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Ashwagandha (Withania somnifera) - Its Antibacterial and Anticancer Activity

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Abstract

Introduction

Ashwagandha, a traditional Indian plant, is gaining increasive attention from scientists worldwide due to its potential health benefits, especially in the context of its antibacterial and anticancer properties.

Aim of the study

The aim of this review was to discuss recent findings regarding the impact of Withania Somnifera on antibacterial effect and its potential applications in anticancer therapy.

Materials and methods:

The paper was created based on the Pubmed and Google Scholar database. The literature was reviewed using the keywards: „Ashwagandha", „Withania Somnifera", „antibacterial ", „anticancer", „side effects", „breats cancer".

Results

Studies have confirmed Withania Somnifera, exhibits significant anticacterial and anticancer effects. It inhibits the growth of both Gram-negative and Gram-positive bacteria. Futhermore, it demonstrates anticancer activity, especially in breast cancer, by stimulating apoptosis, induction of cytotoxic effects on cancerous cells and increasing cell sensitivity to radiation.

Conclusion

Despite promising results, futher research on the benefits of Ashwagandha, particulary in the long term, is essential. There is growing need of more research of the potencial benefits of Ashwagandha in the treatment of various disorders including cancer and bacterial infections and to develop more effective therapeutic strategies. The need for large randomized studies comparing long term clinical effectiveness is emphasized.

Key words: „Ashwagandha"; „Withania Somnifera"; „antibacterial"; „anticancer"; „side effects"; „breast cancer".

1.Introduction

Ashwagandha, also referred to in literature by names such as Withania somnifera, Indian ginseng, winter cherry, is a plant known worldwide since ancient times. The earliest records of its usage trace back to around 6000 BCE in Sanskrit. Since then, it has become a very popular herbal remedy, widely utilized in those regions over the centuries. It is considered one of the most popular Indian plants. [2,8] Withania somnifera has been utilized in traditional medicine as an aphrodisiac, narcotic, tonic, diuretic, antibacterial medication, and stimulant. [1]

It is naturally cultivated in India, but the climate allows for its cultivation in other parts of the world, including Mediterranean countries, the Himalayas, African countries, the Canary Islands, China, Nepal, Afghanistan and Australia. Naturally found in drier regions. [16,37] Around 11,000 hectares of land in India are dedicated to ashwagandha cultivation. [16] The part of the plant most commonly used for the production of medicines and dietary supplements is

the root. Its name "Ashwagandha" originates from the word "ahwa," which means horse, suggesting that consuming the plant will provide strength equal to that of a horse. Additionally, "gandha" means smell, referring to the characteristic scent of the fresh root, making it easily distinguishable from other similar-looking plants devoid of its properties. [1]

1.1. Botanical description

Withania is a green, woody perennial shrub, with an average height of about 2 meters and an average width of about 1 meter. It consists of roots, stems, leaves, flowers, and fruits. The roots are characterized by their bulky shape, fleshy nature, branching, and fibrous structure. The other parts of the plant are more delicate. Withania somnifera is known for its strong odor, described as bitter or even irritating, hence the plant's name alludes to it. The economic worth of ashwagandha roots depends on their physical properties and morphology. [16,26]

1.2. Active compounds found in Ashwagandha

The highest concentration of biologically active molecules is found in the root of Ashwagandha, with smaller amounts also present in the stems and leaves. The majority of extracts from Withania somnifera are obtained from the roots. [3] The active compounds mainly include: flavonoids, tannins, alkaloids, glycosides, and steroidal lactones; saponins, withanolides, withanosides, withanolide glycosides also known as glyco-withanolides, steroid saponins with an additional acyl group, cuscohygrine, anahygrine, salts, coagulin, and many other compounds. [7,37] Withanolides are compounds to which most of the beneficial properties of the plant are attributed. These are steroid lactones with a C-28 structure. There are approximately more than 30 unique compounds of this type. [7,12] The most commonly described ones are withanolide A, withanone, and withanolide D. The structure of withanolides consists of a steroidal skeleton, which includes a lactone ring in the side chain at either C-8 or C-9. In the composition of the plant, numerous alkaloids can be found, at least 12 [16], with the most popular ones being izopelletierine, cuscohygrine, anahygrine, tropine, and withanine. [12] The discovery of novel ergosterol and 1,4-dioxane derivatives, as well as several fatty acids (including octacosane, oleic acid, and stearic acid), steroids, and oleanolic acid in the roots was reported. [26] The concentration of active substances and their percentage distribution can be altered and modified through changes in the growing conditions of the plant, primarily by manipulating the amount of light exposure. [17] Withanolides are important compounds that positively impact human physiology. Their action is similar to hormone precursors. They can indirectly influence hormone balance and contribute to overall well-being. [29]

1.3. Ashwagandha as a dietary supplement

Extract from Withania somnifera, most commonly from its root, is primarily produced and sold in the form of tablets and capsules. In recent years, there has been a significant increase in demand and supply for Ashwagandha in the form of dietary supplements. [4,10] Partly, this aligns with the World Health Organization's (WHO) strategy for 2014-2023, which aimed to support healthcare by incorporating traditional medicine as an alternative and complement to the treatment of various diseases. [4], but it also poses risks associated with the unregulated dietary supplement market and their uncontrolled consumption. These products are widely available, easy to purchase in stationary stores as well as online. However, not all of them undergo rigorous testing for safety and effectiveness. [10] According to research, the global Withania somnifera market is forecasted to grow at a compound annual growth rate, reaching an estimated value of over USD 100 million by the end of 2029. [16]

1.4. Uses of Ashwagandha

Demand for products derived from Ashwagandha is increasing, paralleled by a growing interest among scientists in its properties. For many years, Withania somnifera has been the subject of research all over the world. Analysis of numerous in vitro and in vivo studies has shown that it possesses properties such as anticancer, anti-stress, immunomodulatory, hypoglycemic, hypolipidemic, cardioprotective, antibacterial, and anticancer effects. [5,7,8,9]. Research conducted in vitro has demonstrated antioxidant and anti-inflammatory effects. Due to these properties, the use of this plant has found application in the treatment of stress, anxiety, cognitive disorders, joint inflammation, male infertility, and improvement in physical performance. [5,6,32] However, all these discoveries require verification and further additional research. Indeed, these studies provide hope for more effective treatments and the opportunity to explore new therapies for the aforementioned diseases and conditions. [10] In this study, we will focus on the influence of Ashwagandha on bacteria and cancer cells.

2. Dosage and side Effects, limitations of Withania somnifera intake

According to research, the daily dosage of Ashwagandha ranges from 6 grams to 10 grams in the form of powdered root or from 750 mg to 1250 mg of root/leaf extract. [14] Vaydia et al has examined the safety of using Withania somnifera. They conducted a clinical study involving eighteen healthy male participants, aged 18 to 60 years old. The study lasted four weeks, during which each participant took tablets twice daily, each containing 500 mg of Ashwagandha extract, including 7.5 mg of withanolides. The phisical and biochemical condition of the men remained within normal limits. In controlled studies, the results of

parameters including liver, kidney, and thyroid function remained within normal ranges. The medication was well-tolerated, and no adverse effects were observed. The study showed that healthy men can supplement Withania somnifera at a dose of 1000 mg per day for 4 weeks without the risk of adverse effects. This encourages further research in this direction, considering female participants, longer durations of medication intake, and other dosages.[20]

Langade et al study toxicity of dose administration of Withania somnifera root extract in Wistar rats. The study evaluated the sub-acute toxicity of repeated dose administration of Ashwagandha root extract in rats for 28 days, with 43 days of observation for a satellite group. The study involved thirty male and thirty female Wistar rats. They observed weight gain in all rats. Without any signs of intoxication and without changes in blood biochemistry. There were no abnormal histopathological changes observed in the organs The study shows that Ashwagandha root did not exhibit any major abnormalities at a dose five times higher than the recommended human dose. [19]

There is indeed limited data regarding the potential mutagenic effect of Ashwagandha on human cells. However, Kalaivani et al. conducted studies assessing the genotoxicity, evaluating the potential mutagenic effect of Withania somnifera (WS). In the study, they used a high dose of Withania somnifera root extract. The research was conducted on rats. No mortality, illnesses, or clinical signs of poisoning were observed for 3 days after the administration of Withania somnifera. The extract did not induce mortality even during oral administration at a dose of 2000 mg/kg. No mutations were observed in the examined cells following administration of high concentrations of the extract. [18] Supplementing Withania somnifera during pregnancy should not be recommended due to the limited amount of data regarding the safety of its use during pregnancy. [26]

In numerous clinical and preclinical studies, it has been demonstrated that Ashwagandha is safe, nontoxic, and its use provides benefits for the human body. This confirms the rationale for using this plant in traditional folk medicine. It represents a potential alternative to many synthetic drugs, which can cause dangerous adverse effects on human body. [1]

3. Antibacterial activity

Antibiotic resistance is one of the greatest threats to public health wordwide. In recent years, the escalating resistance has outpaced the development of new synthetic antimicrobial drugs. [21] There is a steady increase in infections caused by antibiotic-resistant strains. One of the main reasons is unjustified antibiotic therapy, the use of antibiotics without indications, and

prescribing therapy that is not in line with current guidelines. Since many years, scientists and doctors have been trying to find substances that can help combat pathogens. Some studies suggest that Ashwagandha could be an important and useful addition to antibiotic therapy [11,24], aiding in the fight against dangerous and potentially life-threatening infections. Withania somnifera is widely available, exhibits minimal side effects, and has demonstrated safety in use. It may also assist in antifungal and antiviral treatment. [1,22] Even without confirmation in scientific research and tests, Ashwagandha has been used as a remedy in folk medicine since ancient times as an anti-inflammatory and antimicrobial agent. [1] A greater antioxidant effect, and consequently, antibacterial effect, was demonstrated in studies for extracts obtained from fresh roots of the plant compared to extracts from fresh stems and leaves. This fact confirms the reason for more frequent use of roots over other parts of the plant. [25]

Compounds extracted from Withania somnifera have been tested for their potential use as antibacterial and antifungal agents. [23] So far, studies have confirmed the antibacterial properties of compounds such as withaferin A and 3-β-hydroxy-2,3-dihydrowithanolide F. Alkaloids and other similar polar compounds also exhibit antibacterial properties through activation of the immune system. [29] In numerous scientific studies, it has been proven that parts of the plant such as the root, leaves, and stem inhibit the growth of bacteria and fungi such as Salmonella typhimurium, Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Raoultella planticola, Pseudomonas aeruginosa, Enterobacter aerogens, Klebsiella pneumoniae, and fungi such as Fusarium oxysporum, Aspergillus flavus, and Fusarium verticilloides and Candida albicans. [23,1,42]

In the study, it was observed that withaferin A also inhibits the growth of dangerous viruses affecting the human body, such as COVID-19. [28,29] The antifungal properties were attributed to a monomeric glycoprotein isolated from the root of Ashwagandha. [29] Studies have shown antibacterial properties through the mechanism of damaging the cell membrane of bacteria. [22] It has also been demonstrated to have immunoprotective, cytotoxic, and gene silencing properties. [1]

Basavraj et al. conducted research against bacteria using the Agar Well Diffusion Method in vitro. They investigated water and alcohol extracts from various parts of the plant. Butanolic subfraction showed the highest inhibitory activity against bacteria, including Salmonella typhimurium. Withania somnifera did not cause lysis of human erythrocytes, unlike synthetic antibiotics used in therapy, which is an argument in favor of the safety of Withania somnifera. The research also showed that water extracts effectively eradicated Salmonella

infection in the organs of the treated animals, consequently enhancing their survival prospects. These results suggest the need for additional experiments in this area. [13]

Ezez et al. investigated the total content of active substances such as phenols and flavonoids, as well as the antioxidant properties of Withania somnifera against 2,2-diphenyl-1-picrylhydrazyl and hydrogen peroxide in solutions obtained by two separate methods: boiling and maceration. The highest content of phenols was observed in methanolic extracts obtained from maceration. The maximum elimination of aqueous radicals was observed in the methanolic extract. High activity against Escherichia coli was observed in the methanolic extract obtained from boiling during the study. The extract from withania somnifera showed antioxidant and antibacterial effect. [15]

In another study, the alcohol extract obtained from Withania somnifera leaves exhibited significant antibacterial activity against methicillin-resistant Staphylococcus aureus (MRSA) and Enterococcus species. [11] Escherichia coli and Pseudomonas aeruginosa were less sensitive to the action of the solutions. [11] The activity against Pseudomonas aeruginosa was also investigated in infected fish larvae. The study observed a reduction in the inflammatory response to the infection caused by this bacterium. This should provide a basis for further research into the inflammatory response. [22]

Sandhiya et al [27] also examined Withania somnifera. They investigated various parts of the plant, obtaining water and alcohol extracts. Antibacterial activity was observed. As in the studies mentioned above, the methanolic solvent extract was the most active against bacteria. Various parts of the plant were examined, and it was observed that the highest concentration of active substances, such as flavonoids and steroids, was present in the aqueous and methanolic solutions. These results may help in the search for new compounds with anti-inflammatory potential and in understanding their mechanisms in combating inflammatory conditions in the body.

Several studies have demonstrated substantial inhibition zones on diffusion discs when using extracts (prepared with both aqueous and alcoholic solvents) from the roots, leaves, and stems of the plant against both Gram-negative and Gram-positive bacteria. [23] These studies require broader scope, confirmation in in vitro and in vivo studies, with particular emphasis on specific substances and their concentrations contained in different parts of the plant. A thorough understanding of Withania somnifera, its active substances, and their mechanisms of action can help in the development of new antibacterial, antiviral, and antifungal therapies.

4. Anticancer effects

Cancer is a group of diseases in which cells divide uncontrollably due to mutations. Cancer remains a leading cause of death worldwide and is the subject of research by scientists from nearly every field. They pose health, economic, and social challenges, with the incidence of cancer continuously increasing. New treatment methods are being sought using all available means and resources, including naturally occurring plants in nature, such as Ashwagandha. [30]

4.1. The mechanism of Ashwagandha's anticancer activity

Research shows that Withania somnifera and its constituents have anticancer properties. [1,29,33] These compounds can be isolated from the roots, stems, or leaves, and the substances with anticancer activity are withanolides and withaferin A. One of their mainly advantages is the lack of toxic effects, unlike most chemotherapeutic agents. Withanolides suppress the inducible and expression of the NF-kappa B signaling pathway implicated in tumor progression. [29,31] In addition, Withania somnifera has shown antiproliferative effects, reducing oxidative stress, inhibiting cyclooxygenase-2, inducing targeted cytotoxic effects on cancer cells, and also exhibiting anti-angiogenic activity. [33]

Withania somnifera and its active ingredients kill mutated cells through at least several distinct mechanisms, including p53 signaling, GM-CSF signaling, death receptor signaling, apoptosis signaling, and DNA damage response in the G2/M phase. [33,30] Research has shown the potential effectiveness of Withania somnifera in treating various cancers such as colorectal, breast, lung, prostate, kidney, blood, liver, and skin cancer. [31,34] Researchers worldwide are investigating the properties and benefits associated with its use, yet its actual usage remains limited. The reason is the high variability in the composition, structure, and concentrations of active ingredients. This affects the pharmacokinetics of the product, resulting in ambiguous and non-reproducible therapeutic outcomes. [38] In this paper, we will focus on the impact of Ashwagandha on cancer treatment using breast cancer as an example, based on available medical literature and the latest scientific research.

4.2. Use of Ashwagandha in breast cancer

Breast cancer is the most common cancer among women and is one of the leading causes of death among cancers. Therefore, there is a growing need for novel therapeutic and preventative strategies, also those derived from medicinal herbs, to alleviate pain, improve survival rate, and decreace mortality rates. [36] Several in vitro and in vivo investigations

indicate that Withania somnifera holds promise for breast cancer treatment, particularly for ER/PR positive and triple-negative breast cancer.[33,30]

Biswal et al. investigated 100 breast cancer patients at various stages of the disease, who were either treated with chemotherapy alone or chemotherapy combined with Withania somnifera at a dose of 6g per day in three divided doses. The chemotherapy regimens used in the therapy were: Taxotere, Adriamycin, and Cyclophosphamide, or 5-fluorouracil, Epirubicin, and Cyclophosphamide. The 24-month overall survival for all stages of the disease in the study group and the control group was respectively 72% compared to 56%. The study group showed statistically significant less fatigue, leading to an improvement in the quality of life. The conclusion is that Ashwagandha demonstrates significant potential in fighting against breast cancer. Randomized studies on the largest possible group of patients are needed to confirm the efficacy of the plant and the results of this study. [35]

Abdulqawi et al [41] also evaluated the anti-cancer activity of alcoholic and water extracts of Withania somnifera against breast cancer cell lines MCF-7 in in vitro tests. Inhibition of tumor cell growth was observed in all extracts regardless of the solvent, depending on the higher concentration of the substances. The results encourage further pharmacological evaluation of active compounds so that they may be tested as potential new drugs for cancer treatment in the future. [41]

Antony et al found that Withaferin A affects the level of tubulin - a protein responsible for the structure of the spindle apparatus during cell division. It was demonstrated that WA induces mitotic arrest in breast cancer cells by reducing the level of β-tubulin. This effect is not observed with other naturally occurring compounds similar to WA. Additionally, the study showed that withaferin A binds to specific sites on β-tubulin, leading to disruption of the spindle apparatus structure. [42]

Joil et al. compiled numerous studies on the compound Withaferin A found in Withania somnifera, which in multiple studies has shown potential in inhibiting breast cancer growth. In the study, information was gathered regarding the effect of the substance on cell lines such as MDA-MB-231, SUM159, MDA-MB-468, SUM149, and 231MFP. [36] In vivo and in vitro studies have shown that Withaferin A exhibits significant inhibition of breast cancer cell growth. [34,39] Withaferin A induces apoptotic cell death in breast cancer cells, preceded by inhibition of the mitochondrial electron transport chain. [39] Withaferin A also sensitizes resistant cancer cells to current chemotherapy treatments. Due to these properties, Ashwagandha may potentially serve as a significant complementary treatment for breast cancer in the future. [36]

According to Kanagaraj et al., it was noted that compounds such as sitoindoside IX, somniferine, and withanone are effective in inhibiting proteins associated with breast cancer. Conversely, anaferine and isopelletierin were found to be less effective. [43] Studies have revealed that Withania somnifera may enhance the efficacy of radiation therapy in combating cancer cells by increasing their sensitivity to gamma radiation, commonly employed in breast cancer treatment. It appears to be a significant agent in combating drug resistance in cancer cells. [40]

5. Conclusions

Ashwagandha, also known as Withania somnifera, is a plant used since ancient times as a remedy with many properties and potential benefits. Extract from the roots of Ashwagandha is commonly used in dietary supplements due to its health-promoting properties. Withania somnifera contains many compounds rich in beneficial properties, such as withanolides, alkaloids, and flavonoids. Research has shown that the use of Ashwagandha extract is safe for health and is not associated with significant adverse effects. The described plant exhibits promising antibacterial activity in in vitro and in vivo studies, especially against antibiotic-resistant bacteria. It also shows high potential in anticancer activity, particularly in breast cancer treatment, by inhibiting the growth of cancer cells and increasing sensitivity to radiation. Withania somnifera may have a range of health benefits, but they require further clinical research to confirm and understand the full potential of this plant.

Author's contribution

Conceptualization, Aleksandra Mazurkiewicz, Wojciech Mądry and Justyna Marcicka; methodology, Joanna Męczyńska; software, Nazarii Saiuk; check, Joanna Męczyńska, Tomasz Seredyński and Michał Andrzej Kozicz; formal analysis, Magdalena Kołodziej and Adriana Wojciechowska; investigation, Adriana Wojciechowska and Weronika Salasa; resources, Magdalena Kołodziej; data curation, Justyna Marcicka; writing - rough preparation, Aleksandra Mazurkiewicz; writing - review and editing, Wojciech Mądry and Nazarii Saiuk; visualization, Michał Andrzej Kozicz; supervision, Tomasz Seredyński; project administration, Joanna Męczyńska; receiving funding, Weronika Salasa

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