INTRODUCTION

Lichens are a group of composite organisms that actually are composite organisms formed by a symbiotic relationship between a fungus (mycobiont) and an algal or cyanobacteria. In the symbiotic cooperation, the bacteria provide the fungus with its organic metabolites and the fungus supply water with dissolved mineral salts.\(^1\)\(^-\)\(^3\) Lichens able to live and survive under excessive life conditions, where they can live in air, rainfall, dew, fog, and soil. It is estimated that lichens cover around 8% of the earth surface with more than 17,000 species and over 800 lichen products are known.\(^4\) A lot of researchers were interested in lichens products, such as polysaccharides, proteins and secondary metabolites regarding their biological activities as medications, food supplements, dyes, where the most famous lichen secondary metabolite is usnic acid (UA).\(^5\) First time to isolate Usnic acid (UA) from lichen metabolite was in 1844, and from that year a lot of studies were done on UA, and it became commercially available in the market. Its wide availability in different lichen species, being isolated easily, and high purity of the isolated product make it an excellent base for producing new pharmaceuticals. In this review the different usage of UA was summarized. It was utilized as an antioxidant, anti-proliferative, antimicrobial and antiprotozoal, larvical and insecticidal, antifungal, antiviral, algidial, anti-inflammatory, pain-relieving and antipyretic agent. Many adverse effects were associated with using usnic acid, especially at high dose, including; hepatotoxicity, genotoxicity, allergenicity, side effects on the cardiovascular system and adipocytes of fatty tissue. This review aimed to throw the light on the updated biological activities, effectiveness and safety and usage of usnic acid during the last decade.

ABSTRACT

Since Usnic acid (UA) was isolated from lichen metabolite in 1844, a lot of studies were conducted on it and now it became commercially available in the market. Its wide availability in different lichen species, being isolated easily, and high purity of the isolated product make it an excellent base for producing new pharmaceuticals. In this review the different usage of UA was summarized. It was utilized as an antioxidant, anti-proliferative, antimicrobial and antiprotozoal, larvical and insecticidal, antifungal, antiviral, algidial, anti-inflammatory, pain-relieving and antipyretic agent. Many adverse effects were associated with using usnic acid, especially at high dose, including; hepatotoxicity, genotoxicity, allergenicity, side effects on the cardiovascular system and adipocytes of fatty tissue. This review aimed to throw the light on the updated biological activities, effectiveness and safety and usage of usnic acid during the last decade.

Keywords: Animal, Cytotoxicity, Human, Hepatotoxicity, Lichen, Nephrotoxicity, Neurotoxicity, Usnic Acid
biological activity of UA aimed to review the publications of UA (effectiveness and safety) during the last decades.

In the Traditional Chinese Medicine, the first used and record of U. species was found in dates to 101 B.C as antimicrobial with the name “Song Lo, Song Lo tea”, it was used for cleaning the liver, treating malaria, healing wounds and snake bite with common dose 6-9 g of dried lichen, it was classified as rare medication.

For long time it was used as antibacterial in many nations and being utilized as a modern pharmaceutical till the penicillin antibiotics were discovered. Where it was used as main agent or as preservative for treating pulmonary tuberculosis, pain, fever, wounds, athlete’s foot, deodorants, and herbal tinctures, with different names regarding the country, in Germany “Monogram”, in Finland, “Rosalina Thrust” and in Argentina, “Barba del la Piedra”. From the end of 2nd world war to 1950s, the work on UA focused on its antimicrobial activity. Then it was activated again in 1980s due to the problem bacterial resistance.

Nature of usnic acid

Usnic acid is produced from several lichen genera such as Cladonia (Cladoniaceae), Usnea (Usneaceae), Lecanora (Lecanoraceae), Ramalina (Ramulinaeace), Evernia, Parmelia (Parmeliaceae), Alectoria (Alectoriaceae). See Figure 1. Ramalina was the first genus to isolate usnic acid in 1843, after one year it was distinguished from other substances and get its name, later by nine decades the chemical structure was determined.

Castle and Kubisch were the first to report that UA is produced within the mycobiont (fungal part) of the lichen and then make a layer on the surface of the photobiont. Lichens’ usnic acid content relies on several factors: geographic location, the time of summer solstice, insolation, and temperature. Where UA represents up to 8% of dry weight regarding seasons with the highest level in late spring and early summer and lower levels in autumn and winter.

Structure of usnic acid

Usnic acid was isolated for the first time as yellow and crystalline a secondary lichen metabolite by the German scientist Knop in 1844. The full scientific name is [2, 6-diacetyl-7, 9-dihydroxy-8, 9 b-dimethyl-dibenzofuran-1, 3(2H, 9bH)-Dione], it has two enantiomers; (+) D-usnic acid and (-) L-usnic acid, due to R or S dropping of the angular-CH3 group at position 9b (Figure 2). Each enantiomer has different biological activities. Two other natural isomers (+) and (-) isousnic acids [2, 8-diacetyl-7, 9-dihydroxy-6, 9b-dimethyl dibenzofuran-1, 3(2H, 9bH)-Dione were found in lichens. On the other hand, UA can be chemically synthesized from methylphloracetophenone first by oxidative coupling, then by hydrolysis in sulfuric acid.

Pharmacokinetics of usnic acid

There is limited number of studies described the in vivo pharmacokinetics of UA including absorption, distribution, metabolism and excretion. A study on rats received parenteral UA (25mg kg1) showed that UA was distributed into different tissues with high levels in liver and lung tissue followed by blood with "mean tissue/plasma ratios of 1.78". Another study on rabbits received UA (25mg kg1) by oral administration showed a plasma concentration of 32.5 6.8μgml -1 in 12.2 3.8 h. This indicated that UA was widely bound to protein with approximately 99.2% of bound. In 2008 a study was conducting to evaluate the metabolism of UA in isolated human liver microsomes pre-incubated with UA and several CYP inhibitors. The authors reported that the oxidative metabolism of UA is mainly mediated by CYP1A2. In this study, it was mentioned that the half-life of UA in human liver microsomes was 19.3 min with an intrinsic clearance of 45.24 ml/min kg1. Similar result was found in another study on 2011.

Studying the effect of UA on the functions of mitochondria was the focus of many studies. One of the early studies was by Johnson and colleagues on 1950 on the homogenates of liver and rat, they found two actions regarding UA concentration, at 1μM, encouraged oxygen consumption in
the existence of several substances. At higher concentrations (8-30μM), phosphate uptake was decreased regardless a parallel fall in oxygen consumption. They induced these actions to uncoupling of oxidative phosphorylation. Similar result was found by Pramyothin and co-workers study, where the results showed that UA encouraged "oxygen consumption" by inducing highest stimulation of respiration (in the absence of ADP, state-4). Han et al, confirmed a similar uncoupling effect of UA in the presence of bovine serum albumin in buffer. While in the absence of BSA in buffer in primary mouse hepatocytes and rat liver mitochondria, UA acted as an uncouple as well as an inhibitor of mitochondria. During 2010 in Brazil and 2013 in Russia two studies were conducting to detect the mechanism on UA on tuberculosis and influenza A, where the authors reported good efficacy of UA on both illnesses; however, they reported unclear mechanism and suggested the need for further studies. Joseph et al. studied the expression rates of 542 genes associated with the mitochondrion structure and liver functioning in female rats. A significant effect on the expression of several genes was induced by UA only at the dose 600μg/mL. It was observed that a significant up-regulation of genes associated with complexes (I) through (IV) in the electron transport chain resulted from UA.

**PROVED BIOLOGICAL EFFECTS OF USNIC ACID**

*Usnic acid as an antioxidant and pro-oxidant*

Odabasoglu et al, reported that usnic acid possesses antioxidant effect when utilized against indomethacin-induced ulcers in rat's stomach. The antioxidant effect of usnic acid is undoubtedly related to the ability to perform peroxyl radical scavenging, reduce hydroxyl radicals and to decrease the synthesis of nitric oxide. Rabelo et al, added that this effect is related to the capacity of usnic acid to increase intracellular ROS synthesis in these cells. In another study performed by Kohlhardt-Floehr et al, low concentrations of UA extracted from Xanthoparmelia farinosa (Vainio) and under physiological UVB intensity, exhibit an antioxidant function.

*Usnic acid as cytotoxic and anti-proliferative agent*

Several studies showed that (-) usnic acid inhabited the murine P388 leukemia assay and revealed cytotoxic activity against cultured L1210 cells, where it was concluded that p-tri-ketone moiety was essential for the optimum activity. Another study showed that (+)-usnic acid (50μg/mL) decreased the cell counts of leukaemic (K - 562) and endometrial carcinoma cell culture (HEC-50). In 2005 reported that "usnic acid has anti-proliferative activity against the wild-type p53 (MCF7) as well as the non-functional p53 (MDA-MB-231) breast cancer cell lines, and the lung cancer cell line H1299, which is null for p53". Where UA worked as either a systemic therapy or as a topical agent for the treatment of tumors. In was concluded that the antineoplastic activity of UA is not related to alterations in the formation and/or stabilization of microtubules.

The cytotoxic activity of purified lichen metabolites was evaluated by Brisdelii et al, in three human cancer cell lines included "MCF-7 (breast adenocarcinoma), HeLa (cervix adenocarcinoma) and HCT-116 (colon carcinoma)". It was found that the highest cytotoxic activity against all cancer cell lines analyzed was higher than 25μM when compared with the other lichen.

*Usnic acid as an antimicrobial and antiprotozoal agent*

Interest in the antibiotic has decreased in the last three decades of the appearance of penicillin and synthetic antibiotics, but has returned to the forefront of interest since 1980 because of the emergence of antibiotic-resistant bacterial strains. Both optical enantiomers of UA are active against Gram-positive bacteria and mycobacteria, where several research studies and clinical trials have established the antibacterial properties of usnic acid.

First studies focused on the sepsis inhabit by UA, where they found that gram positive bacteria are the more sensitive to UA than gram negative second group of the studies focused on the concentration where both enantiomers were tested the last groups focused on the action of (+) and (−) enantiomers on methicillin and mupirocin-resistant St. aureus strains. For example, in preliminary clinical trials, the researchers gave the volunteers a mouthwash with 1% UA was given, then they examine the oral bacterial flora on a regular time. They reported that the growth of Streptococcus mutants was selectively inhibited. Using standardized assesses, the in vitro weakness of pathogenic Gram positive and anaerobic bacteria toward usnic acid has been established. It was reported that usnic acid suppress the growth of Gram-positive organisms responsible for body odor, and the ethoxydiglycol extracts of lichens containing 10% usnic acid on a wet weight basis has used as preserving in moisturizing cream. Several studies were reported the effectiveness of usnic acid against mycobacterium aureum.
with cystic fibrosis patients and its potential use in the sterilization of surgical implants being investigated.\textsuperscript{36} Gupta et al. indicated this efficacy to the disruption of the cell membrane.\textsuperscript{37} Also, reported that the primary effects of UA on \textit{B. subtilis} and \textit{S. aureus} are inhibition of RNA and DNA synthesis. Weakening of protein synthesis in \textit{B. subtilis} and \textit{S. aureus} seem to be indirect, as the effects were late, which suggest that they were reliant on RNA production inhibition.\textsuperscript{1}

Recently, the valuable property of UA activity against protozoa was discovered, where the source of antiprotozoan medications is limited, and the licensed pharmaceuticals are highly toxic.\textsuperscript{3} In a previous study the researchers reported the inhibitor efficacy of usnic acid against the pathogenic protozoan \textit{Trichomonas vaginalis} at reasonably low concentrations (0.4μg/mL) than metronidazole (0.6μg/mL).\textsuperscript{18} The second study reported "leishmanicidal properties both in vitro and in vivo", intraregional administration of UA reduces in lesion weight and parasite body load.\textsuperscript{29} In another study the influence of various doses of (+) UA on the growth of trypanosomes (the epimastigote form), with UA at higher levels, 40 or 80 μg/mL, caused lysis of parasite kinetoplasts and mitochondria without causing fundamental damage of host cells.\textsuperscript{33} Other studies focused on the use of UA against plasmodium falciparum, the causative agent of malaria, where several studies determined the efficacy of the IC\textsubscript{50} of UA, with different concentrations ranged from 15μM, to 75μM.\textsuperscript{38,39}

\textbf{Usnic acid as antiviral agent}

The antiviral activity of UA was not detected till 1995, when Yamamoto et al. confirmed the influence of UA on the replication of carcinogenic Epstein–Barr virus, where inhibition by (+) UA was observed at 1.0μg/mL, while (-) UA was less active at 5.0μg/mL.\textsuperscript{40-43} In another study, it was reported that (+)-usnic acid inhibited the cytopathic effects of herpes simplex type I and polio type I viruses in the infected kidney cells of the African green monkey.\textsuperscript{33}

There is indirect evidence that the inhibition mechanism is related to the ability of UA to repress viral transcription, where the action of UA was specifically targeted at virus specific enzymes mediating the replication of viral nucleic acids.\textsuperscript{44-47} Another study showed good efficacy of UA on influenza A, where anti-influenza activity of native (+)-UA is four times higher than the one of its (+)-counterpart, while activity of newly synthesized derivatives of (+)-UA appeared equal or higher than their (-)-UA counterpart, this confirmed that antiviral activity of usnic acid derivatives can be improved by side moieties introduction, where the modification with chalcones appeared to be the most effective.\textsuperscript{18}

\textbf{Usnic acid algicidal agent}

The toxic action of UA on no symbiotic algae was studied. It was found that UA at the concentration of 10μg/mL suppressed the growth of the single cell green alga \textit{Chlamydomonas Reinhard} by 10–20%. Usnic acid treatment did not affect gametogenesis as it was reported that UA at the concentration 50 μg/mL did not inhibit the growth of another green alga, \textit{Chlorella fusca}.\textsuperscript{48}

\textbf{Usnic acid as antiinflammatory agent}

Inflammation was defined by Riella et al, as “a protective host response to foreign antigenic challenge or tissue injury that, if unopposed, could lead to loss of tissue structure as well as function”.\textsuperscript{49} In two models of inflammation “an acute rat paw edema and a chronic rat cotton pellet with 100 mg/kg oral dose”, the anti-inflammatory action of (+)-UA was similar to ibuprofen with a similar dose.\textsuperscript{46} Another study reported that usnic acid protects lipopolysaccharide produced mice acute lung injury by alleviating the inflammatory reactions and oxidative stress. Assessment of the anti-inflammatory action showed that usnic acid mitigated the expression of: "tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), interleukin-8 (IL-8) and macrophage inflammatory protein-2 (MIP-2)".\textsuperscript{49}

The analgesic and antipyretic actions of UA were estimated in two different studies conducted on mice.\textsuperscript{51} At 100 mg/kg oral dose level, UA showed a substantial analgesic effect as strong as that of acetic acid, while on oral dose levels up to 300mg/kg, UA also expressed notable antipyretic action determined after lipopolysaccharide-induced hyperthermia.\textsuperscript{51}

\textbf{Usnic acid as UV filter}

In the last few years, the natural products from plants and lichens origin showed potential sunscreen effect as they can absorb the UV light and have antioxidant power. Where usnic acid ability to filter UV light and protect against it was assessed in both in vivo and in vitro studies it was found that UA is one of the best UVB filter when compared to references sunscreen named "Nivea sun Spray LSF 5".\textsuperscript{52}

\textbf{Adverse effect of usnic acid and usage}

\textit{Usnic acid induced hepatotoxicity}

Liver is the most organ exposed to toxic injuries, as all substances swallowed then absorbed and go directly to the liver. Adding to that the liver is the organ responsible for the metabolism and excretion of large number of substances.\textsuperscript{2} Usnic acid showed a good property to use as a treatment for different diseases, however it has been accompanied by marked liver injury (hepatotoxicity) when taken as a supplementary medication to induce weight loss.\textsuperscript{2}

Pramyothin et al, studied hepatotoxic effects of UA in isolated rat hepatocytes receiving UA at a dose of 100 or 1000μM and noticed that "UA encouraged the release of
hepatic transaminases (AST and ALT), reduced the content of glutathione, and caused loss of cell membrane integrity.\textsuperscript{15} Treatment with usnic acid and carbon tetrachloride (CCl\textsubscript{4}) the hepatotoxic agent showed similar cellular responses, indicating that usnic acid may have the same hepatotoxic mechanisms as excited by CCl\textsubscript{4}.\textsuperscript{15} In another study, administration of 5 μM UA for 16 hours in a mouse primary hepatocyte induced death in 98% of the cells which seemed to be related to cell necrosis.

They concluded that UA generated free radical that induced oxidative stress that is considered crucial to hepatotoxicity induced by UA.\textsuperscript{16} The toxic effects of usnic acid on human hepatoblastoma – HepG2 cells were described by Sahu et al. suggesting an oxidative mechanism of action of UA.\textsuperscript{13}

Usnic acid induced genatotoxicity

Although the in vitro studies performed on some human tumor cell cultures showed that UA did not damage DNA.\textsuperscript{26} A more recent in vitro study using DNA comet assay showed that usnic acid induced DNA damage at the dose of 60 and 120 μg/mL, although no effect was noticed in the micronucleus test with V79 cells.\textsuperscript{53}

On the other hand, the in vivo study conducted on mice consuming usnic acid at doses 25, 50, 100, or 200μg/kg using the micronucleus and DNA comet showed that UA had no genotoxicity.\textsuperscript{54} Both proteins, genetic materials in mouse spermatozoa showed no changes even after consumption of (+)-UA at the daily dose 200 mg/kg for 35 days.\textsuperscript{54}

Upon assessing the chronic effect of UA on the hepatocytes of at low (100mg/kg) and high dose (300mg/kg) on the adult male rats, it was found that hepatocytes showed an increased lipid droplet, swollen mitochondria and fragmented rough endoplasmic reticulum indicating marked harmful effect of the cellular level induced by UA at high dose (Figure 3).\textsuperscript{55}

Usnic acid induced allergenicity

According to Thune and Solberg, usnic acid ischemically linked to furocoumarin and shows allergic cross-reactivity, however, usnic acid does not classically cause photosensitivity.\textsuperscript{2} Another study showed that only the D-isomer is allergenic.\textsuperscript{56,57} Another study revealed that both the D- and L-isomers of UA were allergenic and that persons may react to one or both enantiomers.\textsuperscript{2} In 2006 study, it was reported that "four patients had positive patch test reactions to lichen acid mix and D-UA". Three of the four patients with patch-tested for the botanical deodorant had positive reactions.\textsuperscript{2}

Usnic acid induced effects on cardiovascular effects

Several studies detect the effect of UA on the CVS, in 2010 study on guinea pig left atrium which in isolated organ bath. The author confirmed that "increasing of UA doses (1-800mM) produces a negative inotropic effect for concentrations more than 100 mM". This effect was associated with a strong diastolic contraction for concentrations above 500mM.

These effects were permanent. Also, the dose more than 100 mM also induced changes in the speed of the cardiac muscle contraction, increasing the time for both systole and diastole.\textsuperscript{58} Another study on human showed no change in cell viability of endothelial cells (Eary926) incubated 24 h with different concentrations of UA.\textsuperscript{2}

Usnic acid induced sideeffects on the adipocytes of fatty tissue

When the chronic effect of UA on the structure of adipocytes of the perirenal fat was assessed at low (100mg/kg) and high dose (300mg/kg) in the adult male rats, it was found that adipocytes became small with the appearance of capillary congestion and intercellular hemorrhages indicating negative impact of these tissues induced by UA at high dose (Figure 4).\textsuperscript{17}
Figure 4: Picture taken by light microscope of adipocytes from UA low dose group (100mg/kg) (A) Small-sized cells compared to the control. There is no signs of necrosis, fibrosis, or vascular changes. Adipocytes from high dose group (300mg/kg) (B) Marked decrease in cell size, marked capillary congestion and intercellular hemorrhage.

CONCLUSION

Even with the good scientific information, it is hard to confirm the use and safety of UA, due to the limitation of preclinical pharmacological studies. Moreover, the toxicological research to support the safety isn’t enough. Hence, further studies are needed to determine the efficacy and safety of UA.

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