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Emerging mycotoxins in botanicals: benefit and risk

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Review

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ABSTRACT

Background: *Fusarium* species are responsible for production of emerging mycotoxins with cyclic hexadepsispeptides structures, like enniatins and beauvericin. Although these mycotoxins have not been regulated yet, their high prevalence in food and feed, as well as their potential toxic effects in humans and animals have made of them a burning issue and a threat to food security. Besides its inophoric properties, these mycotoxins may induce cells damages such as oxidative stress, mitochondrial modifications and the disruption on the cell cycle related to several health adverse effects such as immunotoxicity, genotoxicity, endocrine toxicity and neurotoxicity. Moreover, they showed interesting activity against various microorganisms and insects in several studies, leading to a potential use in pesticide and medicine research, as potential candidates for anticancer therapy. Botanicals can go contaminated by mycotoxins when the harvesting practices or manufacturing conditions are inadequate, which make mandatory their study.

Methods: This review explores emerging mycotoxins occurrence in several botanicals forms and discuss their possible prejudicial and beneficial effects.

Results: Several researchers have reported mycotoxins occurrence at low incidences and levels in botanicals. Despite emerging mycotoxins toxic effects, beneficial properties have been attributed to these mycotoxins by several authors. These compounds are related to insecticidal activity, antibacterial properties, and antifungal and antiviral activities. Cell organelles or enzyme systems are the targets in its antimicrobial activity. Data collected showed also that emerging mycotoxins are involved in capacity of the multidrug transport protein in human cancer cells modulation and apoptotic cell death induction, several researchers are explaining the possibility to employ them with medicinal purpose.

Conclusion: More studies are required to explore enniatins and beauvericin possible applications in medicinal and pesticide research. Furthermore, it is important to highlight that the regulation of emerging mycotoxins in food must be revised and updated.

Keywords: anticancer, antimicrobials, beauvericin, cytotoxicity, enniatins, medicinal plants.

INTRODUCTION

According to WHO, the treatment with extracts of herbal medicine or vegetable is practiced by the 80% of world's population [1]. The use of infusions of leaves, flowers, fruits and seeds of some vegetable spices is widely practiced, and, in many situations, their consumption is associated with cultural aspects based in ethnobotanical knowledge [2]. Nowadays, the market of natural products has increased with the hope of new natural compounds obtained from plants with a commercial potential in the production of energy drinks, capsules, health supplements, energy boosters and food product materials. The phytochemicals of plants that originated interest in the industry consist in alkaloids, anthraquinones, flavonoids, glycosides, phenolics, saponins, steroids, tannins, and terpenes, among others [3,4]. More than 8000 phenolic compounds have been reported in botanicals, half of them are flavonoids presenting as aglycone, glycosides and methylated derivatives. These compounds present antioxidant, anticancer, antibacterial, cardioprotective, anti-inflammatory and immunological properties, and protect the skin from UV radiation, which make botanicals interesting candidates for pharmaceutical and medical application [5].

The attention in the quality and safety of botanicals has increased, because during the plantation, processing and storage, these matrices may be contaminated by pesticide residues, mycotoxins and heavy metals. In this sense, botanicals are susceptible to contamination by mycotoxigenic fungi, during harvesting, manufacturing, transport and storage. Mycotoxins are related to some prejudicial effects such as potential carcinogenicity, teratogenicity, immunotoxicity and neurological dysfunction [6]. The increase in consumption of herbal products may contribute to an increase of mycotoxin intake leading to adverse human health problems [7].

Emerging mycotoxins

Mycotoxins are secondary metabolites produced by filamentous fungi. These contaminants are commonly reported in different commodities such as cereals, nuts, herbal teas, coffee or species. The contamination by mycotoxins frequently occur during field, in the post-harvest stage and throughout the food chain. Significant economic losses are associated with the impact of mycotoxins on human health, animal productivity, domestic and international trade [8, 9]. The Food and Agriculture Organization of the United Nations (FAO) has estimated that up to 25% of the world's food crops are significantly contaminated by mycotoxins [10]. While weather conditions can profoundly affect the growth, distribution and production of mycotoxins in fungi, climate change may also impact on mycotoxins incidence in the coming years [11].

Aspergillus, *Penicillium*, *Alternaria*, *Fusarium* and *Claviceps* are the principal genus involved in the mycotoxin production. *Aspergillus* is responsible of Aflatoxins (AFs) production; *Aspergillus* and *Penicillium,* both produce ochratoxin A (OTA); *Fusarium* species produce trichothecenes (HT-2, T-2, *deoxynivalenol* (DON) as well as nivalenol (NIV)), zearalenone (ZEA), fumonisins (FB1 and FB2) and emerging mycotoxins (fusaproliferin (FUS), moniliformin (MON), beauvericin (BEA) and enniatins (ENs)); *Claviceps* produces ergot alkaloids; *Alternaria* species produce altenuene, alternariol, alternariol methyl ether, altertoxin, and tenuazonic acid [12].

Fusarium species are responsible of ENNs and BEA production in different geographical areas, and their occurrence in some food commodities are high, at levels of mg/kg. Their presence has been highly reported in food matrixes such as maize, corn, wheat, wheat flour, durum wheat, oats but can also contaminate other products including beans, dried fruits, tree nuts, coffee, vegetables oils, botanicals, and feed. Although emerging mycotoxins have not been regulated yet, and maximum levels have not been fixed in food, their high prevalence in food and feed, as well as their potential toxicity in humans and animals has increased their interest and concern. BEA and ENNs are a cyclic hexadepsispeptides structures with alternating D-α-hydroxy-isovaleryl- (2-hydroxy-3-methylbutanoic acid) and amino acid units. In BEA, the three amino acid residues are aromatic N-methyl-phenylalanines, while in ENNs the amino acid residues are aliphatic N-methyl-valine or–isoleucine or mixtures of these two [13].

BEA and ENNs cytotoxic activity

For acute toxicity, EFSA (2014) established the lethal dose (LD_{50}) in mices upon oral administration at 100 mg/kg/bw for BEA and at 350 mg/kg/bw for a mixture of ENNs. The cytotoxicity associated with their exposure to different cell lines revealed inhibitory concentration (IC_{50}) values at 24 h in the range from 11 to 24.6 μM for BEA and from 2.6 to 36 μM for ENNs [14].

In the last years, an increasing number of BEA and ENNs *in vitro* and *in vivo* studies were developed to understand their mechanisms of action [15].

The primary toxic mechanism of action of BEA and ENNs is related to their ionophoric properties, which make them capable of promoting the transport of mono- and divalent cations through membranes resulting in disturbances of the physiological cell cation levels [16]. These evoke changes in the ion intracellular concentration that consequently affects the cell functions. Besides it, ENNs can inhibit acyl-CoA: cholesterol acyl transferase (ACAT) activity and cause oxidative stress. It can also induce mitochondrial modifications and the disruption on the cell cycle that finally can result in apoptotic cell death [17]. In a study conducted on human colon adenocarcinoma cells (Caco-2), Prosperini et al. [18] observed that ENNA, ENNA1, ENNB and ENNB1 induced cytotoxicity involved by early ROS generation that induced LPO oxidative damage, apoptosis and necrosis via the mitochondrial pathway. Furthermore, ENNA and ENNA1 induced DNA damage, corroborated by the arrest of the cell cycle observed. In addition, ENNs produced adrenal endocrine toxicity. Kalayou et al. [19] observed a reduction of hormones and modulation of genes at the lower dose of ENNB (10 μM) in the H295R cells that could suggest that adrenal endocrine toxicity is an important potential hazard. The embryotoxicity has also been related to ENNs. The collected data obtained by Huang et al. [20] suggested that ENNB1 exerted cytotoxic effects on mouse embryos as well as oxidative stress and immunotoxicity during mouse embryo development.

Regarding possible neurotoxic effects, Krug et al. [21] studied the transport of ENN B and ENNB1 across the Blood Brain Barrier (BBB) employing a porcine brain capillary endothelial cells (PBCEC) *in vitro*-model and their influence on cellular viability via cell Counting kit-8 assay (CCK-8) in three different cell types of BBB: PBCEC, human brain microvascular endothelial cells (HBMEC) and human astrocytoma cells (CCF-STTG1). The results obtained revealed high influx rates for ENNB and ENNB across BBB. The cellular viability results showed that ENNB and ENNB1 induced high cytotoxicity in CCF -STTG1 cell line. CCF-STTG1 cells were more sensitive than both endothelial cell types. Furthermore, in CCF-STTG1 especially ENNB, caused induction of apoptosis rather than necrosis.

Regarding toxicogenomic effects, Alonso- Garrido et al. [22] investigated changes in the gene expression profile induced by enniatin B exposure at concentrations of 1.5, 3 and 5 μM to human Jurkat lymphoblastic T-cells after 24 h and observed that 245 genes were differentially expressed and that mitochondria were the organelles with more related differentially expressed genes, that were involved in molecular functions and pathways related to mitochondrial metabolism and cell respiration.

BEA can disturb the normal cell cycle distribution and furthermore, can induce programmed cell death mediated by apoptosis. Moreover, BEA can induce mitochondrial transmembrane depolarization and induce immunotoxicity [23]. Wätjen et al. [24] observed in H4IIE hepatoma cells that BEA produce an inhibition of TNF-α-induced NF-κB activation without inhibiting the basal activity of NF-κB, which is an important modulator in the expression of immunoregulatory genes. BEA is related with oxidative stress, reactive oxygen species (ROS) generation and membrane lipid peroxidation (LPO) has been observed in cells after BEA exposure [23]. Prosperini et al. [25] studied the cytotoxicity of BEA on human colon adenocarcinoma cells (Caco-2) and demonstrated that oxidative stress is one of the mechanisms involved in BEA toxicity. BEA induced cell death by mitochondria-dependent apoptotic process with loss of the mitochondrial membrane potential. Furthermore, BEA

increased LPO level and reduced G0/G1 phase, with an arrest in G2/M. Moreover, DNA damage was observed. Mallebrera et al. [26] studied the injury and the mechanisms of defense in Chinese Hamster ovary (CHO-K1) cell line after exposure to BEA and observed disruption in mitochondrial enzymatic activity and cell proliferation after exposure. BEA inhibited cell proliferation by arresting cells in G0/G1 phase and increased apoptosis. At 48 and 72 h of exposure, BEA induced differentiation of CHO-K1 cells through G2/M arrest and prevented that cells entry into mitosis. After 24 h of exposure at 1 μM DNA strand breaks were observed. On the other hand, BEA exposure increased antioxidants defense mechanisms (catalase and superoxide dismutase activities) that can contribute to eliminate damages produced by BEA.

Juan-García et al. [27] studied the hepatotoxicity of BEA, ENNA1, ENNB at concentrations of 1.5 and 3 μM at 24, 48 and 72 h by flow cytometry in hepatocarcinoma cells (HepG2), and observed that ENNB1 produced a time dependent G1 blockade and that ENNA1 and BEA decreased the apoptotic-necrotic percentage of cells and produced disruptions in the mitochondrial membrane potential (MMP). In the same cell line, Juan-García et al. [28] studied individual and combined cytotoxicity effect of BEA and OTA. The cytotoxic concentrations assayed over 24, 48, and 72 h were from 0 to 25 μM for BEA, from 0 to 100 μM for OTA, and from 3.4 to 27.5 μM for BEA + OTA combinations at a ratio of 1:10. The results obtained by these authors revealed that the toxicity observed for BEA was higher than for OTA. Furthermore, additive and synergistic effects were observed. OTA and BEA + OTA treatments produced cell cycle arrest in the GO/G1 phase, while a decrease in G0/G1 was detected for BEA, revealing induction of cell death. Finally, genotoxicity showed significant effects for BEA, OTA, and their combinations.

Fraeyman et al. [29] evaluated the cytotoxicity of ENNs and BEA towards intestinal porcine epithelial cells of the jejunum (IPEC-J2) using flow cytometric viability assays and observed that all studied mycotoxins resulted in a decline of IPEC-J2 viability, except of ENNB that resulted less cytotoxic, since the exposure at concentrations up to 100 μM resulted in 83% of viable proliferating cells. These authors suggested that ENNB may had minimal effect on intestinal morphometry.

In a work performed on Jurkat T-cells, Manyes et al. [30] studied the effects of both, BEA and ENNB at concentrations from 1 to 15 μM at 24, 48 and 72 h and observed that BEA and ENB produced several toxic responses. IC50 values obtained ranged from 3 to 7.5 μM (72 to 24 h) for BEA while for ENN B 15 μM decreased viability in the range 21-29%. BEA mediated cytotoxicity through mitochondrial alterations, while for ENNB it only occurs at high concentrations and time assayed. Furthermore, BEA affected cell cycle with apoptotic/necrotic cells increase, whereas these effects were not evident for ENNB. BEA and ENNB revealed caspase-3&7 activation, even by different profile activation. No difference in ROS production was observed for both mycotoxins. Finally, BEA produced DNA damage at high concentrations.

BEA can also affect estrogenic activity. García-Herranz et al. [31] determined the cytotoxic effects and the endocrine activities of BEA in two fish and one mammalian hepatoma cell lines and observed that BEA was as toxic to fish as to mammalians cells and showed a weak antagonistic effect at the androgen receptor.

BEA was also related to genotoxicity, internucleosomal DNA fragmentation, chromosomal condensation, membrane blebbing, cell shrinkage, apoptotic body formation and apoptotic morphological changes effects [23]. Çelik et al. [32] studied the genotoxic and cytotoxic effects of BEA on human lymphocytes *in vitro* culture and suggested that BEA is a genotoxic compound producing significant concentration-dependent increase in chromosomal aberrations, sister-chromatid exchanges and micronuclei. It also produced a decrease in the mitotic index at the two highest concentrations employed (5 and 10 μM). Not significant changes in the proliferative and nuclear division indices were observed.

Concerning the toxicogenomic effects, Escrivà et al. [33] investigated gene expression changes triggered by BEA exposure in Jurkat cells at concentrations of 1.5, 3 and 5 μM during 24 h through RNA-sequencing and observed a large number of differentially expressed genes mainly related to respiratory chain, apoptosis, and caspase cascade activation. Molecular functions related to mitochondrial respiratory chain and oxidoreductase activity were over-represented. 77 genes involved in the respiratory chain resulted significantly down regulated. Furthermore, 21 genes related to apoptosis and programmed cell death, and 12 genes related to caspase activity resulted significantly altered. More recently, Escrivà et al. [34] studied the transcriptional effects of combined exposure to BEA and ENNB (1:1) at concentrations of 0.1, 0.5, 1.5 μM h in Jurkat cells at 24 h employing qPCR on 30 selected target genes (10 mitochondrial and 20 nuclear) and observed transcriptional changes, especially at mitochondrial level after BEA-ENNB co-exposure including down-regulation of gens related with antioxidant activity. Differences expression patterns were revealed between individual and combined exposures.

Regarding its possible embryotoxicity, Schoevers et al. [35] investigated the effects of BEA on porcine oocyte maturation and preimplantation embryo development and observed that BEA was toxic in embryos, oocytes and cumulus cells at concentrations >0.5 μM, and that embryos were most vulnerable after the four-cell stage. BEA toxic mechanism is suggested to involve different pathways.

BEA and ENNs bioactivity beneficial properties

Unlike for toxic effects, beneficial properties have been described. BEA and ENNs have different biological properties, which may lead their potential use in medicinal and environmental research.

BEA is a useful tool in combination with chemotherapeutic drugs due its inhibitory capacity of the multidrug transport protein in human cancer cells and the induction of apoptotic cell death. Their anticancer properties can besides in the fact that induces extracellular translocated of Ca2+ into the cytosol, leading the increase of Ca2+ intracellular level, which activate a series of signaling pathways such as MAPK, NF-kB, etc. NF-kB is a transcription factor related with cell survival. BEA also decreases the mitochondrial transmembrane potential, release of Cyt c, and activates caspases, finally promotes cancer cell apoptosis [36].

In this sense, Heilos et al. [37] have observed i*n vivo*, a decrease of tumor size and weight and significant increase of necrotic areas in cervix and colon carcinomas.

BEA also shows anti-inflammatory activities and inhibits inflammatory responses, due its inhibition of NF-$_k$B dependent inflammatory responses by suppressing enzymes Src and Syk. Due its anti-inflammatory properties, BEA can present a useful therapeutic role in colitis and Crohn's disease [38].

Regarding BEA antimicrobial activity, it has shown strong activity against Gram-positive and Gram-negative pathogenic bacteria. Cell organelles or enzyme systems are the targets in its antimicrobial activity [39]. Meca et al. [40] proved BEA biological activity against several pathogenic bacterias: *Escherichia coli*, *Enterococcus faecium*, *Salmonella enterica*, *Shigella dysen-teriae*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Clostridium perfringens*, *Pseudomonas aeruginosa* and two strains of *Staphylococcus aureus* at quantities from 0.1 to 1000 ng. The results revealed that BEA was effective on all pathogenic bacterias tested except of *S.aureus* strains. Regarding their antiviral properties, Shin et al. [41] studied ENNs and BEA potential inhibitory *in vitro* against human immunodeficiency virus type-1 (HIV-1) and observed that BEA was the most effective in inhibiting the 3′-processing activity of HIV-1 integrase with an IC_{50} of 1.9±0.4 μM.

ENNB can be useful in the treatment of atherosclerosis and hypercholesterolemia due their enzyme inhibition activity of ACAT. Furthermore, can be used in combination with chemotherapeutic drugs, because it presents an inhibitor effect of the major multidrug efflux pump Pdr5p$_6$ in *Saccharomyces cerevisiae*.

ENNs can also interact with membrane-located ATP-binding cassette (ABC) transporting, so can cause potential influences on bioavailability of xenobiotics and pharmaceuticals [17].

Regarding, antibacterial, antifungal and insecticidal activities, Zaher et al. [42] observed that methanol extract of fungus *F. tricinctum* that contains the ENNs metabolites (ENNA, ENNA1, ENNB, ENNB1, ENNB2 and ENNQ) showed mild antibacterial and antifungal activities against gram-positive bacteria methicillin-resistant *Staphylococcus aureus* and *Mycobacterium intracellulare*, gram-negative bacteria *E coli* and *Pseudomonas aeruginosa* , and fungus *Candida albicans*, *C. glabrata* , *C. krusei*, *Aspergillus fumigatus* and *Cryptococcus neoformans* with IC_{50} values > 10 µg/ml. Also presented antimalarial activity against *Plasmodium falciparum* by the inhibition of PfTrxR enzyme with IC_{50} of 16.96 µg/ml and antileishmanial activity against *Leishmania donovani*. Wang et al. [43] observed anti -tuberculosis properties of ENNA1. ENNA1 showed an antibacterial effect time-concentration-dependent against *M. tuberculosis* assayed at concentration range from 4 to 64 µg/ml and displayed synergy with anti-tuberculosis drugs (rifamycin, amikacin, and ethambutol). The mechanisms of action can besides in the decreasing of membrane potential and intracellular levels of ATP. Clark et al. [44] also observed antimycobacterial activity against *M. tuberculosis* by the presence of ENNB, ENNB1, ENNB4 in addition to lateropyrone in the extract obtained after fermented *F. acuminatum* in potato dextrose.

Sebastià et al. [45] evaluated the antibiotic effect of ENNJ1 and ENNJ3 at quantities from 0.1 to 1000 ng on several pathogenic strains and lactic acid bacteria, after purified them from the fermentation extract of *Fusarium solani* growth in wheat kamut and observed antimicrobial activity of ENNJ1 and ENNJ3, against *C. perfringens*, *E. faecium*, *E. coli*, *S. dysenteriae*, *S.aureus*, *Y. enterocolitica* and studied lactic acid bacterias, except of *B. adolescentis* that was only inhibited by enniatin J3.

Olleik et al. [46] also observed ENNs and BEA effective activity against gram-positive bacteria (*B. subtilis*, *B. subtilis NR*, *C. perfringens*, *E. faecalis*, *S. aureus*, *S. aureus MRSA*), Mycobacterium, and fungi (*C. albicans*, *F. graminearum*) due these peptides can interacted with bacterial lipids, inducing membrane depolarization and inhibition of macromolecules synthesis. Their structural side chains impact in their interaction with lipids. ENNA was found the most antimicrobial active with minimal inhibitory concentration (MIC) from 3.12 to 12.5 µM for gram positive bacterias, of 6.25 µM for *Mycobacterium* and from 1.5 to < 100 µM for fungus.

During the last years the possible effect of ENNs as anticancer agents has also been suggested. Due to the transport of mono- and divalent cations through the cell membranes can disturbance the physiological homeostasis of cell and lead to apoptotic cells death. Furthermore, present p53-dependent cytostatic and p53-independent cytotoxic activity against several cancer cell types. In lot of studies in various cancer models, after 24 h of treatment at ENNs low concentrations, DNA synthesis stop, cell cycle arrest and apoptotic cell death is induced [47,48].

Moreover, ENNs are few influenced by multidrug resistance transport proteins, leading to therapy resistance and present chemo sensitizing properties which makes them promising compounds as constituent in preparations for cancer therapy [17].

Dornetshuber-Fleiss et al. [49] observed antiangiogenic properties of ENNB and Sorafenib against cervical cancer *in vitro* and *in vivo* due a strong inhibition of human endothelial cell migration and tube formation. The synergism is accompanied by a marked increasing in mitochondrial injury and apoptosis caused by mitochondrial membrane depolarization, caspase-7-activation, and cleavage of PARP. Furthermore, cells stopped DNA synthesis and accumulate in the phases S and G2/M of the cell cycle. The synergism is based on interference with MAPK signaling and angiogenesis inhibition. *In vivo* studies confirmed that the combination treatment is more effective than single treatments against the KB-3-1 cervix carcinoma xenograft model.

In summary, ENNs are known to be insecticidal, antifungal, antibacterial, and antihelmintic compounds. In the last years, have also been proposed as anticancer agents.

Due to their antibiotic properties ENNs can also be effective in the treatment of upper respiratory tract disease such as sinusitis, rhinitis, pharyngitis, tonsillitis, laryngitis, follicular pharyngitis and tracheitis [16].

In general, BEA has numerous biological effects related to ionophobic properties and presents anticancer, anti-inflammatory and anti-cholesterol activities. Moreover, shows insecticidal activity against many insect species, antibacterial properties including human, animal and plant pathogens, and also antiviral and antifungal activity [36].

As constituent in drug preparations, in traditional Chinese medicine, BEA is employed as constituent in anticonvulsant and antineoplastic drugs. BEA has also been used to decrease cholesterol levels in blood. Furthermore, it can be used as chemo sensitizing agent, increasing antibiotic effectiveness, due the inhibition of the active efflux of antibiotics by membrane transport proteins [16].

Therefore, these mycotoxins may be potential candidates for be used in anticancer therapy in combination with other drugs because are cytotoxic to cancer cells, have the capacity to inhibit drug efflux pumps, and inhibit the bone resorption. Furthermore, these compounds have demonstrated interesting activity against several insects and microorganisms in different studies [36, 50].

ENNs and BEA in botanicals products

As has been mentioned above, the demand for botanicals is increasing worldwide due to the preference of the population for natural products. These products are available in the corresponding markets in several forms: the raw botanicals, consumed as infusions or as condiments, like essential oils and like food supplements. Few information is available in literature about the presence of emerging mycotoxins (ENNs and BEA) in botanicals, but the interest in these compounds is growing because their high prevalence in several foods and feed. In order to provide information about emerging mycotoxin contamination in botanicals, this review is focused in ENNs and BEA presence in botanicals as ready for human consumption, such as aqueous infusions, tablets, or capsules.

About the presence of emerging mycotoxins of *Fusarium* in botanical raw materials, Hu & Rychlik, [51] studied ENNs and BEA in 60 Chinese medicinal herbs and observed that 25% of analyzed samples were contaminated with one or more of the ENNs and BEA, with total contents ranging from 2.5 to 751 μg/kg. The mean concentrations of positive samples were 28.9 μg/kg (ENNA), 28.4 μg/kg (ENNA1), 32 μg/kg (ENNB), 3.9 μg/kg (ENNB1) and 33 μg/kg (BEA). Reinholds et al. [52] investigated the presence of 12 mycotoxins in 60 botanicals purchased from Latavia and observed that the 57% of samples were contaminated by emerging mycotoxins (ENNs and BEA). More than one ENNs were found in 13 samples with total contamination levels from 0.35 to 28.4 μg/kg. BEA was detected at concentrations from 4.50 to 5.25 μg/kg. Pallarés et al. [53] analyzed the presence of AFs, ZEA, ENNs and BEA in 224 samples of medicinal plants raw materials and observed for ENNs and BEA incidences between 1 and 15% with mean concentrations ranging from <LOQ (BEA) to 42.43 μg/kg (ENNB), being ENNB the most reported emerging mycotoxins.

In botanical infusions, Pallarés et al. [53] after preparing the resulting beverages from 224 medicinal plants samples (belonging to 56 different species of herbs), observed that ENNB was the only emerging mycotoxin detected at levels > LOQ (with mean concentration of 0.005 μg/L). Also, Pallarés et al. [54] analyzed the multimycotoxin (AFs, 3aDON, 15aDON, NIV, HT-2, T-2, ZEA, OTA, ENNs, and BEA) presence in 44 samples of tea beverages (belonging to black, red, green and green mint tea) and observed that regarding ENNs and BEA, only two samples of green tea resulted positive for ENNB at level <LOQ. Pallarés et al. [55] studied the presence of the 16 mycotoxins mentioned above in 52

samples of botanical beverages belonging to chamomile, chamomile with honey, chamomile with anise, linden, pennyroyal with mint, thyme, valerian and horsetail. For emerging mycotoxins, only two samples of horsetail showed positive, but at levels <LOQ. In botanical dietary supplements, Veprikova et al. [56] studied the presence of 57 mycotoxins in 69 samples of botanical dietary supplements employed to improve liver function (32) (based on milk thistle), reduce the menopause effects (9) (red clover, flax seed, and soya) and support health in general (28) (green barley, nettle, goji berries, yucca, etc.). The mainly detected mycotoxins were *Fusarium (*trichothecenes, zearalenone, enniatins) and *Alternaria* mycotoxins. In milk thistle-based supplements, ENNs were one of the most detected mycotoxins with incidences of (84-91%) and maximum concentrations ranging from 2340 to 10940 µg/kg. BEA was detected with maximum concentration of 2730 µg/kg. In supplements for reduce menopausal effects, ENNs were also one of the most frequently mycotoxins found with incidences from 67 to 78% and maximum concentrations between 89 and 1230 µg/kg. BEA was detected with maximum concentration of 131 µg/kg. In supplements for general health improvement BEA was detected with higher maximum concentration (215 µg/kg) than ENNs (13-136 µg/kg).

Narváez et al. [57] studied the presence of 16 mycotoxins in 10 samples of Cannabidiol botanical supplements made of *Cannabis sativa* L. The results obtained by these authors revealed ENNs presence. One sample was contaminated by ENNB1, ENNA and ENNA1 at levels of 11.6, 4.2 and 5.8 ng/g, respectively. ENNB1 was detected in two other samples at levels below the LOQ (1.56 ng/g).

Contrary to these results Di Mavungu et al. [58] not detected BEA in 62 samples of botanical supplements made of soy, St John's wort, garlic, Ginkgo biloba and black radish.

Risk assessment is an important scientific tool that contributes to risk analysis in the area of food safety. Perform the risk assessment is not possible to emerging mycotoxins, due no TDI value has been set yet. However, some authors have performed an approximate estimation of the risk assessment comparing the EDIs obtained for emerging mycotoxins with the TDIs established for other *Fusarium mycotoxins.*

In teas beverages, Pallarés et al. [54] obtained an EDI of 0.038 ng/kg bw/day for ENNB, that reached less than 0.05% of the TDI established for other *Fusarium* mycotoxins, like DON (1 µg/ kg bw/ day) or the sum of T-2 and HT-2 toxins (0.1 µg/ kg bw/ day). In other study, considering the consumption on botanical beverages of the Spanish population, Pallarés et al. [55] obtained an EDI for ENNB that represented less than 0.05% of the TDI established for the other *Fusarium* mycotoxins. In medicinal plants beverages, Pallarés et al. [53] calculated and EDI for ENNs (ENNB+ENNB1) that reached less than 0.1% of the TDI established for other *Fusarium* mycotoxins, however the percentage increase to 1.42% when were considered high consumers of infusions (3 cups/day). Comparing the percentages of TDI obtained in these matrixes with those obtained in wheat-based products, Stanciu et al. [59] observed percentages of TDI up to 8% for the sum of ENNS, which are higher than those observed in botanicals ready for consumption. In general, not risk was observed for population to emerging mycotoxins through the consumption of botanicals.

CONCLUSION

Although emerging mycotoxins cause cytotoxic effects inducing oxidative stress, mitochondrial modifications, disruptions on the cell cycle, related to several health adverse effects such as immunotoxicity, genotoxicity, endocrine toxicity, neurotoxicity, several studies suggested their potential use in medicinal and pesticide research. Emerging mycotoxins showed potential use in anticancer therapy in combination with other drugs, as they are cytotoxic to cancer cells, also they inhibit drug efflux pumps. Furthermore, they show insecticidal activity, antibacterial properties against human, animal and plant pathogens, and also antifungal and antiviral activities. Their occurrence in raw materials and botanical tablets is reported by several researchers at low incidences and levels, however sometimes their concentrations reached 1000 µg/kg. The levels of these emerging mycotox-

ins are at unconcerning in botanicals beverages, due the little tendency of these mycotoxins to migrate from raw materials. The risk assessment approaches revealed that the population is not much exposed to mycotoxins through botanicals consumption. More studies are required to explore their prejudicial effects and their possible applications in medicinal and pesticide research.

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Author contributions

Houda Berrada, Guillermina Font and Emilia Ferrer: conceptualization, supervision, and writing- review & editing. Noelia Pallarés: writing-original draft. All authors read and approved the final form of the manuscript.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any potential conflict of interest. All authors read and approved the final manuscript.

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