, IκBα, and p65, lowered expression of COX-2 and iNOS and inhibited activation of MAPK signaling (11).

Infection

AG has been used for centuries in treatment of infections. The mechanisms are not completely known, and it might vary in different types of infections. In case of Pseudomonas aeruginosa, it seems that AG has multiple effects. Firstly, is the action oriented towards a QS system, resulting in inhibition of biofilm formation via lowering the expression
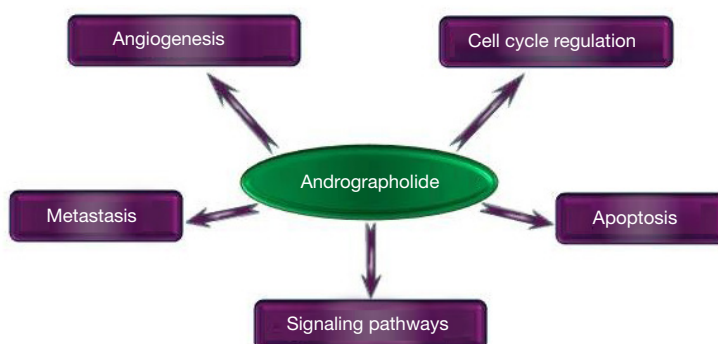
Figure 2 Suggested mechanisms of action.

of Psl polysaccharides (12). Secondary, effects are manifested by inhibition of virulence factors by production obstruction of numerous virulence factors such as pyocyanin (13). Repression of the MexAB-OprM exhaust pump by AG lowers the multidrug resistance (14). Different action has been found in Staphylococcus aureus infection. The major action of AG in protection against this type of infection is the result of regulation of immune reactions. AG can reduce inflammatory reaction via inhibition of formation of inflammatory cytokines such as IL-6 and TNF-α and by expression blockage of apoptotic proteins and NF-kB activation (15). Additional mechanisms involve suppression of the transcriptional regulator SarA, which subsequently reduces the formation of biofilm and expression of various virulent factors (16). In Escherichia coli infection, AG blocks Autoinducer-2, which is a signaling molecule involved in inter- and intra-species communication (17). In addition, AG can lower the bacterial adhesion to the cells (18). Furthermore, AG can damage the integrity of the bacteria directly, probably via damages of the cytoskeleton. For more details about AG effects and its analogs on infection agents, see recent review Zhang *et al.* (19).

An interesting study evaluated the possible effects of increased solubility of AG on its biological results. The authors found significant anti-inflammatory effects and higher phagocytosis by macrophages. When tested for anti-bacterial effects, only very limited activity was found *in vitro*, but using an *in vivo* model, significant improvements in survival have been found. The authors speculate that AG can protect against infection via stimulation of immunity (20). The quality of this study is decreased, however, by omitting the normal AG, making the conclusions about possible improvement via increased solubility impossible.

AG was also tested as a possible anti-viroticum. Using an experimental model of *in vitro* enterovirus D68 infection, AG was found to inhibit viral replication and protein synthesis. Later experiments suggested that this suppression is mediated via changes in the maturation of virus-containing endosomes, particularly by prevention of acidification of endosomes (21).

Cancer

Diterpenoids are known to possess a quite wide range of biological effects and represent an interesting category of molecules suitable even for possible oncological treatments. Anticancer effects of the diterpenoid AG have been tested on various cancer panels. Colon cancer, breast cancer, and head and neck carcinomas were the most investigated, followed by prostate cancer and glioblastoma. A systematic review of over 1,703 articles on AG and cancer found that AG has significant anticancer effects on all types of cancer tested (22). With over 15 suggested mechanisms described in these studies (*Figure 2*), the real mechanisms of action are currently almost impossible to pinpoint but, clearly, AG reflects a promising tool in our fight against cancer.

Neoplasms still represent a relevant problem in their management since their variability in response to the anticancer treatment that, in almost half of the cases, starts in the presence of an already spread disease. The necessity to improve efficacy of treatment and quality of life with interventions having low toxicity and possible enhancement of standard therapeutic effects may find an answer in the derivatives of natural products. Research on the antitumor efficacy of AG has revealed that this molecule can exert interesting pro-apoptotic and anti-inflammatory effects.

It may also be a possible enhancer of radio- and chemo-sensitivity in the treated tumors.

In a study by Lee *et al.* (23), the authors used RAW264.7 macrophages stimulated with LPS. Results showed that AG was able to inhibit the activated inflammatory signaling pathway. Decrease of iNOS and COX-2 protein levels with consequent PGE2 reduction were documented. ROS downregulation was inhibiting NF-κB and AP-1 expression. Decrease of STAT3 phosphorylation, SOCS1 and SOCS3 expression were also found. All these events affected the production of IL-1β, IL-6, and TNF-α, indicating the relevant potential of AG as an anti-inflammatory molecule. The possibility to control inflammatory processes impacting tumor establishment and progression is known from the many studies on tumor microenvironment immunology, with particular interest for head and neck carcinomas and, perhaps, glioblastoma (24-27). The tests with AG performed on these tumors appear promising for controlling the tumor growth.

Nasopharyngeal carcinoma cells, 7,12-dimethyl-1,2-benzanthracene (DMBA) induced oral squamous cell carcinoma in the hamster model of cheek buccal pouch and carcinoma of the tongue cells. This shows the AG treatment was able to inhibit NF-κB. Using a nasopharyngeal carcinoma cell line, AG was found to inhibit proliferation and induce apoptosis. The study also found changes in regulation of the LKB1/AMPK/mTOR signaling pathway, but it is not clear if this regulation really caused the changes in cancer cell proliferation (28). In the *in vivo* model, the dyterpenoid reduced the phosphorylation of p65 and IκBα with consequent block of the NF-κB aberrant activation but also exerted a negative role on angiogenesis. In the *in vitro* model, suppressive activity on NF-κB target genes EGFR, cyclin D1 and survivin. Also, genes regulating proliferation, angiogenesis and migration were negatively affected (MMP-9, ICAM-1, c-Myc, and VEGF), promoting cell apoptosis (27). Similar effects on COX-2 and angiogenesis were found in breast cancer (29) and insulinoma (by interfering with the TLR4 pathway) (30). The anti-angiogenetic properties of AG, tested in multiple models of vascular development (like chick embryo chorioallantoic membrane and yolk sac membrane angiogenesis models and HUVEC cell tube formation), were also found depending on inhibition of the miR-21-5p/TIMP3 signaling pathway (31).

Two other main effects of AG are the inhibition of cell replication and migration. When *Andrographis paniculata* extract was further fractionated into aqueous, methane and ether fractions, three were found to inhibit the proliferation of cancer cells. The only non-active fraction was the aqueous one. Simultaneously, these fractions elevated proliferation of peripheral blood lymphocytes and induced IL-2 formation (32). This suggests simultaneous anticancer and immunomodulatory activities of AG. Breast MDA-MB-231, MCF-7, and HCT-116 cancer cells demonstrated a replication arrest in G1 (33,34), same in colon cancer cells. Another study showed the arrest in G2/M for the HT-29 colon cancer cells but also revealed that, depending on the doses, the low dose treatment was producing the G1 arrest. Higher dosages were arresting the replication in G1/G0 (35). Even in the cancer cell models of liver (HepG2 and SK-Hep1) and bile duct (HuCCA-1 and RMCCA-1) the proliferation block was described as depending on the arrest in G0/G1 and G2/M phases (36). In this tumor, the treatment reduced the expression of cyclin-D1, Bcl-2 and the inactive proenzyme form of caspase-3 and increase of the pro-apoptotic protein Bax. Antiproliferative activity on colon cancer cells (SW-480) was also associated to downregulation of the Notch pathway (37). One of the proposed mechanisms of action is summarized in *Figure 3*.

Inhibition to migration was documented in the glioblastoma cell model where motility was impeded by downregulation of the ERK1/2-mediated matrix metalloproteinase-2 (MMP-2) expression (39,40). This is important as an anti-metastatic effect of AG described for Ras-transformed cells, like the glioblastoma (41). Similar anti-metastatic downregulation of MMP-2, associated also to MMP-9, was found to be induced also in gastric cancer cells (SGC7901). In these cells, the cell cycle arrest in G2/M2 or G1/M (according to the dose of AG and apoptosis consequent to increase of Timp-1/2, cyclin B1, p-Cdc2, Bax and reduction of Bcl-2) was reported (42). Apoptosis is a very common effect of the treatment, observed in many different tumor models (breast cancer, intrahepatic cholangiocarcinoma, colon HNCC, osteosarcoma glioblastoma, prostate cancer, human T-cell acute lymphoblastic leukemia Jurkat cells, bladder cancer, gastric carcinoma) (33-36,41-49). In all models, the apoptosis appears to be a consequence of ROS/JNK pathway or ERK-p53 with caspases activation, PI3K-AKT-p53 signaling pathway, endoplasmic reticulum stress associated to IRE-1 signaling or mitochondrial damage. The increase of ROS levels appears to be the most common apoptotic mechanism induced in colon cancer by the AG (35,44,46).

Another interesting aspect of AG is the capability to act as a therapeutic enhancer of radio- and chemotherapy. Oral carcinoma increased the expression of microRNA-218,
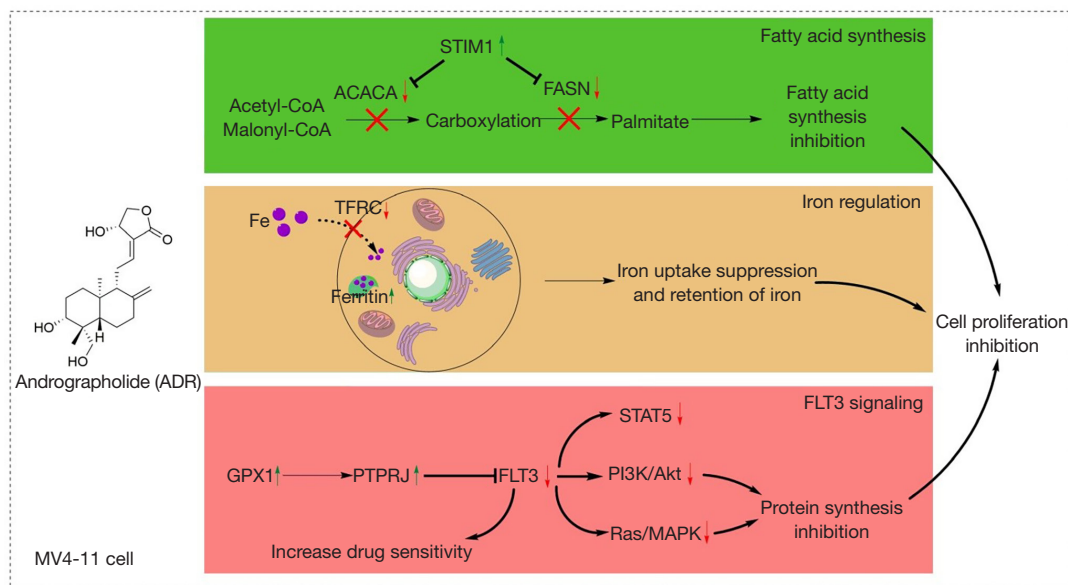
Figure 3 Proposed mechanism for the proliferation inhibitory effects of ADR in MV4-11 cells. From (38).

leading to the downregulation of Bmi1 with impediment to the cancer stemness (50). Radiosensitivity is increased also by the inhibition of ERK–mediated MMP-2 activity (51) in the model of Ras-transformed cells (e.g., glioblastoma). The pro-apoptotic activity of vincristine and carbo-platinum in HNCC, through increase of ROS, are an example of chemo-sensitization finally affecting the AKT-p53 pathway (52-54). Sensitization of colon cancer cells to 5-fluorouracil (5-FU) is mediated by reduction of the p-MET level in the combination treatment (55). Finally, two possible new applications for AG were proposed recently, the first is the preparation of nanofibrous membranes, biodegradable and locally delivering AG for the treatment of cervical cancer (56). The second is the evidence of a protective action on the gut mucosa by AG through regulation of the p38 MAPK pathway (reduction of caspase8/3, Bax and the phosphorylation of p38) (57). Therefore, while sensitizing the tumor to the 5-FU action, the bowel can produce an anti-mucositis effect on the mucosa.

Recently, AG has shown to have anti-proliferative effects in *in vitro* experiments involving induction of apoptosis by activation of pro-apoptotic Bcl2 family in cancer cells (15). Detailed research revealed AG induced mitochondrial-mediated apoptosis in several lymphoma cells with significant involvement of caspases. These effects were blocked by addition of known antioxidant N-acetyl-L-cysteine. Similar results were obtained in cases of human

colon cancer cells, where AG induced apoptosis (42). All these results have been obtained only *in vitro*, so their clinical value is limited. However, the findings of synergistic effects of an AG-5-fluorouracil combination are interesting.

Some effects of AG on cancer development might be caused by stimulation of NK (58) and ADCC cell activity, antibody-dependent complement-mediated cytotoxicity and by inhibition of secretion of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6). It was surprising that the crude extract of *Andrographis paniculata* showed stronger effects than isolated AG (59,60), suggesting the biological activity of yet unknown molecules present in the extract.

Further development of AG as a potential drug is limited to its pharmacological setbacks, leading to preparation of numerous analogs. For a review of anti-cancer effects of these analogs with novel anti-cancer effects, see Soo *et al.* (61).

Immunity

Most of the studies support the hypothesis that AG supplementation stimulates immune system. However, one group found that AG can regulate T-cell activation and down-regulate both cellular and humoral branches of immunity (62). The same group later found that interfering with NF-kB pathway by AG changed the capacity of dendritic cells to induce tolerance in mice with autoimmune encephalomyelitis (63). If confirmed, this study might open

a new window for the therapeutic use of AG for inhibiting development of autoimmune disease by elevating the antigen-specific tolerance.

Immunomodulatory activity of AG showed increased antibody response, stimulation of phagocytosis and improved cellularity in the spleen and thymus after suppression caused by application of cyclophosphamide (64). However, the quality of this study is rather low due to the use of an extract formed by three different AGs and using obsolete techniques such as plaque assay, making any significant conclusions rather difficult.

In vitro addition of AG resulted in reduction of macrophage activation and inhibition of M1/M2 polarization. Supplementation with 1 mg/kg AG caused significant suppression of antibody response. Some data also suggested possible participation of PI3K and MAPK signaling pathways (65). These immunomodulatory effects confirmed that AG modulates both natural and adaptive branch of immune reactions by affecting both macrophages and lymphocytes.

To better approach the mechanisms of action, several AG derivatives were synthesized and analyzed. The results indicated production suppression of pro-inflammatory cytokines via activation of TLR3 and TLR4 ligands. Not all derivatives produced the same effects, some inhibited nuclear translocation of NF-κB p65 subunit. On one hand, the study confirmed significant biological effects of AG on innate immunity, on the other hand demonstrated strong relation between AG structure and its function with only one out of 18 derivatives being recommended for further development (66).

Experiments focused on antibody response effects found that both *Andrographis paniculata* extract and isolated AG have same positive results on elevated antibody formation after Salmonella vaccine application. Subsequent *in vitro* experiments showed elevated production of INF-γ by spleen cells from vaccinated animals, showing that both humoral and cellular response has been raised (67).

Other effects

Numerous experiments demonstrated that AG crosses the blood-brain barrier and moves into different regions of the brain. This discovery led to subsequent studies of the effects on the CNS. Double-blind clinical trials of long-term supplementation with AG, in patients with non-active progressive multiple sclerosis, found potential positive effects on brain atrophy and disease progression (68),

but larger studies are needed to confirm these findings. An interesting approach showed new avenues for treatment development of neurological disorders including memory impairment. *In vitro* experiments revealed AG inhibited experimentally induced overexpression of COX-2, NF-κB, TLR4, and iNOS. In addition, release of inflammatory mediators was inhibited both *in vitro* in primary glial cell cultures and *in vivo* in mouse prefrontal cortex. Further experiments suggested improvements of memory and neuron protection via genomic changes and neuroinflammation suppression (69).

In an animal model, AG was found to improve middle cerebral artery occlusion and brain infarct (70). Among suggested mechanisms, the most promising seems to be the results on endothelial cells and improvement of brain homeostasis [for review see Lu *et al*. (71)]. The other effects are more speculative and involve effects of AG on platelets resulting in improvements of blood supply to the brain. AG supplementation was also found in Parkinson disease, Alzheimer disease and intracerebral hemorrhage, but the action mechanisms are unknown (71). However, the outcomes of AG on CNS are strong enough that they resulted in several currently running clinical trials, often focused on severe anti-depressant-like activities.

AG was found to have hypoglycemic effects in mouse model of diabetes type 1, probably via inhibition of NF-κB pathway (72). A different model of experimental diabetes induced by streptozotocin also found lowering of plasma glucose levels by AG supplementation (73). Subsequent studies suggested that 4-week oral supplementation with AG manifested preventive effects on the development and progression of diabetes. Based on production changes of several cytokines (such as IL-2, IL-10 and IFN-γ) and changes in expression of some mRNA (such as T-bet and GATA3), the authors concluded these preventive effects are mediated via maintaining of Th1/Th2/Th17 homeostasis (74) resulting in protection of β cells. A comprehensive review of possible effects of AG on metabolic treatment suggested promising opportunities, but warned that our recent knowledge is not yet adequate (22).

Another possible role of AG involves protection against lung injury. Using an ovalbumin-induced lung injury model, Peng *et al*., showed that AG application significantly reduced lung injury by inhibition of ROS-mediated NF-κB signaling and activation suppression of NLRP3 inflammasome (75). If confirmed, AG might be developed into a new way to treat asthma. A sulfonated version, AG-S, has been shown to have even stronger effects. In addition,

it is fully soluble and has been widely used for various treatments. A detailed proteomic study showed that AG-S inhibits lung injury via anti-inflammatory mechanisms including suppression of myeloperoxidase and neutrophil-derived proteases (76).

Due to its anti-inflammatory effects, AG was also evaluated as a possible treatment in rheumatoid arthritis. Several studies found significant effects in reduction of serological parameters of the disease and in relief of various symptoms [for review see Hidalgo *et al.* (77)] via reduction of the activation of various transcriptional factors such as AP-1, NFAT, STAT3, and NF-κB.

Much less studied are additional biological effects of AG. Intraperitoneal application of AG into mice, with experimentally induced diabetic retinopathy, showed significant amelioration of this disease by attenuation of retinal inflammation and angiogenesis. In addition, changes in VEGF, NF-κB, and Erg1 signaling pathways were found to be involved (78).

For a summary of additional potential effects, including anti-malaria activity, anti-allergy reactions, cardiovascular protection, hepatic protection, and suppression of skin diseases, see an excellent overview by Dai *et al.* (79).

Conclusions

AG is an extract of *Andrographis paniculata*, traditional Chinese medicinal plant, with various significant biological effects. Among the most pronounced activities are antibacterial and antitumor effects. Significant stimulation and regulation of the immune reactions represent another well-documented biological activity of AG. Based on the recent findings, AG and particularly its analogs, offer a new window for further pharmacological investigations leading to improve our ways of treatment of numerous diseases including cancer and infections. However, more clinical and experimental research is necessary to fully establish the doses, types of treatment and possible negative side effects. In addition, various isolations and compound formulas have been used for treatment of various diseases, making final conclusions problematic. Further research on this molecule to assess its full potential to become a drug is needed.

Acknowledgments

Footnote

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