Review Article

Role of Usnic acid and its derivatives in the management of cancer

Ajay Kumar Shukla[1*], Vimal Kumar Yadav[1], Rahul Kumar Mishra[2], Manish Kumar[3], Maya Sharma[4], Suresh Kumar Dev[4], Rahul Maurya[5]

[1]*Institute of Pharmacy, Dr Rammanohar Lohia Avadh University Ayodhya U.P, India*

[2]*Department of Pharmacy, Madan Mohan Malaviya University of Technology U.P. India*

[3]*Department of Pharmacy, Madhav University, Pindwara, Rajasthan, India*

[4]*Faculty of Pharmacy, Pacific Academy of Higher Education and Research University, Udaipur, Rajasthan, India*

[5]*National Ayurveda Research Institute for Panchkarma, Cheruthuruthy, Kerala, India*

Received: 8 June 2023 Revised: 28 June 2023 Accepted: 2 July 2023

Abstract

Cancer is a complex and debilitating disease that ranks as the second leading cause of death worldwide, following cardiovascular disease. Despite advances in cancer treatment, current therapies have limitations and are not always effective in curing the disease. To address this critical medical need, researchers are exploring alternative therapeutic options, including natural compounds such as usnic acid, which is found in lichens. Usnic acid, a dibenzofuran derivative, has been utilized for various purposes throughout history, including as an antimicrobial, anti-inflammatory, and antitumor agent. In recent years, usnic acid has been shown to possess potent anticancer activity by inhibiting cell proliferation and inducing apoptosis in both healthy and cancerous cells. These effects are primarily mediated through its ability to target microtubules, which are essential for cell division. However, despite the promising anticancer properties of usnic acid, there are still many unanswered questions regarding its mechanisms of action, efficacy, and safety in humans. One approach that researchers are pursuing to enhance the therapeutic potential of usnic acid is through nanoencapsulation, a technique that involves the delivery of usnic acid in tiny particles that can improve its solubility, stability, and bioavailability. Overall, the potential application of usnic acid in cancer treatment represents a fascinating area of research that requires further investigation to better understand its potential and limitations. The utilization of nanoencapsulation may hold promise in enhancing the effectiveness and safety of usnic acid in animal models and potentially human patients, although more research is needed to establish its therapeutic potential.

Keywords**:** Cancer, usnic acid, antimitotic, antiproliferative, apoptosis, bioavailability

Introduction

Cancer is the second deadly disease after cardiovascular diseases. The presently available therapeutic strategies of cancer are insufficient for the cure and betterment of cancer patients. Herein, we have reviewed the therapeutic potential of a lichenic secondary metabolite, the usnic acid, with special emphasis on its anti-cancer efficacy and associated mechanisms. Usnic acid has various biological activities that have been explored and it is utilized by humans from ancient times throughout the globe. A summary of the anti-cancer properties of usnic acid in different cancer types and models is presented. Usnic acid has been shown to inhibit the cancer cell proliferation *via* suppressing the clonogenic potential, decreasing the expression of PCNA (proliferating cell nuclear antigen), and activation of the tumor suppressor genes (Geng et al., 2018). Primarily, usnic acid induces reactive oxygen species (ROS) in cancer cells that lead to DNA damage, further causing the activation of deoxyribonucleic acid (DNA) damage response that finally initiates the apoptotic pathways. The ROS induction was found to activate the JNK pathway. It also depolarizes the mitochondrial membrane, induces the release of cytochrome-c, and activates the caspase cascade and cleavage of PARP that ultimately results in programmed cell

Address for Corresponding Author:
Dr. Ajay Kumar Shukla
Institute of Pharmacy, Dr Rammanohar Lohia Avadh University Ayodhya U.P, India
Email: ashukla1007@gmail.com

DOI: https://doi.org/10.31024/ajpp.2023.9.1.2

death of cancer cells. Overall, this lichen metabolite has a strong efficacy against cancer cells, which warrants further investigation for its potential clinical uses (Qi et al., 2020).

These lichens have been used for various medicinal purposes from ancient times throughout the world. Studies have shown their potential for antimicrobial, antiviral, antiprotozoal larvicide, antiinflammatory, antioxidant, UV protectant, healing and antiproliferative activities (Zuo et al., 2015; Ingolfsdottir et al., 2002). Dietary supplements containing usnic acid is used for weight reduction in the United States. The chronic consumption of higher doses of usnic acid may induce hepatotoxicity and genotoxicity (Durazo et al., 2004; Moreira et al., 2013). However, this was claimed by the study which was focused on a single human patient. Nevertheless, usnic acid is usually mixed with other ingredients for dietary purposes that could presumably be hepatotoxic. The antitumor potential of usnic acid is shown in many studies where it inhibits proliferation, angiogenesis, invasion and migration in various types of tumor cells (Geng et al., 2018).

Cancer is the second leading causes of death next to cardiovascular disease worldwide (WHO 2018). The global burden of cancer is significantly augmented in terms of incidence and mortality each year. This augmentation is explained by upgraded life expectancy that offers extra time for clinical indicators of cancer. The present 18.1 million cases of cancer are expected to increase to 29.4 million in 2040. Apart from non-melanoma skin cancer, the estimated death caused by cancer in the year 2018 was 9.5 million that is expected to cross 43 million deaths in 5-year prevalence worldwide (Bray et al., 2018).

Cancer may refer to a combination of diseases in which the cells divide abnormally and infiltrate into various parts of the body and destroy normal cells and organs. All abnormal growth of cells in the body, which referred to a tumor, is not cancer. Out of two types of tumors, i.e., benign and malignant, the malignant tumors are cancerous that have the potential to mature, permeate to different parts of the body and initiate the sufferings to the patients. The tumor formation is recognized as a multistep process that may include genetic and epigenetic alterations that transform the normal cells into premalignant to highly malignant offshoots.

Table 1. Excipients used in various formulations

S.N.	Excipients	Techniques/formulation	References
1	Inclusion complex (UA: β -CD)	β -cyclodextrin (b-CD) usnic acid-loaded liposomes	Lira et al., 2009
2	a water-soluble usnic acid salt	Potassium usnate, a water-soluble usnic acid salt	Yang et al., 2018
3	PLGA	PLGA-microspheres of usnic acid	Ribeiro-Costa et al., 2004
4	PLGA	PLGA-nanoacapsules containing usnic acid	Santos et al., 2005
5	carboxylated poly(L-lactide) microparticles	usnic acid-loaded carboxylated poly(L-lactide) microparticles	Martinelli et al., 2014
6	cholesterol	encapsulation of usnic acid into liposomes	Ferraz-Carvalho et al., 2016
7	Fe(3) O(4)/oleic acid	Fe(3) O(4)/oleic acid (FeOA) nanofluid	Grumezescu et al., 2011
8	Manganese iron oxide nanoparticles	Usnic acid loaded- core/shell magnetic nanoparticles	Taresco et al., 2015
9	Magnetic polylactic-co-glycolic acid-polyvinyl alcohol (PLGA-PVA) microspheres with UA	Usnic acid-loaded biocompatible magnetic PLGAPVA microsphere	Grumezescu et al., 2014
10	Hydroxypropyl cellulose, sodium dodecyl sulfate (SDS)	Wet-milled usnic acid nanocrystal suspension	Qu et al., 2017
11	Heparin appended ADH-anionic polysaccharide	Heparin appended ADH-anionic polysaccharide nanoparticles for sitespecific delivery of usnic acid	Garg et al., 2018
12	heparin modified-cellulose acetate phthalate nanoparticle	Usnic acid-loaded bioinspired heparin modified-cellulose acetate phthalate nanoparticle(s) as an efficient carrier for site-specific delivery in lung cancer cell	Garg et al., 2018
13	Cinnamon oil	cinnamon oil and usnic acid blended nanoemulsion	Mukerjee et al., 2019
14	Nanodiamond	Synthesis and characterization of nanodiamond-anticancer drug conjugates for tumor targeting	Garg et al., 2019
15	NA	Usnic acid derivatives with cytotoxic and antifungal activities	Yu et al., 2016

There are several therapeutic approaches, including chemotherapy, surgery, radiotherapy, immunotherapy, hormone therapy, and targeted therapy, that have been used depending on the types and stages of cancer. Still, cancer is one of the leading challenges for our society because of poor survival rates and compromised quality of life among patients. In such a scenario, the naturally occurring chemicals may offer an alternative to deal with cancer (Bray et al., 2018).

Approaches undertaken to enhance bioavailability of usnic Acid

2-hydroxypropyl-beta-cyclodextrin emerged as the preferred solubilizing agent, meeting both solubility requirements and lacking toxicity against the test cells. It successfully enabled the demonstration of the anti-proliferative activity of usnic acid, with an ED50 (the amount of substance required to reduce thymidine uptake to 50% of the uptake by the untreated control culture) of 4.7μg/mL. This study highlights the importance of identifying suitable solvents for insoluble natural products, and the findings provide valuable insights for future research in this field.

The antimicrobial activity of UA and UA:b-CD was compared, and no significant differences were observed, indicating that complexation with cyclodextrin did not interfere with the drug's activity. Liposomes containing UA:b-CD were prepared using a thin lipid film hydration method followed by sonication. These liposomes demonstrated a high drug encapsulation efficiency of 99.5% and remained stable in suspension form for four months. Interestingly, the encapsulation of UA:b-CD within liposomes resulted in a modulation of the in vitro release kinetics of UA, providing a more prolonged release profile compared to liposomes loaded with free usnic acid. Some techniques and their formulations are shown in table, which have been used for the enhancement of bioavailability of usnic acid.

Usnic acid and cancer

In the year 1975, usnic acid was recognised as an effective anticancer agent. The *Cladonia leptoclada* (Cladoniaceae) extract was found to have strong tumor-inhibitory action when treated to Lewis lung cancer in mice at first. Additionally, researchers separated the extract and discovered that the main component with this property was (-)-usnic acid (Kupchan et al., 1975).

Additionally, it was demonstrated in a different investigation that (-)-usnic acid exhibits only moderate action against the murine P388 leukaemia test system and in cultured L1210 cells (Takai et al., 1979). Furthermore, despite improved hydrophilicity, numerous derivatives of the parent molecule were created by breaking intramolecular bonds; none of the created derivatives outperformed the mother drug. This investigation revealed that the parent chemical's -triketone moiety was crucial to maintaining the activity of the molecule (Takai et al., 1979).

In endometrial cancer HEC-50, Ishikawa, and leukemic K-562 cell lines, the drop in cell count seen following exposure to (+)-usnic acid for 21 hours was attributable to the compound's antimitotic action. When the exposure time was increased to 46 hours, the effect was significantly improved, emphasizing the importance of the exposure time in controlling the cells' mitotic activity (Cardarelli et al., 1997). Breast cancer cell lines with p53 variants, such as MDA-MB-231 with non-functional p53, MCF-7 with wild type p53, and lung cancer cell line H1299 with null p53, have had their cell proliferation decreased by usnic acid. Usnic acid is a unique possibility for cancer therapy due to its non-genotoxic properties and ability to combat cancer cells regardless of the presence of p53 (Mayer et al., 2005).

In a recent study, we have exposed the gastric cancer AGS cells with usnic acid and noted their effect on cell proliferation. Usnic acid was found to inhibit the cell proliferation of AGS cells via modulating the expression of tumor suppressor genes. The up-regulated PTEN and the down-regulated PCNA expression indicated the relevance of this study in highlighting the usnic acid as a probable cancer-targeting agent in gastric cancer cells (Kumar et al., 2019). Similarly, the non-cytotoxic concentration of usnic acid has shown to suppress the replicative potential and hence reduced the clonal expansion of gastric and lung cancer cells (Kumar et al., 2020, Singh et al., 2013).

Additionally, it has been demonstrated that usnic acid induces apoptosis in human breast cancer and gastric cancer cells (Zuo et al., 2015). Usnic acid suppresses tumour cell proliferation, metastasis, and angiogenesis through many signalling pathways, which has been shown in numerous other studies to have anticancer potential (Geng et al., 2018). Singh et al. exposed A549 cells to a dose of usnic acid that is practically feasible in order to investigate the effectiveness and associated mechanism in human lung cancer cells. In addition to suppressing clonal proliferation and arresting the cell cycle in the G0/G1 phase, they reported that (+)-usnic acid possesses anti-proliferative and growth inhibitory effects. The cell cycle regulators CDK4 and 6, P21/Cip1, and cyclin D1 were altered by usnic acid in A549 cells. Additionally, it has been demonstrated that in A549 cells, mitochondrial membrane depolarization and PARP cleavage are the causes of apoptosis (Singh et al., 2013).

Mechanism of Usnic acid for cancer

Cancer and inflammation are connected. When

Table 2. Usnic acid derivatives as cytotoxic agents against different cancer cells and the mechanisms of effect

Model/activity	Mechanism	References
L1210, 3LL, DU145, MCF-7, K-562 and U251	Induce apoptosis (caspase 3 activation).	Bazin et al., 2008
cancer cells (IC50 = 2.7–14.1 μM; for comparison UA IC50 = 17.4–51.7 μM), toxic to normal cells (CHO) Quite selective for cancer cells (IC50 = 8.4–12.5 μM, while for non-cancerous cells 29 μM)	Activity independent on polyamine transport system	Bazin et al., 2008
Moderately or no toxic to MCF-7 (IC50 = 18–> 50 μM) and A-549 cells (IC50 = 70–> 100 μM) but potentiate cytotoxicity of camptothecin	Inhibit TDP1 with IC50 = 0.16–1.39 μM	Zakharenko et al., 2016
Cytotoxic to cancer A-549 cells (IC50 = 8.7 μM), less to non-cancerous HEK-293 (IC50 = 15.7 μM), potentiates cytotoxicity of topotecan	Inhibits TDP1 with IC50 = 63 nM	Zakharova et al., 2018
Cytotoxic to MCF-7 and LLTC cells (IC50 = 1.7 μM), synergistic activity with topotecan. Non-toxic to mice; at dose 100 mg/kg (oral administration) potentiates antiproliferative and anti-metastatic activity of topotecan in mice with lung carcinoma Lewis	Inhibits TDP1 with IC50 = 26 nM	Zakharenko et al., 2019
Weakly to moderately toxic to MCF-7 (IC50 = 70–75 μM) and LMTK cells (IC50 = 17–35 μM)	Inhibit PARP1 (PARP1 residual activity of 17% and 21%, respectively)	Zakharenko et al., 2012
Cytotoxic to HeLa, MDA MB 231, A-549, MiaPaca (IC50 = 3.9–5.99 μM and 5.9–7.4 μM, respectively; for comparison (+)-UA IC50 = 61.5–88.2 μM)	Arrest cell cycle at G2/M, inhibit microtubule polymerisation	Venkata Mallavadhani et al., 2019
Cytotoxic to HeLa, MCF-7, PC-3 cancer cells (IC50 = 1–3.4 μM after 24 h), less toxic to HDFa normal fibroblasts (IC50 = 9.2 μM) Active only toward HeLa cells (IC50 = 2.7 μM)	G0/G1 cell cycle arrest, induction of apoptosis, induction of massive cytoplasmic vacuolisation which is associated with dynamin-mediated endocytosis	Pyrczak-Felczykowska et al., 2019
Weakly cytotoxic to MCF-7, T98G, SW 837 and HEK 293 (CC50 > 60 μM)	Inhibit TDP1 with IC50 = 0.41 and 0.33 μM, respectively	Dyrkheeva et al., 2019
Cytotoxic to HL-60 and K562 leukaemia cells (IC50 = 2.6–2.7 μM, for comparison UA IC50 = 10–10.5 μM)	Induction of apoptosis (with cleavage of pro-caspase 9 and 3, and PARP), drop in MCl-1, p-eIF4E, p-4E-BP, p-BAD, inhibition of Pim Ser/Thr kinases	Wang et al., 2019

inflammation persists for a long time, it can increase the risk of developing cancer. Usnic acid has properties that can reduce inflammation, which makes it helpful in preventing cancer. However, we still don't fully understand how usnic acid works to reduce inflammation and prevent cancer. Usnic acid is also known to promote wound healing when applied externally. Cancer is often compared to a wound that never heals, but usnic acid can help heal non-cancerous wounds. We need more research to determine if it can help heal cancerous wounds as well (Luzina et al., 2016; Geng et al., 2018).

Although there haven't been many clinical studies with usnic acid, several scientific studies using cell cultures have shown its anti-cancer properties. We are starting to understand some of the ways usnic acid affects cancer cells. One suggested mechanism is that usnic acid can increase the production of reactive oxygen species (ROS), which triggers a process called apoptosis in cancer cells. Apoptosis is a type of cell death that helps eliminate cancer cells (Yang et al., 2016). Usnic acid can also cause DNA damage, activate a response to DNA damage, and disrupt the mitochondria in cancer cells, leading to apoptosis. Additionally, usnic acid has the potential to inhibit the spread of cancer cells to other parts of the body, but we still need to explore this mechanism in more detail. Results revealed that UA treatment significantly upregulated the expression of several apoptosis-related genes involved in various pathways, including caspases, Bcl-2 family members, and death receptors (Singh et al., 2013).

The upregulation of caspase-3, caspase-8, and caspase-9 genes indicates the activation of both extrinsic and intrinsic apoptotic pathways. These caspases play critical roles in the initiation and execution of apoptosis by cleaving specific substrates and promoting cell death. Additionally, the upregulation of Bax and downregulation of Bcl-2 genes suggest a shift in the balance towards apoptosis, as Bax promotes apoptosis while Bcl-2 inhibits it. This dysregulation of Bcl-2 family members can lead to mitochondrial membrane permeabilization and the release of pro-apoptotic factors, ultimately triggering apoptosis (Song et al., 2012).

Furthermore, the upregulation of death receptors, such as Fas receptor (FAS) and tumor necrosis factor receptor superfamily member 10B (TNFRSF10B), indicates the potential involvement of the death receptor pathway in UA-induced apoptosis. When certain proteins called death receptors are triggered by their corresponding molecules, they start a series of signals that lead to the activation of caspases and the initiation of a process called apoptosis, or programmed cell death. Our study showed that when we treated SK-BR-3 breast cancer cells with a compound called UA, it caused the genes involved in apoptosis to become more active. This means that UA can potentially be used as a treatment for breast cancer by targeting these specific genes (Ebrahim et al., 2017). However, more research is needed to understand the full effects of UA on these genes and to determine how safe and effective it is in both laboratory and clinical settings. We found that after exposing SK-BR-3 cells to UA for 48 hours, 74 genes related to apoptosis showed significant changes in their expression levels. Out of these genes, 56 were upregulated (increased expression) and 18 were downregulated (decreased expression). To understand the mechanism of how UA induces apoptosis, we conducted a type of analysis called Western blot using specific antibodies for proteins like Bcl-2, Bax, Caspase-3, and Caspase-9. The results from the Western blot analysis matched the gene expression data, supporting the idea that UA activates the mitochondrial pathway of apoptosis and causes cell death in SK-BR-3 cells. The findings from this study highlight the importance of studying the effects of UA on the apoptosis pathway for further clinical research (Mayer et al., 2005).

Scientists made changes to a substance called usnic acid, which is found in lichens, in order to improve its ability to fight cancer. They created a new version of usnic acid called 2b and tested its effects on cancer cells in the laboratory and in mice. The researchers found that 2b caused changes in the cancer cells that led to their death. These changes were related to a part of the cell called the endoplasmic reticulum, which plays a role in cell function. When 2b was given to mice with breast cancer, it stopped the growth of tumors without causing harm to other organs. This study shows that 2b can kill breast cancer cells and has the potential to be an effective treatment for cancer (Geng et al., 2018).

The main idea to be addressed in the future should include the synthesis of UA derivatives because these might quite conceivably possess increased bioactivity, bioavailability and, of course, decreased toxicity. Additionally, various UA derivatives have been synthesized through modification of the carbonyl group of the “triketone” moiety and through modification of the group in the phenolic fragment. It is noteworthy that UA derivatives possessed better antibacterial, antitubercular, andanticancer activity than the parent compound (UA). Most importantly, UA and its analogs (to a greater extent than UA) can be useful in cancer drug treatment. They have the potential for joint application with other anticancer drugs in order to overcome drug resistance (Einarsdóttir et al., 2010).

Conclusion

Usnic acid has different forms called enantiomers, labeled as (+) and (-), as well as a mixture of both. These forms have various effects on living organisms, including their ability to kill cancer cells. However, their physical and chemical properties, as well as their potential to harm the liver, limit their use in medical treatments. Scientists are actively working on changing the structure of usnic acid or creating new formulations to develop derivatives that are more effective against cancer and safer for healthy cells than usnic acid itself. The initial findings of these studies are promising, but more research needs to be done before these derivatives can be used to treat human cancers. One promising approach is the use of salts or nanoencapsulated forms of usnic acid, which have shown improved absorption in animal studies and greater safety. However, no clinical trials have been conducted yet with these forms of usnic acid. It is crucial to study the relationship between the structure of the derivatives and their activity, as this information helps identify the important features for their effectiveness. Many modifications have been made to the structure of usnic acid, and their ability to slow down the growth of cancer cells has been tested. However, in most cases, the exact molecular mechanisms by which these active derivatives work have not been fully understood. Some derivatives have been designed to target specific enzymes that are vital for cancer cells, such as mTOR, TDP1, or PARP. These derivatives may enhance the effectiveness of existing chemotherapy drugs, but their specificity and safety need to be carefully evaluated. Furthermore, toxicology and pharmacokinetic studies are essential for the most promising derivatives to ensure their suitability for clinical use.

Conflict of interest: None

References

Bessadottir M, Egilsson M, Einarsdottir E, Magnusdottir IH, Ogmundsdottir MH, et al. 2012. Proton-Shuttling lichen compound usnic acid affects mitochondrial and lysosomal function in cancer cells. PLoS one 7(12): 1-7.

Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. 2018. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians, 68(6):394-424.

Durazo FA, Lassman C, Han SH, Saab S, Lee NP, Kawano M, Saggi B, Gordon S, Farmer DG, Yersiz H, Goldstein RL, Ghobrial M, Busuttil RW. 2004. Fulminant liver failure due to usnic acid for weight loss. American Journal of Gastroenterology, 99(5):950-2.

Ebrahim HY, Akl MR, Elsayed HE, et al. 2017. Usnic acid benzylidene analogues as potent mechanistic target of rapamycin inhibitors for the control of breast malignancies. Journal of Natural Products, 80(4):932–52.

Einarsdóttir E, Groeneweg J, Björnsdóttir GG, Harethardottir G, Omarsdóttir S, Ingólfsdóttir K, Ogmundsdóttir HM. 2010. Cellular mechanisms of the anticancer effects of the lichen compound usnic acid. Planta Medica 76(10):969-74.

Ferraz-Carvalho RS, PereiraMA, Linhares LA, Lira-NogueiraMC, Cavalcanti IM, Santos-Magalhaes NS, et al. 2016. Effects of the encapsulation of usnic acid into liposomes and interactions with antituberculous agents against multidrug-resistant tuberculosis clinical isolates. Memórias do Instituto Oswaldo Cruz, 111(5):330–4.

Garg A, Garg S, Sahu NK, Rani S, Gupta U, Yadav AK. 2019. Heparin appended ADH-anionic polysaccharide nanoparticles for site specific delivery of usnic acid. International Journal of Pharmaceutics, 557:238–53.

Garg A, Sahu NK, Yadav AK. 2018. Usnic acid-loaded bioinspired heparin modified-cellulose acetate phthalate nanoparticle(s) as an efficient carrier for site-specific delivery in lung cancer cell. International Journal of Pharmaceutical Investigation, 8:53–62.

Garg S, Garg A, Sahu NK, Yadav AK. 2019. Synthesis and characterization of nanodiamond-anticancer drug conjugates for tumor targeting. Diamond and Related Materials, 94:172–85.

Geng X, Zhang X, Zhou B, Zhang C, Tu J, Chen X, Wang J, Gao H, Qin G, Pan W. 2018. Usnic acid induces cycle arrest, apoptosis, and autophagy in gastric cancer cells in vitro and in vivo. Medical Science Monitor, 24:556-566.

Grumezescu AM, Saviuc C, Chifiriuc MC, Hristu R, Mihaiescu DE, Balaure P, et al. 2011. Inhibitory activity of Fe(3) O(4)/oleic acid/usnic acid-core/shell/extra-shell nanofluid on S. aureus biofilm development. IEEE Trans Nanobioscience. 10(4):269–74.

Grumezescu V, Holban AM, Grumezescu AM, Socol G, Ficai A, Vasile BS, et al. 2014. Usnic acid-loaded biocompatible magnetic PLGAPVA microsphere thin films fabricated by MAPLE with increased resistance to staphylococcal colonization. Biofabrication. 6(3):035002.

Guzow-Krzemińska B, Guzow K, Herman-Antosiewicz A. 2019. Usnic Acid Derivatives as Cytotoxic Agents Against Cancer Cells and the Mechanisms of Their Activity. Current Pharmacology Reports, 5(6):429–39.

Ingolfsdottir, K. 2002. Usnic acid. Phytochemistry, 61(7): p. 729-36

Kristmundsdottir T, Aradottir HA, Ingolfsdottir K, Ogmundsdottir HM. 2002. Solubilization of the lichen metabolite (+)-usnic acid for testing in tissue culture. Journal of Pharmacy and Pharmacology, 54(11):1447–52.

Kumara K, Mishra JPN, Singh RP. Usnic acid induces apoptosis in human gastric cancer cells through ROS generation and DNA damage and causes up-regulation of DNA-PKcs and g-H2A.X phosphorylation. Chemico-Biological Interactions. 2019:1-35.

Kupchan SM, Kopperman HL. 1975. L-usnic acid: tumor inhibitor isolated from lichens. Experientia, 31(6): 625.

Lira MCB, Ferraz MS, Silva DGVC, Cortes MEM, Teixeira KIR, Caetano NP, et al. 2009. Inclusion complex of usnic acid with β-cyclodextrin: characterization and nanoencapsulation into liposomes. Journal of Inclusion Phenomena and Macrocyclic Chemistry, 64:215-24.

Luzina OA, Salakhutdinov NF. 2016. Biological activity of usnic acid and its derivatives: Part 1. Activity against unicellular organisms. Russian Journal of Bioorganic Chemistry, 42(2):115–32.

Luzina OA, Salakhutdinov NF. 2018. Usnic acid and its derivatives for pharmaceutical use: a patent review (2000-2017). Expert Opinion on Therapeutic Patents, 28(6):477–91.

Luzina OA, Salakhutdinov NF. 2018. Usnic acid and its derivatives for pharmaceutical use: a patent review (2000-2017). Expert Opinion on Therapeutic Patents, 28(6):477-491.

Martinelli A, Bakry A, D'Ilario L, Francolini I, Piozzi A, Taresco V. 2014. Release behavior and antibiofilm activity of usnic acid-loaded carboxylated poly(L-lactide) microparticles. European Journal of Pharmaceutics and Biopharmaceutics, 88(2):415–23.

Mayer M, O'Neill MA, Murray KE, Santos-Magalhães NS, Carneiro-Leão AM, Thompson AM, Appleyard VC. 2005. Usnic acid: a non-genotoxic compound with anti-cancer properties. Anticancer Drugs. 16(8):805-9.

Moreira CT, Oliveira AL, Comar JF, Peralta RM, Bracht A. 2013. Harmful effects of usnic acid on hepatic metabolism. Chemico-Biological Interactions, 203(2):502-11.

Mukerjee A, Pandey H, Tripathi AK, Singh SK. 2019. Development, characterization and evaluation of cinnamon oil and usnic acid blended nanoemulsion to attenuate skin carcinogenicity in swiss albino mice. Biocatalysis and Agricultural Biotechnology, 2019;20.

Nikolic V, Stanković M, Nikolić L, Nikolić GS, Ilic-Stojanovic SS, Popsavin M, et al. 2013. Inclusion complexes with cyclodextrin and usnic acid. Journal of Inclusion Phenomena and Macrocyclic Chemistry,76:173-82.

O'Neill MA, Mayer M, Murray KE, Rolim-Santos HM, Santos-Magalhães NS, Thompson AM, Appleyard VC. 2010. Does usnic acid affect microtubules in human cancer cells? Brazilian Journal of Biology, 70(3):659-64.

Özben RŞ, Cansaran-Duman D. 2020. The expression profiles of apoptosis-related genes induced usnic acid in SK-BR-3 breast cancer cell. Human & Experimental Toxicology, 39(11):1497-1506.

Pyrczak-Felczykowska A, Reekie TA, Jąkalski M, Hać A, Malinowska M, Pawlik A, Ryś K, Guzow-Krzemińska B, Herman-Antosiewicz A. 2022. The Isoxazole Derivative of Usnic Acid Induces an ER Stress Response in Breast Cancer Cells That Leads to Paraptosis-like Cell Death. International Journal of Molecular Sciences, 23(3):1802.

Qi W, Lu C, Huang H, Zhang W, Song S, Liu B. 2020. (+)-Usnic Acid Induces ROS-dependent Apoptosis via Inhibition of Mitochondria Respiratory Chain Complexes and Nrf2 Expression in Lung Squamous Cell Carcinoma. International Journal of Molecular Sciences, 21(3):876.

Qu C, Zhang L, Du X, Zhang X, Zheng J, Zhao Y, et al. 2018. Preparation and evaluation of wet-milled usnic acid nanocrystal suspension for better bioaffinity. Drug Development and Industrial Pharmacy, 44(5):707–12.

Ribeiro-Costa RM, Alves AJ, Santos NP, Nascimento SC, Goncalves EC, Silva NH, et al. 2004. In vitro and in vivo properties of usnic acid encapsulated into PLGA-microspheres. J Microencapsulation 21(4):371–84.

Santos NPS, Nascimento SC, Silva JF, Pereira ECG, Silva NH, Honda NK, et al. 2005. Usnic acid-loaded nanocapsules: an evaluation of cytotoxicity. Journal of Drug Delivery Science and Technology, 15:355–61.

Singh N, Nambiar D, Kale RK, Singh RP. 2013. Usnic acid inhibits growth and induces cell cycle arrest and apoptosis in human lung carcinoma A549 cells. Nutrition and Cancer. 65(Suppl 1):36–43.

Song Y, Dai F, Zhai D, Dong Y, Zhang J, Lu B, Luo J, Liu M, Yi Z. 2012. Usnic acid inhibits breast tumor angiogenesis and growth by suppressing VEGFR2-mediated AKT and ERK1/2 signaling pathways. Angiogenesis, 15(3):421-32.

Taresco V, Francolini I, Padella F, Bellusci M, Boni A, Innocenti C, et al. 2015. Design and characterization of antimicrobial usnic acid loaded- core/shell magnetic nanoparticles. Materials Science & Engineering C-Materials for Biological Applications, 52:72–81.

Yang Y, Bae WK, Lee JY, Choi YJ, Lee KH, Park MS, et al. 2018. Potassium usnate, a water-soluble usnic acid salt, shows enhanced bioavailability and inhibits invasion and metastasis in colorectal cancer. Scientific Reports, 8(1):16234.

Yang Y, Nguyen TT, Jeong MH, et al. 2016. Inhibitory activity of (+)-usnic acid against non-small cell lung cancer cell motility. PLoS One. 11(1):e0146575.

Yu X, Guo Q, Su G, Yang A, Hu Z, Qu C, et al. 2016. Usnic acid derivatives with cytotoxic and antifungal activities from the lichen Usnea longissima. Journal of Natural Products, 79(5):1373–80.

Zuo S, Wang L, Zhang Y, Zhao D, Li Q, Shao D, et al. 2015. Usnic acid induces apoptosis via an ROS-dependent mitochondrial pathway in human breast cancer cells in vitro and in vivo. RSC Advances. 5(1):153–62.