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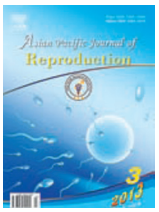

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Pharmacological and analytical aspects of withaferin A: A concise report of current scientific literature

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ABSTRACT

Withaferin A is an important phytoconstituents of *Withania somnifera* (*W. Somnifera*) belonging to the category of withanolides, that are a group of naturally occurring C_{28}-steroidal lactone triterpenoids. Withaferin A has been used in the traditional and indigenous system of medicine for the treatment of various disorders. In view of its unique therapeutic potential, it has gained much attention in the modern science. In the couple of the years, Withaferin A has been scientifically validated for different pharmacological activities including anti-cancer, adaptogenic, anti-stress, anti-convulsant, immunomodulatory, neurological, anti-inflammatory, anti-tumor, cardioprotective, and neuroprotective activities. Pharmacological and analytical aspects of Withaferin A were highlighted in the present article. From the literature review it was found that Withaferin A has a very impressive pharmacological profile especially against cancer and could be useful for the development of the new drug in the future for the treatment of cancer and other metabolic disorders.

1. Introduction

Plants produce different kind of phytoconstituents during their development and in response to various environmental stimuli. Different enzymatic pathways produce secondary metabolites that alter by growth conditions such as climates, locations, microenvironments, *etc*. and different stresses (Physical and chemical stimuli). These changes cause a variation of the active phytochemicals present in the plant *i.e*. alkaloids, terpenoids, and phenylpropanoids *etc*. [1]. More than 12 alkaloids, 40 withanolides, and several sitoindosides have been isolated and reported from aerial parts, roots and berries of *Withania* species. Withaferin A was the first member of withanolide group to be isolated from the South-Asian medicinal plant, *Withania somnifera* (*W. Somnifera*) . Lavie′s [2]. Withanolides are groups of secondary metabolites that contain a steroid backbone attached with lactone or one of its derivatives. Withanolides are mainly found in the Solanaceae group of plants such as *W. somnifera*. Withaferin A (Figure 1) is present in the roots and leaves of *W. somnifera* also known as Ashwagandha in Ayurvedic medicine and chemically classified in the steroidal compound (highly oxygenated $C-_{28}$ steroid derivatives). Withaferin A can be easily extracted from roots and leaves of *W. somnifera* using different chromatographic techniques [3]. Use of withaferin A in the ancient times against various ailments has been reported in the literature. It showed antitumor, antiangiogenic and radiosensitizing activities. The anticancer activity of withaferin A have been investigated in prostate, breast and pancreatic cancers [4, 5]. Withaferin A is found to be a natural proteasome inhibitor, it induces actin microfilament aggregation and apoptosis by inhibiting Topoisomerase-IDNA complex. It acts as a mitotic poison (arrest tumor cells at metaphase), inhibits the umbilical vein endothelial cell sprouting, and promotes the formation of dentrities. Withaferin A showed antimitotic properties, suppress adjuvant arthritis in rats, activate the peritoneal macrophages, increase the activity of lysosomal enzymes secreted by macrophages and exerts immunosuppressant activities. It also showed antimicrobial activities against both gram positive and gram negative bacteria [4].

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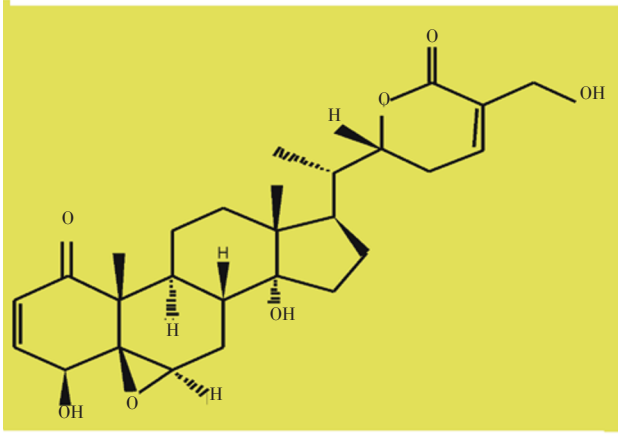

Figure 1. Chemical structure and overview of withaferin A.

2. Overview of withanolides

Withanolides are a group of naturally occurring C_{28} steroidal lactones characterized by the presence of an ergostan skeleton and frequently a delta lactone in C-17 [6, 7]. Withanolides are produced mainly by the Solanaceae family, in particular by the genera *Withania*, *Physalis*, *Datura*, *Nicandra*, *Dunalia*, *Lycium*, *Tubocapsicum* and *Jaborosa*. It has also been reported in Lamiaceae, Taccaceae and Fabaceae families and in some marine organisms. Withanolides display significant pharmacological activities including antimicrobial, antitumor, anti-inflammatory, hepatoprotective, immunomodulatory, antibacterial, insect-antifeedant, insect-repellent, antistress and immunosuppressive activities [6, 8]. Ecologically, the withanolides exhibit activity as feeding deterrents, as insecticides and ecdysteroid antagonists, and appear to be significant as a part of the chemical defense armamentarium of solanaceous plants [9].

3. Pharmacological significance of withaferin A

Withaferin A, a steroidal lactone purified from the Indian medicinal plant *W. somnifera* has attracted the attention of chemists as well as biologists due to its interesting structure and anti-cancer, adaptogenic, anti-stress, anti-convulsant, anti-inflamatory, cardioprotective, immunomodulatory and neurological effects. Withaferin A potently inhibits NF-κB activation by preventing the TNF-induced activation of IκB kinase β via a thioalkylation-sensitive redox mechanism. Withaferin A induces apoptosis through reactive oxygen species (ROS) generation, Par-4 induction and p38 MAP kinase activation [2, 10, 11]. Withaferin A exerts anticancer effect on prostate, colon, breast, leukemia, pancreatic, renal, head and neck cancer cells of human. It activates p38 MAP kinase, inhibits the Notch signalling pathway and nuclear factor-lB activation, induces Akt inactivation, death receptor 5 (DR5) up-regulation and down-regulation of c-FLIP [12]. The anticancer activity of withaferin A has been demonstrated in prostate cancer cells, breast cancer cells, leukemia cells, and melanoma cells. Withaferin A induces apoptosis in prostate cancer cells through Par-4 induction, inhibits the chymotrypsin- like activity of proteasome, and targets the intermediate filament protein vimentin by covalently modifying the cysteine residue. In addition, Withaferin A also targets Annexin II to induce Actin microfilament aggregation [13]. Anti-proliferative effect by G2/M phase cell cycle arrest, inhibition of cell adhesion molecules by inactivation of Akt and NF-B, and inhibition of colon cancer survival by down-regulating Notch, and induction of apoptosis by reactive oxygen species (ROS) generation has been reported for withaferin A. In addition, it also inhibits cell migration/invasion through down-regulation of STAT3 activity and induces apoptosis in human breast cancer cells [14].

Withaferin A alters cytoskeletal architecture by covalently binding annexin II, exerts antitumor activity by inhibiting proteasomal chymotrypsin-like activity, and induces apoptosis throughthe inhibition of protein kinase C [15]. Withaferin A triggered apoptosis through the modification of gene expression, mitochondrial depolarization, induction of oxidative stress or triggering of endoplasmic reticulum stress[16]. Withaferin A induced early reactive oxygen species (ROS) generation that caused the failure of nuclear factor-κB (NF-κB) binding to DNA as well as nuclear cleavage by activated caspase-3, leading to apoptosis. Withaferin A was also shown to suppress NF-κB activation and its regulated genes' expression, including COX-2, intercellular adhesion molecule, inhibitor of apoptosis protein 1, and cellular FADD-like IL-1 β -converting enzyme inhibitory protein (c-FLIP), in cancer cells [17].

4. Pharmacological activities of withaferin A

The effect of withaferin A treatment on pancreatic islets cell viability was examined using the fluorescein diacetate-propidium iodide dye exclusion test and glucose stimulation assay. Withaferin A was found to be not toxic to the islet cells and inhibits the inflammatory response of islet cells to cytokine exposure [18]. The effects of withaferin A on MCF-7 viability and proliferation were evaluated by 3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay and trypan blue exclusion assays. Withaferin A was found to show growth inhibition and decreased viability in MCF-7 cells [19]. Treatment of TDP-43(TAR DNA-binding protein 43) mice with withaferin A, an inhibitor of NF-κB activity reduced denervation in the neuromuscular junction and disease symptoms [20]. In another study effect of withaferin A on Heat Shock Factor 1 (HSF1)-dependent stress response was evaluated and found to be active [21]. The effect of withaferin A on corneal angiogenesis and retinal gliosis was investigated. Withaferin A treatment potently inhibited corneal neovascularization and downregulated the expression of soluble and filamentous GFAP [22]. Effects of pure withaferin A on the isolated skin melanophores of frog was investigated. Withaferin A induced powerful dose-dependent physiologically significant melanin dispersal effects in the isolated skin melanophores, which were completely blocked by atropine and hyoscine [23]. Withaferin A inhibited lipopolysaccharide (LPS)-induced cyclooxygenase (COX)-2 mRNA and protein expression and prostaglandin E2 (PGE (2)) production in BV2 murine microglial cells. These results suggest that withaferin A inhibits LPS-induced PGE (2) production and COX-2 expression [24]. In computational analysis, the large value

of binding energy involved in binding of withaferin A to the active Hsp (Heat shock proteins) 90/Cdc37 complex consolidates the thermodynamic stability of the binding [25].

The effect of withaferin A in NF-κB signalling pathway modulating capability was investigated and found that the trajectories of the native protein and the protein complexes with withaferin A are stable over a considerably long time period [26]. Proteasome inhibition capability of withaferin A was investigated and found that withaferin A can inhibit the mammalian proteasomes irreversibly and with a high rate through acylation of the N-terminal Thr1 of the β-5 subunit [27]. The effect of withaferin A on inhibition of Hsp90/Cdc37 chaperone/co-chaperone association complex was investigated. Molecular docking studies reveal that withaferin A in combination with 17-DMAG can act as potent chaperone system inhibitors [28]. In another study it was found that withaferin A markedly inhibit *Escherichia coli* induced polymorphonuclear neutrophil (PMN) transmigration, a dual inhibitor of vimentin and proteasome [29]. Gliosis is a biological process that occurs during injury repair in the central nervous system and is characterized by the overexpression of the intermediate filaments glial fibrillary acidic protein and vimentin. Withaferin A is a novel chemical probe of glial fibrillary acidic protein [30]. Oral administration of withaferin A to 7,12-dimethylbenz(a) anthracene (DMBA)-treated animals significantly reduced the formation of tumors and synchronized the status of lipid peroxidation and antioxidants potential [31]. Growth inhibition and differentiation potential of the withaferin A, on glioma (C6 and YKG1) cell lines was investigated and found that withaferin A markedly inhibited the proliferation of glioma cells in a dose-dependent manner [32]. The effect of withaferin A in *in vitro* model of CF-related inflammation was also investigated [33]. Cotreatment with subtoxic doses of withaferin A and tumor necrosis factor-related apoptosis-inducing ligand was investigated and found that withaferin A induces apoptosis in human renal cancer cells and Caki cells [17].

The role of withaferin A on airway inflammation and its mechanism was investigated and found that withaferin A inhibit the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in human lung epithelial A549 cells [10]. The effect of withaferin A on the development and decay of thermotolerance in B16F1 melanoma was studied in C57BL mice. Withaferin A increases the tumor response during repeated hyperthermia by reducing the magnitude of thermotolerance developed and by decreasing the recovery time from thermotolerance [34]. The effect of withaferin A on monosodium urate crystal-induced inflammation in mice was investigated. Paw volume, the levels of lysosomal enzymes, lipid peroxidation, and inflammatory mediator tumour necrosis factor-alpha were found to be reversed in the withaferin A treated group as compared to control group [35]. Withaferin A induces apoptosis in association with the activation of caspase-3. Results indicated that the JNK and Akt pathways and inhibition of NF-kappaB activity were key regulators of apoptosis in response to withaferin A in human leukemia U937 cells [36]. Withaferin A caused increased levels of Bax in response to MAPK signaling, which resulted in the initiation of a mitochondrial death cascade. It can be used as new, alternative, inexpensive chemotherapeutic agent for the treatment of leukemia of both lymphoid and myeloid origin [37]. Administration of withaferin A with human filarial parasite *Brugia malayi*, offers differential protection in Mastomys coucha with chemotype 101R offering better protection as compared to other chemotypes [38].

The effect of withaferin A in inhibiting viral DNA polymerase was investigated and found to bind very similarly to that of the previously reported 4-oxo-DHQ inhibitor [39]. The effects of withaferin A was examined in CaOV3 and SKOV3 ovarian carcinoma cell lines using the MTS assay, clonogenic assay, annexin V/propidium iodide flow cytometry and cell cycle analysis. Withaferin A inhibits CaOV3 and SKOV3 ovarian carcinoma cell growth [40]. Withaferin A was evaluated for antitumor activity in pancreatic cancer cells with confirmatory RT-PCR and Western blotting assay. Withaferin A analog showed dose and time dependent apoptosis and inhibits proliferation [41]. Withaferin A enhances the ionizing radiation (IR)-induced apoptosis in human lymphoma U937 cells which is associated with the PARP cleavage, caspase-3 activation, as well as specifically down-regulation of anti-apoptotic protein Bcl-2 [30]. Effects of withaferin A on human cervical cancer cells *in vitro* and *in vivo* were investigated. Withaferin A potently inhibited proliferation of the cervical cancer cells, and was found to downregulate expression of HPV E6 and E7 oncoproteins [4]. Withaferin A-induced apoptosis is mediated by reactive oxygen species (ROS) production which is mainly due to inhibition of mitochondrial respiration [43]. Imaging of breast cancer cell lines revealed that withaferin A induces perinuclear vimentin accumulation followed by rapid vimentin depolymerization revealed its potent breast cancer anti-metastatic agent [44]. Withaferin A, an anti-estrogen and the proapoptotic effect is partially attenuated by p53 knockdown and E2-ER-α [45]. Withaferin A induced a dose-dependent apoptotic cell death in several types of human cancer cells, as measured by FACS analysis and PARP cleavage [46]. The effect of Withaferin A on radiation-induced apoptosis in human renal cancer cells (Caki cells) were investigated and found that compared with withaferin A or radiation alone, the combination of both resulted in a significant enhancement of apoptosis [14]. Fifteen gene-targets were identified and were investigated for their role in specific cancer cell killing activity of withaferin A by undertaking the shRNA-mediated gene silencing approach [47].

The anticancer potential of withaferin A tested against the head and neck squamous cell carcinoma (HNSCC) cell lines, MDA1986, JMAR, UM-SCC-2, and JHU011and found that it had antiproliferative activity against head and neck squamous carcinoma [48]. Withaferin A inhibits constitutive as well as interleukin-6 (IL-6)-inducible activation of signal transducer and activator of transcription 3 (STAT3), which is an oncogenic transcription factor activated in many human malignancies including breast cancer [49]. The tumor sensitizing effect of withaferin A with or without local hyperthermia on the response of B16F1 melanoma to fractionated and acute radiotherapy was investigated. Withaferin A is a better radiosensitizer than HT in fractionated regimen and the response of radioresistant tumors like melanoma [50]. Withaferin-A elicited marked apoptosis and vimentin cleavage in vimentin-expressing tumor cells but significantly less in normal mesenchymal cells. Moreover, withaferin-A significantly blocked soft tissue sarcoma

growth, local recurrence, and metastasis in a panel of soft tissue sarcoma xenograft experiments [51]. Withaferin A is a bioactive compound which inhibits Notch-1 signaling and downregulates prosurvival pathways, such as Akt/NF-kappaB/Bcl-2 in three colon cancer cell lines (HCT-116, SW-480, and SW-620) [5]. Efficacy and the mechanism of Hsp90 inhibition of withaferin A in pancreatic cancer *in vitro* and *in vivo* were investigated. Withaferin A binds Hsp90, inhibits Hsp90 chaperone activity through an ATP-independent mechanism [13]. Withaferin A was found to repress IL-6 gene transcription in metastatic breast cancer cells upon dual inhibition of NF-kappaB and AP-1 Fra-1 transcription factors and silencing of IL-6 promoter chromatin accessibility [52]. The protective effect of withaferin-A in 7,12-dimethylbenz[a]anthracene (DMBA) induced oral carcinogenesis in Syrian golden hamsters was investigated. Oral administration of withaferin-A for 14 weeks completely prevented the tumour incidence, tumour volume and tumour burden [53]. The protective effect of withaferin-A on molecular pathogenesis of oral cancer by evaluating the immunoexpression of p53 and bcl-2 proteins were investigated. Withaferin-A has significant protective role against 7,12-dimethylbenz(a)anthracene (DMBA) induced molecular alterations in the buccal mucosa of golden Syrian hamsters [54]. Withaferin A treatment causes G2 and mitotic arrest in human breast cancer cells [55].

5. Analytical aspects of withaferin A

Withaferin A was isolated from *W. somnifera* (Solanaceae). All structures were elucidated on the basis of spectroscopic methods (IR, HRESIMS, 1D/2D NMR) and X-ray crystallography [56]. A rapid and sensitive high-performance liquid chromatography method for determination of withaferin A in human plasma was developed using reverse-phase C_{18} column, water and acetonitrile as a mobile phase with UV-visible detection at 225 nm [57]. Withaferin A was isolated from *Ajuga bracteosa* Wall using ethanolic extract by silica gel column chromatography [58]. Metabolic characterization of *W. somnifera* leaves, stems, and roots collected in six different regions in India was performed using ^{1}H NMR spectroscopy followed by principal component analysis and hierarchical clustering analysis using withaferin A as a marker compound [59]. Phytochemical studies on the aerial parts of *W. somnifera* L. Dunal. led to the isolation of withaferin A with some other compounds [60]. In another study, withaferin A was extracted using a microwave-assisted extraction technique from the air-dried aerial part of *W. somnifera* Duna [61].

The hydroalcoholic fraction of *Withania coagulans* Dunal was standardized by withaferin A using high performance thin layer chromatography [62]. In another study *W. somnifera* extract was analyzed by liquid chromatography-serial mass spectrometry and found to be present numerous withanolide derivatives including withaferin A [63]. Modified and validated HPLC-DAD method for quantitative measurement of withanolides including withaferin A and fingerprint analysis was developed and validated [64]. Reverse-phase preparative HPLC analysis of the n-butanol fraction of *W. somnifera* showed the presence of a novel chlorinated withanolide namely withanolide Z and withaferin A [65]. A sensitive, selective, specific, robust, and validated densitometric high-performance thin-layer chromatographic method for the simultaneous determination of withaferin A in *W. somnifera* using dichloromethane-methanol-acetone-diethyl ether as the mobile phase was developed [66].

6. Conclusion

Medicinal plants were used for the treatment of different type of diseases in several ways in the very early age [67]. The plant contains different type of phytoconstituents which showed different pharmacological activities. Treatment of various health ailments by plant material has been in practice from time immemorial [68]. More than 80% of the World's population in 2001 used herbal medicine for their primary health care [69]. Before the development of the synthetic drug natural products were used as alternative therapy for the treatment of various diseases [70, 71]. Herbal medicines are used for treatment of various ailments in the world due to the belief of its fewer side effects. Many synthetic and natural compounds have been obtained from different natural sources such as plants, minerals and organic matter. Natural materials are an emerging field of food science because of their increasing popularity with consumers concerned about health. To maintain proper growth, the pharmaceutical industries need to innovate and access to high output rate on low-cost materials with reasonable safety. The combination of modern chemistry with bio-based starting materials can be the scope of revolutionizing pharmaceutical industries. Withaferin A is found in many plants and have an important place in the different system of medicine due to its unique property and pharmacological activities. Many investigations have been carried out to explore the hidden potential of withaferin A and result showed that it has significant anticancer and other pharmacological activities. The information presented in this review will be helpful to the researcher to explore the hidden potential of withaferin A.

Declare of interest statement

The authors report no conflict of interest.

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