Journal of Medicinal and Aromatic Plant Sciences 45(3-4), 2023, 108-122

REVIEW ARTICLE

Review on chemistry, pharmacology, toxicity and future prospectives of withaferin A

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Article History

Received: Dec 07, 2023
Revised: Dec 18, 2023
Accepted: Dec 19, 2023

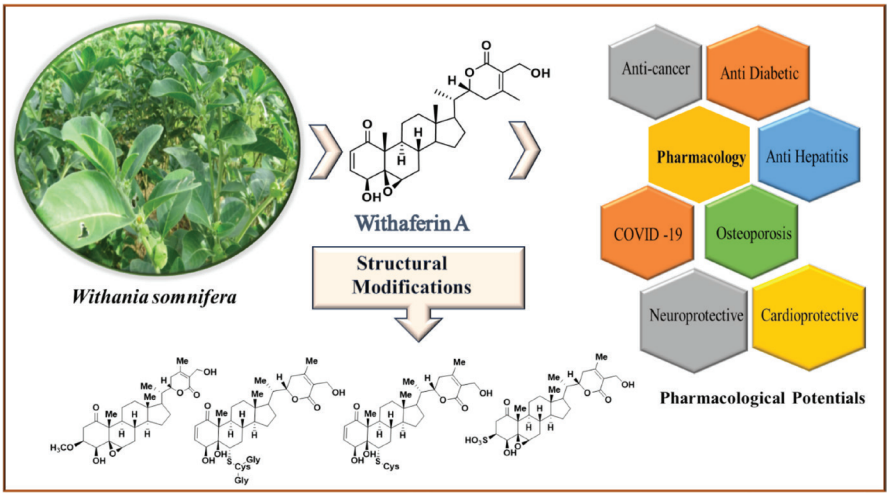
Key Words

Biosynthesis
Pharmacology
Toxicity
Withaferin A
Withania somnifera

ABSTRACT

The medicinal plant Withania somnifera is widely researched for its anti-inflammatory, cardioprotective, and anticancer effects. W. somnifera preparations are specifically used to treat menstruation issues and arthritis in Ayurveda, the main traditional Indian medical system. The bioactive substance withaferin A (WA), a steroidal lactone, was first isolated from the leaves of W. somnifera. Numerous studies showed that WA contains various pharmacological properties, making it a good choice for treating various diseases. Its structural modification targets several signal transduction pathways and improves the effectiveness of treatment for a range of illnesses. We comprehensively reviewed withaferin A and its pharmacological activities against various diseases. Among the most well-studied effects of withaferin A are anticancer properties, including breast cancer, ovarian cancer, prostate cancer, lung cancer, colorectal cancer, multiple myeloma, neuroblastoma, leukaemia, and glioblastoma, where WA have shown the potential anticancer effect. The studies demonstrated that WA inhibits the growth and metastasis of several cancer cell lines. The current review thoroughly analyses the pharmacokinetics, biological activities, and changes of several disease targets linked to WA.

Graphical Abstract



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Institutional Communication No. CIMAP/PUB/2023/180
Doi: https://doi.org/10.62029/jmaps.v45i3.shukla

ABBREVIATIONS

Alzheimer's disease (AD); Alpha-synuclein (α-Syn); Cancer stem cells (CSCs); Colorectal cancer (CRC); Cycloartenol synthase (CAS); 1-Deoxy-D-xylulose-5-phosphate reductase (DXR); 1-deoxy-D-xylulose-5-phosphate synthase (DXS); Deoxyglucose pathway (DOXP); Double-strand break (DSB); Epithelial-mesenchymal transition (EMT); Farnesyl diphosphate synthase (FPPS); Heme oxygenase 1; Homo vanillic acid (HVA); 6-Hydroxydopamine (6-OHDA); Second gap phase/ mitosis (G2/M phase); Insulin receptor substrate 1 (IRS-1); Interleukin 6 (IL-6); c-Jun-N-terminal kinase (JNK); Lactate dehydrogenase (LDH); Leucine-rich repeat kinase 2 (LRRK2) ; Michigan Cancer Foundation-7 (MCF-7); Mevalonate (MVA); 2-Methyl-D-erythritol-4-phosphate (MEP); NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) ; Non-alcoholic steatohepatitis (NASH); Neurogenic locus notch homolog protein 3 (Notch 3); Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB); Nuclear factor erythroid 2-related factor 2 (NRF2); NAD(P)H quinone oxidoreductase 1 (NQO1); Osteoprotegerin (OPG); Proliferating cell nuclear antigen (PCNA); Phosphatidylinositol-3 kinase (PI3K); Parkinson's disease (PD); Pharmacokinetics (PK); Protein kinase B (PKB)- Akt; Phosphatase and tension homolog (PTEN); Reactive oxygen species (ROS); Receptor activator of nuclear factor Kappa-B ligand (RANKL); Signal Transducer and Activator of Transcription 3 (STAT3); Squalene synthase (SQS)/SS; Squalene epoxidase (SE); Tartrate-resistant acid phosphatase (TRAP); Type 1 diabetes mellitus (T1DM); Withaferin A (WA).

INTRODUCTION

Ayurvedic therapy is one of the oldest and still-practiced approaches to herbal medicine. More recently, Western health sciences have become interested in bioactive compounds' use and mode of action in medicinal plants. One of these bioactive compounds is Withaferin A (WA), a steroidal lactone first isolated and purified by Israeli chemists Asher Lavie and David Yarden in 1962 from the leaves of the Indian medicinal plant *Withania somnifera* Dunal, also known by its Sanskrit name ''Ashwagandha'' or Indian ginseng or Indian winter cherry (Fig. 1 Withaferin A). It belongs to the Solanaceae family and is widely distributed in the Mediterranean, North Africa, the Middle East, and India. The key ingredient in Southeast Asian Ayurvedic medicine is *W. somnifera* Dunal roots, used for life-extending properties. It has long been used to treat various diseases, including rheumatism, chronic tiredness, and dehydration. The health benefits of ashwagandha also include the treatment of inflammation and immunological regulation, anxiety reduction, and arthritic pain attenuation. In the *W. somnifera* plant, several phytocompounds, including tannin, alkaloids, flavonoids, and steroidal lactones (withanolides), have been reported.

The biological activities of *W. somnifera* extracts have been thoroughly investigated, and several analytical techniques have been revealed to investigate the content of the compounds present in the *W. somnifera* plant (Berghe *et al*., 2012).

Withaferin A was first isolated withanolide with the unsaturated lactone in the side chain, allylic primary alcohol, and highly oxygenated rings A and B (Fuska *et al*., 1984). The cytotoxicity of WA is due to the presence of α, β-unsaturated carbonyl moiety, and 5,6-epoxide ring in the skeleton (Santagata *et al*., 2012). In addition, it also possesses a broad range of activities, such as anti-inflammatory, tumor-preventive, cytotoxic, radio-sensitizing, antiangiogenic, and Covid-19 infection. Various biological studies have shown that the mechanism of action of WA includes the acylation or alkylation of significant macromolecules or the covalent attachment to enzyme activities (Moncrief and Heller, 1967). The current review is an extensive survey of WA chemistry, biosynthesis, toxicity studies, potential pharmacological activities, structural modifications, pharmacokinetics, WA formulations, and prospects in natural product research to the withanolides.

Structure of Withaferin A

Withaferin A is a group of naturally occurring C28-steroidal lactone and an arrangement of four cycloalkane ring structures in which three cyclohexane rings and one cyclopentane ring are joined to each other to form a specific pattern in steroids. The lactone part is a cyclic ester, which, in the case of WA, is characterized by a closed ring consisting of 5 carbon atoms and a single oxygen atom (Fig. 1).

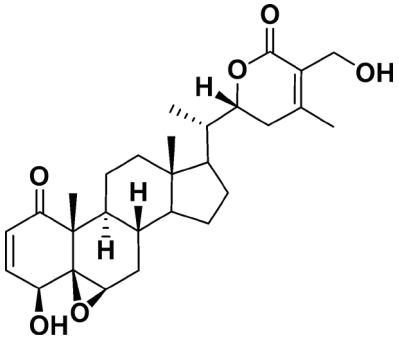

Figure 1: Chemical structure of Withaferin A

Alternatively, *W. frutescens*, a plant native to Europe whose leaves contain a high concentration of withaferin A and which has demonstrated a phytochemical profile resembling that of *W. somnifera*, can also be used to extract withaferin A (Zomborszki *et al.*, 2016).

Three locations may be implicated in alkylation reactions with nucleophilic sites, such as sulfhydryl groups of cysteine residues in target proteins, as suggested by the chemical structure of WA. These consist of the epoxide structure at positions 5 and 6, the unsaturated A-ring at C3, and the C24 in its E-ring of WA. The capacity of WA to form adducts with cysteine residues was confirmed by UV spectrophotometry due to the hypochromic shift that happened when WA and L-cysteine were incubated together (Santagata *et al.*, 2012). The primary nucleophilic target site for ethyl mercaptan, thiophenol, and L-cysteine ethyl ester *in vitro* was determined to be C3 in the unsaturated A-ring using NMR spectral analysis. The Michael addition alkylation reaction covalently attaches all of these withaferin A sites to the cysteine residues of protein, which causes the target protein's activity to be lost. Nevertheless, the C27 hydroxy group can be conjugated with biotin to enable it to recognize a variety of target proteins and is not physiologically significant for withaferin A action (Yokota *et al.*, 2006).

Structural Modifications of Withaferin A

The pharmacological properties may be enhanced by chemical modifications such as hydroxylation or acetylation. Therefore, understanding the relationship between structure and function may spur the development of new drugs. With more significant bioactivity, alkylated secondary metabolites (methyl or ethyl) have more excellent bioactivity, chemoprotective potential, and stability (Walle, 2009). A mortalin chaperone promotes carcinogenesis by deregulating apoptosis and inactivating the tumour suppressor protein p53. Numerous investigations have shown that stimulating p53 by complexly abrogating it with mortalin stops the proliferation of cancer cells (Deocaris *et al.*, 2013). WA obstructs a mortal's

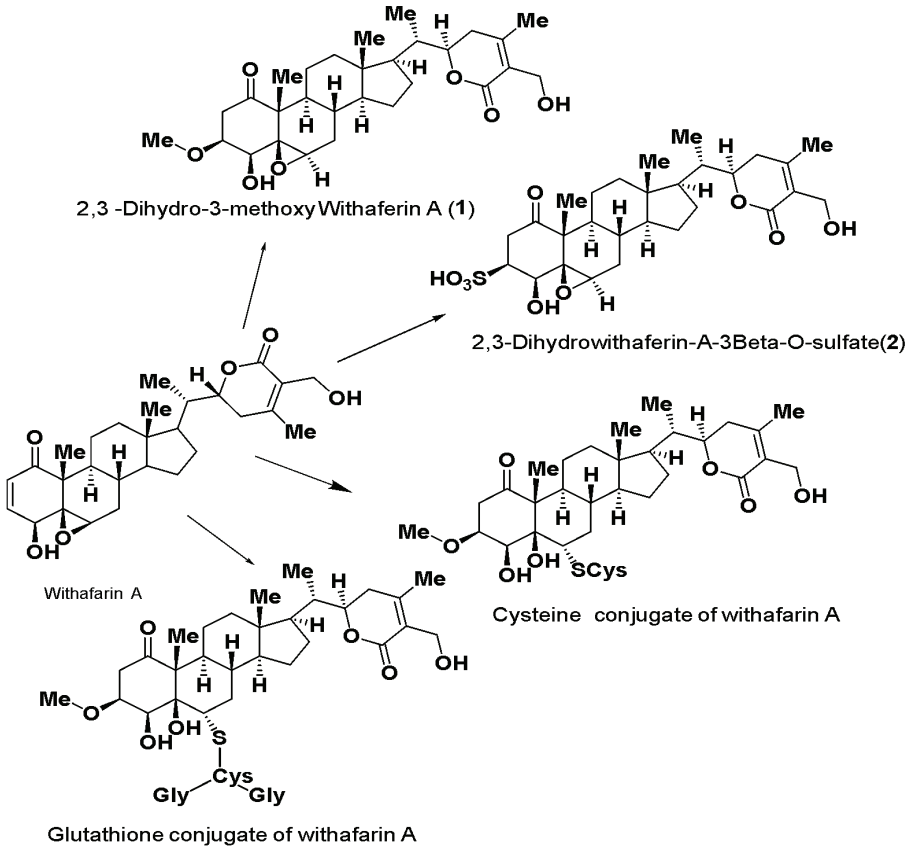

Figure 2: Chemically modified analogues of Withaferin A

ability to interact with p53. The binding domain of the mortal in the substrate was used to dock 3*β*-methoxy-withaferin-A. Beyond establishing drug potency, methylation of WA significantly impacts its protein binding efficacy and attenuates its chemotherapeutic potency (Huang *et al*., 2015). According to the study, Two conjugates, glutathione (CR-777) and cysteine (CR-591), conjugates revealed neuroprotective effects in a variety of neurodegenerative disorders. The damage to mesencephalic neurons caused by 1-methyl-4-phenylpyridinium (MPP+), alpha-synuclein (α-Syn), and 6-hydroxydopamine (6-OHDA) which was reversed by a nanomolar dosage of WA CR-777 conjugate. Furthermore, the addition of the WA CR-777 compound prevented the overexpression of α-Syn by 6-OHDA and preserved neurite integrity. By activating the PI3K/mTOR pathway. It also possesses neuroprotective effects by downregulating oxidative stress, suppressing TAU phosphorylation, caspase-3 expression, and α-Syn aggregation (Rabhi *et al*., 2019). 2,3-dihydro-3*β*-methoxy (3βmWi-A), an analogue with *β*-methoxy group substitution of WA, did not cause cell cytotoxicity and was well tolerated at greater dosages. Healthy cells protect against oxidative, chemical, and ultraviolet (UV) stressors and pro-survival signals (Chaudhary *et al*., 2019). Furthermore, compared to WA, the analog 2,3-dihydrowithaferin A-3β-O-sulphate has shown a 35-fold increase *in vitro* cytotoxicity against various human cancer cell lines (Yousuf *et al*., 2011). The different analogs of WA are shown in Fig. 2.

Biosynthesis of Withaferin A

Withanolides are a broad class of natural compounds with various molecular configurations, primarily of terpenoid origin. Based on the ergostane framework, withanolides, also known as 22-hydroxy ergostane-26-oic acid 26, 22-δ-lactones, are C-28 steroidal lactones that form a δ-lactone ring when oxidized at C-22 and C-26 (Chatterjee *et al*., 2010). Several withanolides, like withaferin A, withanone, and withanolides A and D, have been identified from different parts of *W. somnifera* (Mirjalili *et al*., 2009). Withanolides are produced by the isoprenoid route, referred to as the deoxyglucose pathway (DOXP), which involves the mevalonate (MVA) and non-mevalonate (MEP) processes. The MVA pathway is found in the cytoplasm (Bhat *et al*., 2012). In the MVA method, endophyte inoculation increases the expression of HMGR with the relevant isopentenyl pyrophosphate (IPP). In this instance, 1-deoxy-D-xylulose-5-phosphate reductase (DXR) and 1-deoxy-D-xylulose-5-phosphate synthase (DXS) are unaffected by the transcription process's gene-encoding enzymes, which may be the leading cause of the withanolide components' maximal productivity (Singh *et al*., 2014). The five-carbon precursor is opentenyl diphosphate (IPP), a common component in MVA and MEP processes (Thirugnanasambantham, Roy, Charles, & Senthil, 2014). Cholesterol is the relevant precursor for producing withanolides in the MVA pathway. Through metabolization, two units of acetyl-CoA become mevalonate. Then, it changes into isopentenyl pyrophosphate (IPP) by losing one carbon atom. Geranyl pyrophosphate (GPP) is produced by head-to-tail condensation of IPP with 3,3-dimethylallyl pyrophosphate (DMAPP). Furthermore, trans-geranyl pyrophosphate condenses with another IPP molecule to produce farnesyl pyrophosphate (FPP). Similar to this, squalene is produced when two molecules of farnesyl pyrophosphate are condensed head-to-head and are catalyzed by the enzyme squalene synthase in the presence of NADPH (Yousefian *et al*., 2018). NADPH-linked oxide catalyzes the oxidation process that forms squalene 2,3-epoxide from ambient oxygen. Afterwards, ring closure produces lanosterol, which can then be transformed into a few obtusifoliol skeletons and steroidal triterpenoid (Delta 8, 14-sterol) compounds. In addition, the biotransformation of lanosterol into 24-methylene cholesterol remains incompletely understood. 24-methylenecholestrol, on the other hand, might be a biosynthetic precursor of steroidal lactones. Regarding this, it has been proposed that hydroxylation of C22 and δ-lactonization between C22 and C26 of 24-methylenecholestrol create WA. The study suggested that WA, or the α, β-unsaturated ketone in ring A of common withanolides, can be made by the 20–23 sequence (Mirjalili *et al*., 2009). The unsaturated A-ring at position C3, the epoxide structure at position C5, and the E-ring at position C24 are the three sites that are vulnerable to nucleophilic assaults and Michael addition alkylation reactions (Kabir *et al*., 2021). Depicted in Fig. 3 is the biosynthesis of WA.

Pharmacological potentials of Withaferin A

It has been discovered that certain withanolides and their derivatives have shown several interesting

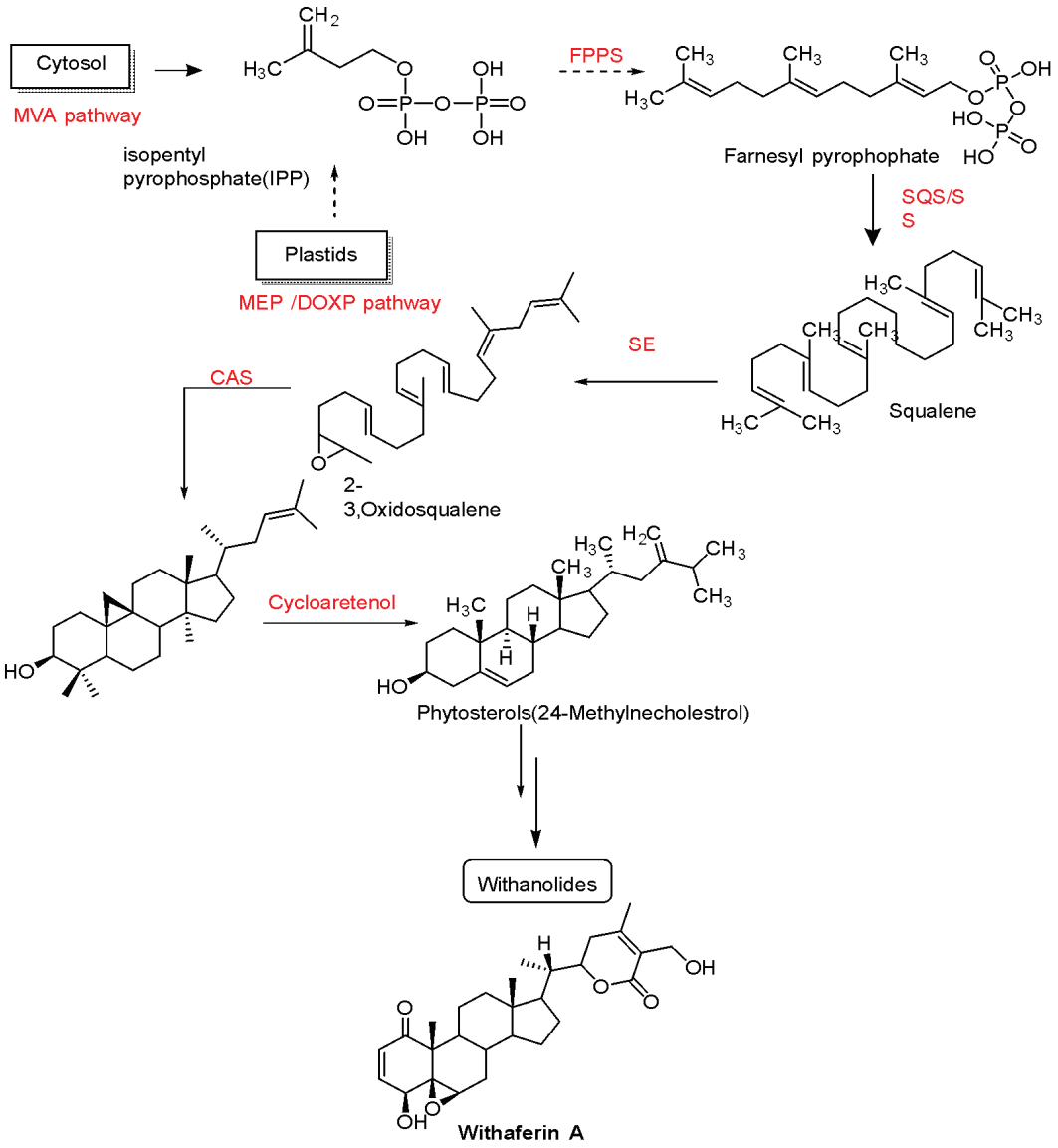

Figure 3: Biosynthetic pathways of Withaferin A

biological activities, including antibacterial, antioxidant, adaptogenic, antitumor, and antistress properties etc. The most significant withanolide that has been shown to exhibit these properties without being toxic is WA, which has been considered for use in the treatment of cancer. According to the reported activities described, much research is being done on plants containing withanolides. Below, we describe the pharmacological activities of withaferin A in detail.

Anticancer

In Western Australia, anticancer efforts began in the 1970s (Sohat *et al.*, 1967). Since then, numerous cancer cell lines, including those from multiple myeloma, neuroblastoma, leukaemia, glioblastoma, ovarian, breast, colon, head, and neck cancer, have shown proof of WA's anticancer activity (Hassannia *et al.*, 2018). The numerous organ chemopreventive effects of WA.

i. Breast Cancer

Breast cancer is one of the leading causes of cancer mortality in women worldwide. Breast cancer depends on the sex hormone estrogen and participates in tumour growth. Estrogen receptors (ER) are two types, Erα and Erβ, primarily involved in tumour formation in breast cancer. Erα targeted

by many pharmacological therapies and to develop medicines against breast cancer. Endocrine therapy can slow tumour growth by inhibiting Erα stimulation or lowering endogenous estrogen levels (O'Regan and Jordan, 2002). A breast cancer study found that WA cytoplasmic action compacts DNA molecules and splits the enzyme poly-(ADP-ribose)-polymerase. Another study discovered that WA attenuates IL-6, modifies the signal transducer pathway, and activates transcription 3 in constitutive (MDA-MB-231) and inducible (MCF-7 and MDA-MB-231) cell lines. The induction of apoptosis and obstruction of cell migration through the regulation of STAT 3 demonstrated therapeutic potential by WA exposure in MDA-MB-231 and MCF-7 cells, regardless of whether IL-6 was activated (Lee *et al.*, 2010). A study showed that WA investigated the mitochondrial dysfunction associated with reactive oxygen species (ROS) generation, resulting in apoptosis of cells. Oxidative phosphorylation equally inhibits the complex III treatment with WA. The mitochondrial Rho 0 cell line with DNA impairment and 40 embryonic fibroblasts generated from Bax/Bak knockdown cells showed higher resilience than wild-type cells (Hahm *et al.*, 2011) by reducing the expression of the proliferating cell nuclear antigen (PCNA) in human breast cells (Stan *et al.*, 2008). Phosphorylation of vimentin at the serine-56 residue inhibits the proliferation of 4T mouse mammary tumour cells, and downregulation of heat shock protein (HSP90) due to DNA double-strand break (DSB) inhibits the single-strand annealing sub-pathway (SSA). To restrict autophagy flow, WA blocks lysosomal activity and induces apoptosis in breast cancer cells (Liu *et al.*, 2019). WA activity causes autophagosomes to cluster. The phosphorylation impairment resulting from autophagic flux limits leads to inadequate fuel recycling and tricarboxylic acid substrate. Adenosine triphosphate is reduced, AMP protein kinase activation is encouraged, and lactate dehydrogenase (LDH) synthesis is reduced when WA is administered (Sivasankarapillai *et al.*, 2020).

ii. **Ovarian Cancer**

Ovarian cancer is the most lethal of all gynaecological malignancies, affecting over 22,000 women annually in the United States alone. Due to late-stage diagnosis of ovarian cancer, in most cases, cancer cells disseminate into the peritoneal cavity, which imposes clinical challenges. In human ovarian cancer cell lines SKOV3 and CAOV3, the WA exhibited G2/M phase cell cycle arrest (Fong *et al.*, 2012). The downregulation of Notch-3/Akt/Bcl-2 signalling mediated cell survival and resulted in caspase-3 activation and consequent apoptosis. Inadequate dosages of doxorubicin and cisplatin cause WA to create ROS and cause cell death (Zhang *et al.*, 2012). In another study using the A2780 cell line, Xenografting and mortality decreased by WA. It reduces the quantity of NF-κB-related phospho-p65 cytokines in the nucleus and cytoplasm of xenografted tumours (Straughn and Kakar, 2019). In ovarian cancer, xenografting induces cardiac cachexia and causes loss of the heart's normal functioning, such as systolic or diastolic dysfunction. In addition, WA also improved heart weight and preserved systolic function. In ovarian cancer, tumour cells were induced AT1R pathway-mediated by pro-inflammatory markers and formed the MHCβ isoform, ameliorated by WA (Kelm *et al.*, 2020).

iii. **Prostate Cancer**

Prostate cancer is the most common cancer and the second leading cause of cancer death among men. It occurs in the prostate of men. Initially, it was believed that prostate cancer has an androgen-related pathogenesis, but many individuals also develop an androgen-independent (metastatic castration-resistant) etiology (Cereda *et al.*, 2014). Androgen-dependent PCa cells, such as LNCaP, display stem-like properties, while androgen-independent PCa cells (mCRPC) DU-145 and PC-3 display none of these traits. Comparatively more violent and displaying more epithelial-mesenchymal transition (EMT) than homologous bulk population cells were the side-populating cells of xenograft tissues and human PC cell lines (Luo *et al.*, 2014). fibroblasts showed no signs of cell death, while vimentin-bound prostate tumour cells did. It results in a reduction in FLIP and an increase in the production of ROS and c-Fos, which most likely causes the cytoskeletal framework to break down. Thus, WA may be a pharmaceutical intervention that effectively eliminates cancer stem cells (CSCs). These CSCs, in contrast to other cancer cells, can reproduce tumours inside the tumour bulk, which facilitates chemoresistance (Kreso and Dick, 2014). Vimentin and WA interact directly by changing the cysteine residue (Cys328), which leads to an accumulation of vimentin filaments and,

when combined with F-actin, disrupts the vimentin cytoskeleton (Bargagna-Mohan *et al*., 2007). They were followed by alteration in cell shape, reduced motility, and upregulated vimentin phosphorylation (Grin *et al*., 2012). The effectiveness of WA against the CaP iPten-KO model has been shown in a different investigation. It has often been found that the Akt pathway, PTEN loss, mutation, and EMT are involved in metastatic prostate tumours. WA inhibited the production of HG-PIN and delayed the transformation of the PTEN-deficient tumour into an adenocarcinoma. WA stopped the PI3K/AKT pathway. WA supplementation increased the AKT-mediated proapoptotic proteins Par-4 and FOXO3A levels in Pten-KO mice. Immunohistochemical analysis revealed decreased expression of pAKT and the epithelial-to-mesenchymal transition markers N-cadherin and β-catenin in control WA-treated tumours (Moselhy *et al*., 2017). Telomere shortening triggers the DNA damage response and causes senescence and apoptosis (Bartkova *et al*., 2005). WA severely damaged telomeres in cancer cells by drastically reducing the expression of pAKT and promoting FOXO3a/Par-4-mediated tumour inhibition in TRAMP mice (Suman *et al*., 2016). In vivo prostate tests, a different study showed that the intraperitoneal dose of WA (0.1 mg) significantly suppressed the production of ATP citrate lyase, carnitine palmitoyl transferase 1A, and circulatory free fatty acid and fatty acid synthase (Kim *et al*., 2020).

iv. **Colorectal Cancer**

Globally, colorectal cancer (CRC) ranks as the fourth most common cause of death (Ferlay *et al*., 2010). Reactive oxygen species (ROS) produced by WA cause human colorectal cancer cells' Nrf2, HO-1, and NQO1 pathways to become activated. Furthermore, WA increases the expression of the upstream regulator of Nrf2, c-Jun-N-terminal kinase (JNK). This stopped the stimulation of cell death induced by the loss of the tumour suppressor gene Tap73. HCT116 and SW480 colorectal cancer cells undergo a cell cycle transition into the G2/M phase due to WA. Two crucial spindle complex components delayed mitosis by interfering with the proteasomal degradation of Mad2 and Cdc20 (Das *et al*., 2014). Moreover, it decreased the expression and growth Notch-1 in human colon cancer cells. Notch1 and AKT are activated by EMT in colorectal cancer. Since this type of cancer is caused by EMT, treatments that target AKT/Notch1 pathways and prevent metastasis are at the top of the current research paradigm. The WA impact was examined using various mice models of spontaneous intestinal carcinogenesis and the colitis-regulated colon mouse model. WA effectively suppresses intestinal polyp and colon carcinogenesis; proliferative markers are decreased, pro-survival signalling markers (NFκB, Notch1, and pAKT) are downregulated, and proliferative indicators are reduced (Pal *et al*., 2018).

v. **Lung Cancer**

Substance A549 and H1299 lung cancer cells demonstrated reduced cell adhesion, migration, and invasion following pre-treatment with WA. It was demonstrated by immunofluorescence, qRT-PCR, and western blot analysis that WA downregulated EMT in both cells, which were brought on by the production of TNFα and tumour growth factor beta 1 (TGFβ1). Additionally, WA inhibits the phosphorylation of Smad2/3, NF-κB, and nuclear translocation (Kyakulaga *et al*., 2018). Moreover, WA causes dose-dependent apoptosis in A549 cells. The primary players in apoptosis, caspase-9 and caspase-3, were activated along with reduced MMP when WA-treated cells were stained with JC-1. WA halted lung cancer (A549) cells in the G0/G1 phase, further inhibiting the PI3K/Akt pathway and lowering Bcl-2 synthesis. Furthermore, WA observed a decrease in dose-dependent distribution in lung nodules. As a result, WA is highly effective against lung cancer and preventive medication that also stops CSC from proliferating. Furthermore, it inhibits the mTOR/STAT3 pathway, which stops lung cancer from producing spheroids (Li *et al*., 2015).

Anti-diabetic Activity

An imbalance between the supply and utilization of glucose characterizes a metabolic and endocrine disorder, diabetes. The elevated level of glucose in diabetes has several pathophysiologies that ultimately lead to beta-cell death and pancreatic histological disruption. The only effective treatment for insulin-dependent diabetes mellitus, often known as type I diabetes, is insulin replacement therapy. Diabetes has several subtypes of consequences, including organ infections, obesity, and micro- and macrovascular problems (Vesa *et al*., 2020). More evidence suggests that WA may modify lipid profiles and glucose metabolism. It reduces

inflammation and promotes weight reduction in diabetic mice, which raises insulin sensitivity (Khalilpourfarshbafi *et al.*, 2019). The anti-diabetic drug rosiglitazone increases insulin sensitivity but does not appear to help with weight loss or liver function. It also lessens hepatic steatosis in mice. Inflammatory mediators play a significant role in obesity. They promote insulin receptor substrate 1 (IRS-1) phosphorylation, which prevents insulin signalling. The WA treatment significantly reduced it. Diabetes has been linked to downregulated insulin-signalling gene expression, according to an earlier study. While rosiglitazone therapy enhanced the expression of INSR, IRS1, and SLC2A4, WA treatment upregulated the mRNA expression of INSR, PI3K, and IRS1. The anti-inflammatory properties of WA appear to have contributed to insulin-sensitizing capacity by indirectly influencing insulin signalling events, upregulating adiponectin, and inhibiting PPARγ phosphorylation. WA mitigated streptozotocin-induced type 1 diabetes. Following the production of inflammatory mediators that lead to the demise of pancreatic islet cells, the NF-κB axis is activated by oxidative stress. The ultimate cause of the malfunctioning β-cell is the DNA alkylation mediated by STZ. Apoptotic morphological changes caused by overexpression of caspase 3 include cytoplasmic and nuclear condensation, membrane blebbing, DNA fragmentation, and the development of apoptotic bodies (Riboulet-Chavey *et al.*, 2008). Hyperglycaemia arises from the breakdown of β-cells in the pancreas that secrete insulin. By reducing tissue nitrile levels, WA intervention eliminates nitrosative stress. With its ability to reduce fragmented DNA, which increases caspase three expression, and lower TNF-α and IL-6 concentrations, WA was thought to be a possible chemical to treat T1DM (Tekula *et al.*, 2018).

Neuroprotective Activity

Accumulation of Aβ in the central nervous system (CNS) leads to neurodegeneration diseases like Alzheimer's disease. Amyloid beta (Aβ) aggregation is produced by cocaine and is inhibited by WA 0.5–2 μM without causing cytotoxicity in cell cultures. Cytoplasmic vacuoles and dendritic beading are reduced by WA therapy. Additionally, this buildup of Aβ in the brain of an HIV patient is a contributing factor to a series of neurological conditions that cause ageing or associated dementias (Tiwari *et al.*, 2018). WA decreases the action of acetylcholesterinases and butyrylcholinesterase, according to *in vitro* experiments. When acetylcholinesterase's hydrolytic activity interferes with the neurotransmitter acetylcholine, acetate and choline are produced. The mechanism of butyrylcholinesterase is still unknown. Increasing acetylcholine levels improve Alzheimer's disease (AD) cognitive deficits because acetylcholine plays a vital role in cognitive disorders (Choudhary *et al.*, 2004). In the substantia nigra and striatum, the neuroprotective potential of WA (50 mg/kg b.w.) showed a resurgence of dopamine (DA) and homo vanillic acid (HVA). Motor impairments result from these catecholamines' lowered levels. The elevated DA and HVA levels point to WA's potential for neuroprotection (Raziya *et al.*, 2020). When Aβ oligomers and Aβ fibrils interact with microglia in AD, it activates the microglia. It triggers an inflammatory response by activating the NLRP3 and nuclear factor NF-κB pathway, which releases pro-inflammatory cytokines and chemokines (Heneka *et al.*, 2013). Microglia absorb Aβ fibrils by phagocytosis, and neprilysin and insulin-degrading enzymes break these fibrils down. In AD patients, activation of the NF-κB and NLRP3 pathways inhibits Aβ phagocytosis, increasing Aβ fibril buildup. This creates a self-reinforcing loop that leads to neuroinflammation. Treatment with WA reduced the production of NF-κB, an essential component in the cascade of inflammatory cytokines. JAK and STAT were observed to be downregulated, but IKBKB and IKBKG were upregulated (Atluri *et al.*, 2020). Parkinson's disease (PD) and Alzheimer's disease (ADL) neurological patients have significant mutations in leucine-rich repeat kinase 2. Both co-chaperone Cdc37 and chaperone HSF90 stabilize this protein. The N9 microglial cell line treated with WA reduces LRRK2 and alters HSP90 and Cdc37, destabilising and decreasing LRRK2 concentration (Narayan *et al.*, 2015). All of the information indicates that WA has great promise as a natural neurotherapeutic agent to improve cognitive deficiencies linked to AD, PD, and ALS; hence, further research should be done on its use in various neurodegenerative disease models.

Cardioprotective Activity

Heart attacks, or myocardial infarctions, are the leading cause of death and a global health concern. A dosage of one milligram per kilogram of WA induced the mitochondrial antiapoptotic pathway,

increased the expression of Bcl-2 protein, and decreased the death of apoptotic cells. A low amount of WA—1 mg/kg—protected mice against MI harm, according to the in vivo experiment, while a bigger dose—5 mg/kg—did not offer any protection at all and instead caused damage to the animals' cardiac cells. WA, which has shown better cardiac function by promoting AMPK activation and lowering mitochondrial apoptosis, may affect the Bcl-2/Bax ratio. As such, WA is administered therapeutically to cancer patients who also have issues with their circulatory systems (Guo *et al*., 2019). Type I collagen production and deposition are induced by the activation of collagen-synthesizing cells connected to fibroproliferative diseases. The elevated half-life of collagen I mRNA and the transcription of the collagen I gene are responsible for this outcome (Rona, 1985). WA disrupts endothelial cells and is present in astrocytes where the vimentin filament is found. Vimentin filament interactions stabilize type I collagen. WA plays a transcriptional and post-transcriptional role in regulating type I collagen.

Moreover, it inhibits NF-kB activation and TGF stimulation. Collagen fibre accumulation in the cardiac interstitium is linked to myocardial fibrosis, seen in several cardiac disorders, such as hypertensive heart disease, idiopathic interstitial cardiac fibrosis, hypertrophic cardiomyopathy, and decompensated congestive heart failure. Heart function is disrupted by fibrosis, which leads to heart failure (Lombardi *et al*., 2003).

Anti-Hepatitis Activity

The advanced form of non-alcoholic fatty liver disease, known as non-alcoholic steatohepatitis (NASH), increases the risk of liver cirrhosis and cancer when combined (Michelotti *et al*., 2013). When fatty acids are consumed in excess, toxic lipids are formed, which cause ER stress, hepatic oxidative stress, inflammation, and hepatic cell death (Friedman *et al*., 2018). One kind of lipid that accumulates in the blood and tissues is called ceramides. WA's reduction of oxidative stress, as evidenced by heme oxygenase (HO-1) synthesis and its nuclear factor erythroid-related factor 2 pathway, mitigates acetaminophen-induced liver damage. WA at a dosage of 5 mg/kg decreased hepatic steatosis, fibrosis, and inflammation pathologies connected to non-alcoholic fatty liver disease (NASH). Kelch plays a role in multiple processes, such as ECH-associated protein 1 and glycogen synthase kinase 3(D. P. Patel *et al*., 2019). These results imply that WA successfully shields cells from NASH, although more research is still needed to determine the precise mechanism. Given that WA may be used to treat NASH, this could aid in repurposing.

Osteoporosis

Osteoporosis is a skeletal bone disorder characterized by an imbalance in bone resorption and formation (Tit *et al*., 2018). WA promotes the growth and differentiation of osteoblasts by upregulating the production of a transcription factor specific to osteoblasts. WA inhibits the cytokines that produce inflammation. WA decreases the number of osteoclasts, or bone-resorbing cells, by downregulating the expression of receptor activator of nuclear factor kappa-B ligand (RANKL), osteoprotegerin (OPG), and tartrate-resistant acid phosphatase (TRAP). In addition, WA suppresses NF-kB signalling, stabilizes RunX2, and activates the nuclear p65 subunit of NF-kB. Thus, it promotes the proliferation of osteoblastic bone-forming cells (Khedgikar *et al*., 2013). These results suggested that WA inhibits osteoclast development by downregulating RANKL and TRAP, which prevents osteoporosis.

COVID-19

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) that is currently causing the COVID-19 pandemic has caused a global health crisis. Various in silico and biochemical studies have shown that the bis-electrophiles WA and the medicinal plant *W. somnifera* are effective and safe Mpro inhibitors. It was discovered through docking experiments that WA may bind to Mpro. Under physiological circumstances, WA can combine with Cys and GSH to produce thermodynamically stable adducts. Fluorescence-based kinetic assays and 2- and 3-dimensional cell culture experiments demonstrated that WA can inhibit Mpro, with IC50 values of 0.54 and 1.8 μM for WA covalently binds to Mpro, according to LC-HRMS/MS analysis and equilibrium dialysis tests. Furthermore, according to docking studies, the GSH adducts of WA can bind Mpro and create covalent adducts. Research revealed that the commonly used medicinal plant *W. somnifera* in Washington can bind to Mpro in covalent adducts to prevent the replication of SARS-CoV-2 (Chakraborty *et al*., 2022).

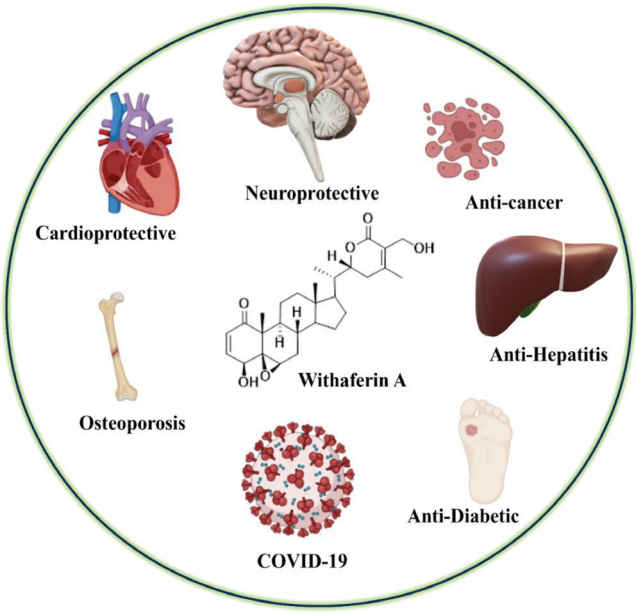

Figure 4: Pharmacological potentials of Withaferin A

Pharmacokinetics and Bioavailability

Research on pharmacokinetics (PK) provides valuable information about the bioactive components of herbal medicines. The profile of targeted or untargeted metabolites that develop following oral administration of a single chemical component of the crude drug forms the basis of PK analysis. Following oral administration of 1000 mg/kg of *W. somnifera* root aqueous extract, the mice's plasma was determined to contain 0.4585 mg/kg of WA. The PK data showed that WA could be absorbed quickly orally; the Cmax, Tmax, and T1/2 values were 16.69 ± 4.02 ng/mL, 20 min, and 59.92 ± 15.90 min, respectively. According to one study, the relative bioavailability of *W. somnifera*, WA, was 1.5 times that of other withanolides (Patil *et al*., 2013). Following permeability measurements, WA was found to have an extraordinarily impermeable probability (Peff) value of 4.05 × 10−5 (Devkar *et al*., 2015). The oral bioavailability was found to be 32.4 ± 4.8% following the intravenous dose of 5 mg/kg and the oral administration of 10 mg/kg of WA.

An *in vitro* study revealed that WA may traverse colorectal adenocarcinoma (Caco-2) cells and does not have a P-glycoprotein substrate. The stability studies of WA in human and male rat stomach contents, liver microsomes, and intestinal microflora solution produced the same outcomes. In addition, WA declined quickly; after just one hour, 27.1% left (Dai *et al*., 2019). Research on the PK and safety of WA in patients with metastatic cancer was also noted. The formulation dose of 4800 mg, or 216 mg of WA, was well tolerated and did not show any dose-limiting toxicity, according to the phase I trial on WA. The highest dosage cohort patients could take was four WA regimen pills (TID). Combining the data showed that administering WA to patients with high-grade osteosarcoma that was in an advanced stage had a good safety profile and resulted in rapid oral absorption.

Moreover, Phase II clinical trials may employ 216 mg/day (Pires *et al*., 2020). Consequently, there is great potential for this natural chemical. As a result, novel, customized drug delivery strategies can be developed to treat various human ailments.

Toxicity of Withaferin A

Determining a medicine's or chemical's safety before human consumption is very important. This review details the safe dosages of withaferin A or enriched extract of *W. somnifera*, which different research groups studied during the studies. In one such investigation, the hydroalcoholic extract of *W. somnifera* was tested for sub-acute toxicity in Wistar rats, and the results showed that the compounds were not toxic (Prabu *et al*., 2013). Another study used *W. somnifera* extract (WSE) oral administration to test the acute and subacute toxicity of Withaferin A in Wistar rats. The sub-acute toxicity study of Wistar rats was divided into groups based on the amount of oral administration of the *W. somnifera* extract (WSE), which included control, 500, 1000, and 2000 mg/kg of body weight/day for 28 days. A dose of 2000 mg/kg was given for the acute toxicity study.

Additionally, there were no treatment-related histopathological changes or observable variations in body or organ weight, and no statistically significant changes in serum chemistry indicated any toxicologically important changes (Patel *et al*., 2016). The ADMET study showed no evidence of hepatotoxicity, mutagenicity, or carcinogenicity for any components of *W. somnifera* extract, including Withaferin A. Even though withaferin A was shown to have anti-proliferative effects on endometrial cancer KLE cells, a 48-hour treatment period decreased normal THESCs cells, with viability dropping from 100% to 25%. When compared, the toxicity of withaferin A therapy was higher for

KLE cells from endometrial cancer than for normal THESC cells (Xu, Shi, Du, & Ou, 2021). In a different investigation, six tumour cell lines representing different types of cancer and various standard and multidrug-resistant bacterial strains were used to test Withaferin A for cytotoxicity and antibacterial activity. Lung cancer, glioblastoma, neuroblastoma, uterine colon cancer, murine melanoma, and chronic myeloid leukaemia were among them. Effective cytotoxic and antibacterial action was discovered for withaferin A; cytotoxicity was demonstrated by increased ROS generation (Atteeq, 2022).

Formulations Prepared from Withaferin A

Dexamethasone and WA gold nanoparticles could inhibit the epithelial-mesenchymal transition in tumour cells, preventing metastasis by inhibiting mouse melanoma tumours, thereby reducing mice's mortality rate. Glucocorticoid receptor-dependent selective cytotoxicity occurs using this metallic nanoparticle formulation (Agarwalla *et al*., 2016). In one study, mannosylated liposomes were used for the encapsulation of WA to target synovial macrophages in a rat model of arthritis. With the help of confocal microscopy, ML-WA showed robust internalization of synovial macrophages. Moreover, osteoprotegerin production was upregulated after the treatment, and there was no degradation of cartilage or bone erosion. The study suggested that ML-WA has enormous potential for reducing bone resorption and inflammation (Sultana *et al*., 2017).

A new liposomal-efficient drug delivery system was developed to target angiogenic endothelial cells and CD13-positive cancer epithelial cells using homing peptides (NGR). NGRKC16-lipopeptide liposomes are encapsulated with WA, which leads to the apoptosis of CD13-positive pancreatic cancer cells and angiogenic endothelial cells. Therefore, the reported WA-encapsulated liposomal formulation could be a therapeutic strategy for treating aggressive pancreatic cancer (Aminu *et al*., 2020). The WA nano vesicular system novel formulation showed higher anticancer activity against HeLa cells in the SRB assay, followed by flow cytometry and comet assays. So, this study provides an opportunity to use natural materials as cancer treatment agents (Shah *et al*., 2020). Polycaprolactone implants embedded with WA were prepared for controlled systemic release to overcome the problems associated with oral bioavailability and decrease the dose requirement. The WA implant inhibits nearly 60% of lung cancer in A549 cell xenografts, but no cancer suppression was seen when the same dose was given intraperitoneally (Gupta *et al*., 2012). The WA formulation has proved beneficial in arthritis and cancer, although this formulation still requires more process optimization for efficient clinical translation. This could also be beneficial for pharmaceutical and translational researchers.

CONCLUSION AND FUTURE PERSPECTIVES

This review comprehensively explores the chemistry, pharmacology, biosynthesis, and toxicity of withaferin A. The unique 28-carbon-containing steroid skeleton of Withaferin A has α, β- unsaturated double bonds and an epoxide ring, which have been used for further chemical modifications to prepare novel analogues with improved pharmacological properties. Withaferin A possesses various pharmacological properties like anticancer, anti-osteoporotic, Neuroprotective properties, etc. It has been widely studied for its anticancer properties. The pharmacokinetics research showed that withaferin A has a quick oral absorption rate, making it helpful in developing medication delivery systems that address a range of illnesses. Withaferin is a promising anticancer agent with numerous other therapeutic benefits, including cardioprotective, neuroprotective, osteoporotic, and antiviral properties. The molecule is gaining attention on a global scale. According to the current review, Withaferin A overcomes drug resistance, targets several targets, and enhances therapeutic effects.

Furthermore, pharmacokinetic and bioavailability studies must be conducted in depth to determine this compound's active dose. With the development of new chemical methodologies, it is possible to prepare new analogues that could provide lead compounds from Withaferin A. A comprehensive toxicity evaluation is imperative to elucidate its safety profile.

ACKNOWLEDGMENTS

The authors thank the Director, CSIR-CIMAP, Lucknow, for providing essential facilities. SS extends thanks to DST for the DST-Inspire fellowship (IF200046). Additionally, we acknowledge CSIR, India, for funding the NMITLI project (FC2020-23/NMITLI/TLP0002).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Agarwalla P, Mukherjee S, Sreedhar B, Banerjee R. 2016. Glucocorticoid receptor-mediated delivery of nano gold–withaferin conjugates for reversal of epithelial-to-mesenchymal transition and tumor regression. *Nanomed* **11**: 2529-2546.

Aminu N, Bello I, Umar NM, Tanko N, Aminu A, Audu MM. 2020. The influence of nanoparticulate drug delivery systems in drug therapy. *J Drug Delivery Sci Technol* **60:** 101961.

Atluri VSR, Tiwari S, Rodriguez M, Kaushik A, Yndart A, Kolishetti N, Nair M. 2020. Inhibition of amyloid-beta production, associated neuroinflammation, and histone deacetylase 2-mediated epigenetic modifications prevent neuropathology in alzheimer's disease *in vitro* model. *Front in Aging Neurosci* **11:** 342.

Atteeq M. 2022. Evaluating anticancer properties of Withaferin A: A potent phytochemical. *Front Pharmacol* **13:** 975320.

Bargagna-Mohan P, Hamza A, Kim Y-e, Ho YKA, Mor-Vaknin N, Wendschlag N, Zhan C-G. 2007. The tumor inhibitor and antiangiogenic agent withaferin A targets the intermediate filament protein vimentin. *Chem Biol* **14**: 623-634.

Bartkova J, Hoøejší Z, Koed K, Krämer A, Tort F, Zieger K, Lukas C. 2005. DNA damage response as a candidate anticancer barrier in early human tumorigenesis. *Nat* **434**: 864-870.

Berghe WV, Sabbe L, Kaileh M, Haegeman G, Heyninck K. 2012. Molecular insight in the multifunctional activities of Withaferin A. *Biochem Pharmacol* **84**: 1282-1291.

Bhat WW, Lattoo SK, Razdan S, Dhar N, Rana S, Dhar RS, Vishwakarma RA. 2012. Molecular cloning, bacterial expression, and promoter analysis of squalene synthase from *Withania somnifera* (L.) Dunal. *Gene* **499**: 25-36.

C Deocaris C, Lu W.-J, C Kaul S, Wadhwa R. 2013. Druggability of mortal in for cancer and neurodegenerative disorders. *Curr Pharm Design* **19**: 418-429.

Cereda V, Formica V, Massimiani G, Tosetto L, Roselli M. 2014. Targeting metastatic castration-resistant prostate cancer: Mechanisms of progression and novel early therapeutic approaches. *Expert Opin Invest Drugs* **23**: 469-487.

Chakraborty S, Mallick D, Goswami M, Guengerich FP, Chakrabarty A, Chowdhury G. 2022. The natural products withaferin A and withanone from the medicinal Herb *Withania somnifera* are covalent inhibitors of the SARS-CoV-2 main protease. *J Nat Prod* **85**: 2340-2350.

Chatterjee S, Srivastava S, Khalid A, Singh N, Sangwan RS, Sidhu OP, Tuli R. 2010. Comprehensive metabolic fingerprinting of *Withania somnifera* leaf and root extracts. *Phytochem* **71**: 1085-1094.

Chaudhary A, Kalra RS, Malik V, Katiyar SP, Sundar D, Kaul SC, Wadhwa R. 2019. 2, 3-Dihydro-3â-methoxy Withaferin-A lacks anti-metastasis potency: Bioinformatics and experimental evidence. *Sci Rep* **9**: 17344.

Choudhary MI, Yousuf S, Nawaz SA, Ahmed S. 2004. Cholinesterase inhibiting withanolides from Withania somnifera. *Chem Pharm Bull* **52**: 1358-1361.

Dai T, Jiang W, Guo Z, Wang Z, Huang M, Zhong G, Dai R. 2019. Studies on oral bioavailability and first-pass metabolism of withaferin A in rats using LC–MS/MS and Q-TRAP. *Biomed Chromatogr* **33**: e4573.

Das T, Roy KS, Chakrabarti T, Mukhopadhyay S, Roychoudhury S. 2014. Withaferin A modulates the spindle assembly checkpoint by degradation of Mad2–Cdc20 complex in colorectal cancer cell lines. *Biochem Pharmacol* **91**: 31-39.

Devkar ST, Kandhare AD, Sloley BD, Jagtap SD, Lin J, Tam YK, Hegde MV. 2015. Evaluation of the bioavailability of major withanolides of *Withania somnifera* using an *in vitro* absorption model system. *J Adv Pharm Technol & Res* **6**: 159.

Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. 2010. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* **127**: 2893-2917.

Fong MY, Jin S, Rane M, Singh RK, Gupta R, Kakar SS. 2012. Withaferin A synergizes the therapeutic effect of doxorubicin through ROS-mediated autophagy in ovarian cancer. *Plos One* **7**: 1-16.

Friedman SL, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. 2018. Mechanisms of NAFLD development and therapeutic strategies. *Nat Med* **24**: 908-922.

Fuska J, Fuskova A, Rosazza J, Nicholas A. 1984. Novel cytotoxic and antitumor agents. IV. Withaferin A: Relation of its structure to the *in vitro* cytotoxic effects on P388 cells. *Neoplasma* **31**: 31-36.

Grin B, Mahammad S, Wedig T, Cleland MM, Tsai L, Herrmann H, Goldman RD. 2012. Withaferin alters intermediate filament organization, cell shape, and behavior. *PloS One* **7**: e39065.

Guo R, Gan L, Lau WB, Yan Z, Xie D, Gao E, Wang Y. 2019. Withaferin A prevents myocardial ischemia/reperfusion injury by upregulating AMP-activated protein kinase-dependent B-cell lymphoma2 signaling. *Circulation J* **83**: 1726-1736.

Gupta RC, Bansal SS, Aqil F, Jeyabalan J, Cao P, Kausar H, Vadhanam MV. 2012. A controlled-release systemic delivery new concept in cancer chemoprevention. *Carcinog* **33**: 1608-1615.

Hahm E.-R, Moura MB, Kelley EE, Van Houten B, Shiva S, Singh SV. 2011. Withaferin A-induced apoptosis in human breast cancer cells is mediated by reactive oxygen species. *PloS One* **6**: e23354.

Hassannia B, Wiernicki B, Ingold I, Qu F, Van Herck S, Tyurina YY, Choi SM. 2018. Nano-targeted induction of dual ferroptotic mechanisms eradicates high-risk neuroblastoma. *The J Clin Invest* **128**: 3341-3355.

Heneka MT, Kummer MP, Stutz A, Delekate A, Schwartz S, Vieira-Saecker A, Tzeng T.-C. 2013. NLRP3 is activated in alzheimer's disease and contributes to pathology in APP/PS1 mice. *Nat* **493**: 674-678.

Huang C, Vaishnavi K, Kalra R, Zhang Z, Sekar K, Kaul S, Wadhwa R. 2015. 3beta-methoxy derivation of Withaferin-A attenuates its anticancer potency: Bioinformatics and molecular evidence. *Med Aromat Plants* **4**: 2167-0412.100021.

Johny L, Conlan X, Cahill D, Adholeya A. 2015. *In vitro* and situ screening systems for morphological and phytochemical analysis of *Withania somnifera* germplasms. *Pl Cell Tissue Organ Cult* **120:** 1191-1202.

Kabir MT, Uddin MS, Jeandet P, Emran TB, Mitra S, Albadrani GM, Simal-Gandara J. 2021. Anti-alzheimer's molecules derived from marine life: Understanding molecular mechanisms and therapeutic potential. *Mar Drugs* **19**: 251.

Kakar SS, Parte S, Carter K, Joshua IG, Worth C, Rameshwar P, Ratajczak MZ. 2017. Withaferin A (WFA) inhibits tumor growth and metastasis by targeting ovarian cancer stem cells. *Oncotarget*, **8**: p.74494.

Kelm NQ, Straughn AR, Kakar SS. 2020. Withaferin A attenuates ovarian cancer-induced cardiac cachexia. *PloS One* **15**: e0236680.

Khalilpourfarshbafi M, Devi Murugan D, Abdul Sattar MZ, Sucedaram Y, Abdullah NA. 2019. Withaferin A inhibits adipogenesis in the 3T3-F442A cell line, improves insulin sensitivity, and promotes weight loss in high-fat diet-induced obese mice. *PloS One* **14**: e0218792.

Khedgikar V, Kushwaha P, Gautam J, Verma A, Changkija B, Kumar A, Trivedi P. 2013. Withaferin A: A proteasomal inhibitor that promotes healing after injury and exerts an anabolic effect on osteoporotic bone. *Cell Death Dis* **4**: e778-e778.

Kim S.-H, Hahm E.-R, Singh KB, Shiva S, Stewart-Ornstein J, Singh SV. 2020. RNA-seq reveals novel mechanistic targets of withaferin A in prostate cancer cells. *Carcinog* **41:** 778-789.

Kreso A, Dick JE. 2014. Evolution of the cancer stem cell model. *Cell Stem Cell* **14**: 275-291.

Kyakulaga AH, Aqil F, Munagala R, Gupta RC. 2018. Withaferin A inhibits epithelial to mesenchymal transition in non-small cell lung cancer cells. *Sci Rep* **8**: 15737.

Lee J, Hahm ER, Singh SV. 2010. Withaferin A inhibits the activation of signal transducer and activator of transcription 3 in human breast cancer cells. *Carcinog* **31**: 1991-1998.

Li X, Zhu F, Jiang J, Sun C, Wang X, Shen M, Peng F. 2015. Synergistic antitumor activity of withaferin A combined with oxaliplatin triggers reactive oxygen species-mediated inactivation of the PI3K/AKT pathway in human pancreatic cancer cells. *Cancer Lett* **357**: 219-230.

Liu W, Wang G, Palovcak A, Li Y, Hao S, Liu ZJ, Zhang Y. 2019. Impeding the single-strand annealing pathway of DNA double-strand break repair by withaferin A-mediated FANCA degradation. *DNA Repair* **77**: 10-17.

Lombardi R, Betocchi S, Losi MA, Tocchetti CG, Aversa M, Miranda M, Chiariello M. 2003. Myocardial collagen turnover in hypertrophic cardiomyopathy. *Circ* **108**: 1455-1460.

Luo Y, Cui X, Zhao J, Han Y, Li M, Lin Y, Lan L. 2014. Cells susceptible to epithelial-mesenchymal transition are enriched in stem-like side population cells from prostate cancer. *Oncol Rep* **31**: 874-884.

Meena AK, Rekha P, Perumal A, Gokul M, Swathi K, Ilavarasan R. 2021. Estimation of Withaferin-A by HPLC and standardization of the Ashwagandha lehyam formulation. *Heliyon* **7**.

Michelotti GA, Machado MV, Diehl AM. 2013. NAFLD, NASH, and liver cancer. *Nat Rev Gastroenterology & Hepatology* **10**: 656-665.

Mirjalili MH, Moyano E, Bonfill M, Cusido RM, Palazón J. 2009. Steroidal lactones from *Withania somnifera*, an ancient plant for novel medicine. *Mol* **14**: 2373-2393.

Moncrief JW, Heller KS. 1967. Acylation: A proposed mechanism of action for various oncolytic agents based on model chemical systems. *Cancer Res* **27**: 1500-1502.

Moselhy J, Suman S, Alghamdi M, Chandarasekharan B, Das TP, Houda A, Damodaran C. 2017. Withaferin A inhibits prostate carcinogenesis in a PTEN-deficient mouse model of prostate cancer. *Neoplasia* **19**: 451-459.

Narayan M, Zhang J, Braswell K, Gibson C, Zitnyar AC, Lee DK, Jinwal U. 2015. Withaferin A regulates LRRK2 levels by interfering with the Hsp90-Cdc37 chaperone complex. *Curr Aging Sci* **8**: 259-265.

O'Regan RM, Jordan VC. 2002. The evolution of tamoxifen therapy in breast cancer: selective estrogen-receptor modulators and downregulators. *The Lancet Oncol* **3**: 207-214.

Pal D, Tyagi A, Chandrasekaran B, Alattasi H, Ankem MK, Sharma AK, Damodaran C. 2018. Suppression of Notch1 and AKT mediated epithelial to mesenchymal transition by Verrucarin J in metastatic colon cancer. *Cell Death Dis* **9**: 798.

Patel DP, Yan T, Kim D, Dias HB, Krausz KW, Kimura S, Gonzalez FJ. 2019. Withaferin A improves non-alcoholic steatohepatitis in mice. *J Pharmacol Exp Ther* **371**: 360-374.

Patel SB, Rao NJ, Hingorani LL. 2016. Safety assessment of *Withania somnifera* extract standardized for Withaferin A: Acute and sub-acute toxicity study. *J Ayurveda Integr Med* **7**: 30-37.

Patil D, Gautam M, Mishra S, Karupothula S, Gairola S, Jadhav S, Patwardhan B. 2013. Determination of withaferin A and withanolide A in mice plasma using high-performance liquid chromatography-tandem mass spectrometry: Application to pharmacokinetics after oral administration of *Withania somnifera* aqueous extract. *J Pharm Biomed Anal* **80:** 203-212.

Pires N, Gota V, Gulia A, Hingorani L, Agarwal M, Puri A. 2020. Safety and pharmacokinetics of Withaferin-A in advanced stage high-grade osteosarcoma: A phase I trial. *J Ayurveda Integr Med* **11**: 68-72.

Prabu P, Panchapakesan S, Raj CD. 2013. Acute and sub-acute oral toxicity assessment of the hydroalcoholic extract of *Withania somnifera* roots in Wistar rats. *Phytotherapy Res* **27**: 1169-1178.

Rabhi C, Arcile G, Le Goff G, Da Costa Noble C, Ouazzani J. 2019. Neuroprotective effect of CR-777, a glutathione derivative of Withaferin A, obtained through the bioconversion of *Withania somnifera* (L.) Dunal extract by the fungus Beauveria bassiana. *Mol* **24**: 4599.

Raziya Banu M, Ibrahim M, Prabhu K, Rajasankar S. 2020. Withaferin-A protects the nigral dopamine neuron and recovers motor activity in aged rats. *Cells Tissues Organs* **208**: 59-65.

Riboulet-Chavey A, Diraison F, Siew LK, Wong FS, Rutter GA. 2008. Inhibition of AMP-activated protein kinase protects pancreatic â-cells from cytokine-mediated apoptosis and CD8+ T-cell–induced cytotoxicity. *Diabetes* **57**: 415-423.

Rona G. 1985. Catecholamine cardiotoxicity. *J Mol and Cell Cardiol* **17**: 291-306.

Santagata S, Xu Y.-m, Wijeratne EK, Kontnik R, Rooney C, Perley CC, Whitesell L. 2012. Using the heat-shock response to discover anticancer compounds that target protein homeostasis. *ACS Chem Biol* **7**: 340-349.

Shah HS, Usman F, Ashfaq–Khan M, Khalil R, Ul-Haq Z, Mushtaq A, Iqbal J. 2020. Preparation and characterization of anticancer liposomal withaferin–A formulation for improved delivery to cancer cells: *In vitro, in vivo*, and *silico* evaluation. *J Drug Delivery Sci Technol* **59:** 101863.

Singh S, Pal S, Shanker K, Chanotiya CS, Gupta MM, Dwivedi UN, Shasany AK. 2014. Sterol partitioning by HMGR and DXR for routing intermediates toward withanolide biosynthesis. *Physiol Plant* **152**: 617-633.

Sivasankarapillai VS, Madhu Kumar Nair R, Rahdar A, Bungau S, Zaha DC, Aleya L, Tit DM. 2020. Overview of the anticancer activity of withaferin A, an active constituent of the Indian ginseng *Withania somnifera*. *Environ Sci and Pollut Res* **27**: 26025-26035.

Sohat B, Gitter E, Abraham A, Lavie D. 1967. Antitumor activity of withaferin A. *Cancer Chemother Rep* **51***:* 51271-51276.

Stan SD, Zeng Y, Singh SV. 2008. Ayurvedic medicine constituent withaferin causes G2 and M phase cell cycle arrest in human breast cancer cells. *Nutr Cancer* **60**: 51-60.

Straughn AR, Kakar SS. 2019. Withaferin A ameliorates ovarian cancer-induced cachexia and pro-inflammatory signaling. *J Ovarian Res* **12**: 1-14.

Sultana F, Neog MK, Rasool M. 2017. Withaferin-A, a steroidal lactone encapsulated mannose decorated liposomes ameliorates rheumatoid arthritis by intriguing the macrophage repolarization in adjuvant-induced arthritic rats. *Colloids Surf B: Biointerfaces* **155**: 349-365.

Suman S, Das TP, Moselhy J, Pal D, Kolluru V, Alatassi H, Damodaran C. 2016. Oral administration of withaferin A inhibits carcinogenesis of the prostate in the TRAMP model. *Oncotarget* **7**: 53751.

Tekula S, Khurana A, Anchi P, Godugu C. 2018. Withaferin-A attenuates multiple low doses of streptozotocin (MLD-STZ) induced type 1 diabetes. *Biomed Pharmacotherapy* **106**: 1428-1440.

Thirugnanasambantham P, Roy IM, Charles SN, Senthil K. 2014. Ontogenetic assessment of withanolide biogenesis and expression of selected pathway genes in *Withania somnifera*, a traditional medicinal herb. *J Pharm Res* **8**: 1344-1351.

Tit DM, Bungau S, Iovan C, Nistor Cseppento DC, Endres L, Sava C, Furau C. 2018. Effects of the hormone replacement therapy and soy isoflavones on bone resorption in postmenopause. *J Clin Med* **7**: 297.

Tiwari S, Atluri VSR, Yndart Arias A, Jayant RD, Kaushik A, Geiger J, Nair MN. 2018. Withaferin a suppresses beta-amyloid in APP expressing cells: studies for tat and cocaine-associated neurological dysfunctions. *Front Aging Neurosci* **10**: 291.

Vesa CM, Popa L, Popa AR, Rus M, Zaha AA, Bungau S, Zaha DC. 2020. Current data regarding the relationship between type 2 diabetes mellitus and cardiovascular risk factors. *Diagnostics* **10**: 314.

Walle T. 2009. Methylation of dietary flavones increases their metabolic stability and chemopreventive effects. *Int J Mol Sci* **10**: 5002-5019.

Xu K, Shi H, Du Y, Ou J. 2021. Withaferin A inhibits the proliferation of human endometrial cancer cells via transforming growth factor-â (TGF-â) signaling. *Biotech* **11**: 323.

Yokota Y, Bargagna-Mohan P, Ravindranath PP, Kim KB, Mohan R. 2006. Development of withaferin A analogs as probes of angiogenesis. *Bioorg Med Chem Lett* **16**: 2603-2607.

Yousefian Z, Hosseini B, Rezadoost H, Palazón J, Mirjalili MH. 2018. Production of the anticancer compound withaferin from genetically transformed hairy root cultures of *Withania somnifera*. *Nat Prod Commun* **13**: 943-948.

Yousuf SK, Majeed R, Ahmad M, lal Sangwan P, Purnima B, Saxsena A, Taneja SC. 2011. Ring A structurally modified derivatives of withaferin A and the evaluation of their cytotoxic potential. *Steroids* **76**: 1213-1222.

Zhang X, Samadi AK, Roby KF, Timmermann B, Cohen MS. 2012. Inhibition of cell growth and induction of apoptosis in ovarian carcinoma cell lines CaOV3 and SKOV3 by natural withanolide Withaferin A. *Gynecologic Oncol* **124**: 606-612.

Zomborszki ZP, Peschel W, Boros K, Hohmann J, Csupor D. 2016. Development of an optimized processing method for withania frutescens. *Acta Alimentaria* **45**: 452-456.