Original article DOI: 10.2478/aiht-2021-72-3541

The role of pumpkin pulp extract carotenoids against mycotoxin damage in the blood brain barrier *in vitro*

Manuel Alonso-Garrido[1], Noelia Pallarés[1], Guillermina Font[1], Paola Tedeschi[2], Lara Manyes[1], and Manuel Lozano[1,3]

[1] *University of València Faculty of Pharmacy, Laboratory of Food Chemistry and Toxicology, Burjassot, Spain*
[2] *University of Ferrara, Department of Chemistry and Pharmaceutical Sciences, Ferrara, Italy*
[3] *Jaume I University and University of València Foundation for the Promotion of Health and Biomedical Research in the Valencian Region (FISABIO), Epidemiology and Environmental Health Joint Research Unit, València, Spain*

[Received in February 2021; Similarity Check in February 2021; Accepted in August 2021]

Some mycotoxins such as beauvericin (BEA), ochratoxin A (OTA), and zearalenone (ZEA) can cross the blood brain barrier, which is why we tested the anti-inflammatory action of a pumpkin carotenoid extract (from the pulp) against these mycotoxins and their combinations (OTA+ZEA and OTA+ZEA+BEA) on a blood brain barrier model with co-cultured ECV304 and C6 cells using an untargeted metabolomic approach. The cells were added with mycotoxins at a concentration of 100 nmol/L per mycotoxin and pumpkin carotenoid extract at 500 nmol/L. For control we used only vehicle solvent (cell control) or vehicle solvent with pumpkin extract (extract control). After two hours of exposure, samples were analysed with HPLC-ESI-QTOF-MS. Metabolites were identified against the Metlin database. The proinflammatory arachidonic acid metabolite eoxin (14,15-LTE4) showed lower abundance in ZEA and BEA+OTA+ZEA-treated cultures that also received the pumpkin extract than in cultures that were not treated with the extract. Another marker of inflammation, prostaglandin D2-glycerol ester, was only found in cultures treated with OTA+ZEA and BEA+OTA+ZEA but not in the ones that were also treated with the pumpkin extract. Furthermore, the concentration of the pumpkin extract metabolite dihydromorelloflavone significantly decreased in the presence of mycotoxins. In conclusion, the pumpkin extract showed protective activity against cellular inflammation triggered by mycotoxins thanks to the properties pertinent to flavonoids contained in the pulp.

KEY WORDS: beauvericin; ECV304; metabolomics; ochratoxin A; zearalenone

Several mycotoxins such as beauvericin (BEA), ochratoxin A (OTA), zearalenone (ZEA), and enniatins (ENNs) can cross the blood brain barrier (BBB) and affect brain function, mostly through oxidative stress (1–3). Their neurotoxic action can mediate mitochondrial dysfunction associated with chronic diseases such as Parkinson and Alzheimer (4–7).

Applying low mycotoxin concentrations and short time of exposure to an *in vitro* BBB model we wanted to study the molecular basis of the early events triggering mycotoxin toxicity. Considering that carotenoids have been evidenced to protect genes against oxidative action of mycotoxins (8–12) and are depleted in Alzheimer's and Parkison's disease (13, 14), the second aim our study was to establish if pumpkin extract protects against BBB damage induced by BEA, OTA, and ZEA in differentiated human ECV304 cells applying the metabolomics approach, which provides an insight into how organisms interact with the environment (15). It looks into metabolites (intermediates and end products of cellular processes) that mediate the body's response to contaminants (metabolome), which are identified and quantified by spectrometry.

Corresponding author: Lara Manyes, University of València Faculty of Pharmacy, Laboratory of Food Chemistry and Toxicology, Avenida Vicent Andrés Estellés s/n, 46100, Burjassot, Spain, E-mail: *lara.manyes@uv.es*

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MATERIALS AND METHODS

Reagents

Cell culture components including Hanks' Balanced Salt Solution (HBSS), Dulbecco's Modified Eagle Medium (DMEM) (1X) supplemented with GlutaMAX, DMEM F-12 nutrient mixture [DMEM/F-12 (1:1) (1X)], phosphate buffer saline (PBS), foetal bovine serum (FBS), trypsin-EDTA (0.05 %), streptomycin, and penicillin were all of the Gibco brand (Fisher Scientific, Loughborough, UK). Dimethyl sulphoxide (DMSO) (99.7 %) was supplied by Fisher Bio Reagents (Geel, Belgium) and phenol red by Sigma-Aldrich (St. Louis, MO, USA).

Mycotoxin standards – ZEA (≥99 % purity), OTA (≥98 % purity), and BEA (≥97 % purity) – were supplied by Sigma-Aldrich. Stock solutions at 1 mg/mL of each mycotoxin were prepared in methanol and stored in darkness at -20 °C until analysis. They were used at a working concentration of 100 nmol/L as described

elsewhere (10). Methanol was evaporated with nitrogen flux and then the standards diluted in DMSO.

Carotenoid extract from pumpkin pulp was provided by Nicola Marchetti from the University of Ferrara (16). The working concentration of 500 nmol/L is based on beta-carotene as reference carotenoid to simulate a real-life scenario (17).

Solvents for high-performance liquid chromatography (HPLC), namely acetonitrile (Optima LC/MS grade) was acquired from Fisher Scientific, deionised water (resistivity >18 MΩ/cm) was obtained with a Milli-Q SP® Reagent Water System (Millipore Corporation, Bedford, MA, USA), and formic acid (reagent grade ≥95 %) was supplied by Sigma-Aldrich.

Cell culture

The function of the blood-brain barrier was simulated with an *in vitro* model based on human umbilical vein endothelial (ECV304) and rat glioma (C6) cell lines (18). C6 cells were maintained in DMEM/F-12 medium supplemented with 100 U/mL penicillin, 100 mg/mL streptomycin, 10 % (v/v) FBS, and 2 mmol/L L-glutamine. ECV304 cells were kept in DMEM supplemented with 100 U/mL penicillin, 100 mg/mL streptomycin, and 10 % (v/v) FBS. Culture medium was changed every two days. Incubation conditions were: pH 7.4, 37 °C, atmosphere of 5 % CO_2, and constant relative humidity of 95 %.

C6 cells were added to a 24-well PET plate (Falcon) at a density of 5×10^4 cells/mL and incubated for 2–3 h to attach. They provide an appropriate environment for ECV304 cells to differentiate but make no part of the exposure experiment. Then we placed the Millicell-PET culture inserts with a polyethylene terephthalate membrane (0.4 μm pore size, surface 0.3 cm^2; Millipore Corporation, Bedford, MA, USA) into the wells and covered them with ECV304 cells at a density of 5×10^4 cells/mL. This ECV304/C6 co-culture was maintained under incubation conditions for 9–10 days until the barrier was formed.

Monolayer integrity checks

From day 4 of co-culture, we measured transepithelial/transendothelial electrical resistance (TEER) across the ECV304 cell monolayer with epithelial voltohmmeter (Millicell® ERS-2, Millipore Corporation, Bedford, MA, USA) to check BBB integrity (18). The cell monolayer had to be preconditioned for the measurements in HBSS for 30 min by removing the culture medium by aspiration and replacing it with 200 μL of HBSS in the apical compartment and with 1300 μL in the basal compartment. After TEER measurement, HBSS was aspired and replaced with fresh C6 cell medium. The TEER value was calculated using the following formula:

$TEER=(R_{total}-R_{control})*A$

where R_{total} is the resistance of the cell monolayer, $R_{control}$ the resistance of blank or control inserts, and A is the Millicell insert surface**.** TEER reached its maximum around day 9–10 of the experiment. After the exposure, samples were collected, HBSS added, and TEER measured again to check for barrier damage. We found none regardless of treatment.

Monolayer integrity was also verified with phenol red permeation on three control inserts. The medium was aspired and the inserts washed three times with PBS and transferred to a new plate where 1300 μL of PBS were added to the basal compartment. The apical compartment received 200 μL of a phenol red solution in PBS at a concentration of 42 μmol/L, and the plate was incubated at 37 °C for one hour. Then we collected the content of the basal compartment, adjusted pH to 11 with a solution of NaOH, and measured the amount of phenol red in the basal compartment with a spectrophotometer at 558 nm. The finding of <6 % of phenol red in the basal compartment evidenced the integrity of the cell monolayer.

BBB exposure experiment

After we confirmed ECV304 cell confluence with TEER around day 9 or 10, we could proceed with exposing the cells to mycotoxins. Cells were first incubated at 37 °C for 30 min in 800 μL HBSS added to the basal compartments in a new 24-well plate by transferring the ECV304 cell monolayer to this new plate to exclude C6 cells from exposure (18). This step is important in order to exclude metabolites not resulting from ECV304 cell exposure. After this preconditioning, the apical compartments of the inserts were exposed to a 400 μL mixture of HBSS and OTA, ZEA, or BEA alone or OTA+ZEA (1:1) or OTA+ZEA+BEA (1:1:1) combinations (100 nmol/L each) with DMSO in the final concentration of 0.5 %.

All these mycotoxins were also assayed in parallel with the pumpkin extract at the beta-carotene concentration of 500 nmol/L as reference carotenoid.

In control samples the cells were incubated with 0.5 % of DMSO in HBSS. In positive control samples the cells were incubated with 500 nmol/L of beta-carotene in HBSS diluted with 0.5 % of DMSO. All analyses were done in triplicate on cell cultures at passages between the P20 and P30.

After 2-hour exposure at 37 °C simulating mycotoxin transfer across the BBB, the media of the apical and basal compartments were collected separately for each insert/well and kept at -70 °C until metabolite extraction (Figure 1).

Metabolite extraction procedure

The basal compartment was selected for metabolite extraction because it represents the brain in the model. Extracellular metabolites were extracted from the basal culture medium according to the Agilent protocol for preparation and extraction of biological samples for wide coverage in untargeted metabolomics experiments (19).

Briefly, samples were defrosted in an ice bath and kept cold throughout the extraction procedure. Hydrophilic and lipid metabolites were extracted separately. From each sample, two aliquots of 320 µL of media each were collected in low-binding Eppendorf tubes with 800 µL of MeOH and sonicated in an ice/MeOH bath 3–5 times for 1 s each time. After sonication, 400 µL of $CHCl_3$ were added and the Eppendorf tubes vortexed. Then 400 µL of H_2O and 400 µL of $CHCl_3$ were added and vortexed. Finally, the Eppendorf tubes were centrifuged at 2800 *g* at 4 °C for 30 min. Centrifugation serves to separate the aqueous phase containing hydrophilic metabolites at the top layer from protein disc in the middle layer and from the organic phase with lipid metabolites at the bottom layer. The aqueous and organic phase were collected separately and dried. The organic phase was dried under the nitrogen stream, and the aqueous phase was lyophilised. Finally, the dried aqueous and organic phases were reconstituted in 100 µL of chromatographic mobile phase A (H_2O, 0.1 % formic acid). At the end of the process we obtained two replicates of aqueous and two replicates of organic phases per basal sample. This procedure allows to obtain three biological replicates and two technical replicates for each extraction type (organic or aqueous) and statistically analyse sample data.

Liquid chromatography-electrospray ionisation quantitative time of flight-mass spectrometry (LC-ESI-QTOF-MS)

For chromatographic analysis we used an Agilent 1200-LC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a vacuum degasser, autosampler, and binary pump. For the column we used a Luna Omega Polar C18 (100 mm × 2.1 mm, 3 µm particle size; Phenomenex, Torrance, CA, USA). Mobile phase A contained 0.1 % formic acid water, and mobile phase B 0.1 % formic acid acetonitrile. The injection volume and flow rate were to 10 µL and 0.3 mL/min, respectively. Samples were injected in both positive and negative electrospray ionisation (ESI) modes. In the positive mode the injection lasted 20.1 min and the gradient programme was as follows: 0–3 min, 95 % A; 3–3.5 min, 80 % A; 3.5–6 min, 75 % A; 6–8 min, 35 % A; 8–14 min, 20 % A; 14–17 min, 0 % A, and finally 17.1–20.1 min, 95 % A to re-equilibrate the column. In the negative mode, injection lasted 15.5 min, and the gradient programme was as follows: 0–1 min, 95 % A; 1–3.5 min, 50 % A; 3.5–8.5 min, 15 % A; 8.5–9 min, 2 % A; 9–12 min, 0 % A, and 12.5–15.5 min, 95 % A to re-equilibrate the column.

Spectrometry analysis was done with a 6540 Agilent Ultra- High-Definition Accurate-Mass QTOF-MS coupled to the HPLC part equipped with an Agilent Dual Jet Stream for electrospray ionisation (Dual AJS ESI). In the positive mode, the following conditions were used: drying gas flow (N_2) 8.0 L/min; nebuliser pressure 40 psi [275.8 kPa]; gas temperature 300 °C; capillary voltage 4000 V; fragmentor voltage 100 V, skimmer 65 V; and Oct 1 RF Vpp 750 V. The scan range was 50–1500 m/z. Automatic MS/MS experiments were carried out using the following collision energy values: 10, 20, and 30 eV. In the negative mode the conditions were as indicated above, but the capillary voltage was 3500 V. Sample data were acquired using the MassHunter Workstation software version B.08.00 (Agilent).

Feature extraction and chromatographic alignment

Total ion chromatograms generated with LC-QTOF-MS were processed with the MassHunter Profinder batch recursive feature extraction algorithm (Agilent) for small molecules/peptides , which, combined with chromatographic alignment across multiple data files allows peak finding and minimising false positive and negative features.

Statistical analysis

The obtained feature data were then imported to the Mass Profiler Professional (MPP) software, version 15.0 (Agilent) (Figure 1) for statistical data comparison between mycotoxin groups, alone or in combination, with or without the pumpkin extract, and controls. MPP uses the fold change (FC) as the ratio between the groups, and the log ratios are calculated for better visualisation of fold changes (Log2FC). To determine differences in features between mycotoxin and control samples, we used the unpaired t-test. Differences in features between mycotoxin groups were tested with the one-way analysis of variance (ANOVA). *Post-hoc* Tukey tests were used to identify features differing between each mycotoxin group. Two-way ANOVA was used to compare mycotoxin and pumpkin extract data. The level of significance was set at $p<0.05$ for all cases using the Benjamini-Hochberg multiple testing correction. Finally, to identify significant features as compounds we relied on the Metlin database, which contains experimental MS/MS data for more than 500.000 compounds (20).

Bioinformatics

Once we identified relevant compounds (metabolites), we could put to use the Reactome Knowledgebase (21) to establish biological processes likely to occur in humans. This knowledge base maps molecular features of transport, signal transduction, metabolism, DNA replication, and other cellular processes to provide a user-friendly tool for discovering functional relationships between metabolites.

RESULTS

Differential analysis of metabolite abundances

Metabolomics analysis identified 181 features in the basal phase, of which 159 were statistically significant

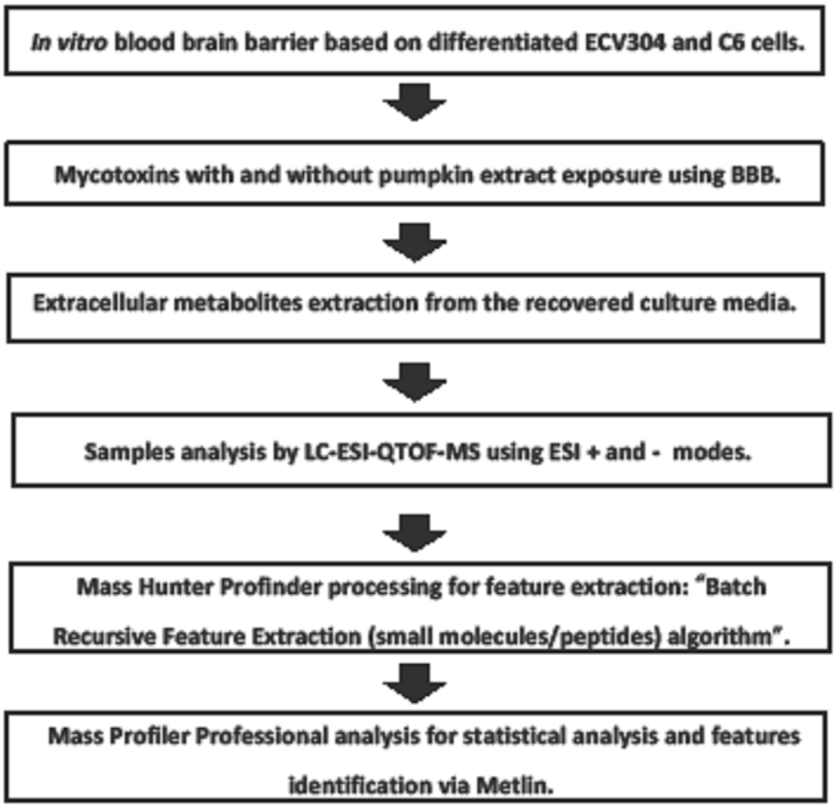

Figure 1 Workflow used to assess the metabolic profile of the blood-brain barrier model exposed to mycotoxins with and without pumpkin extract. BBB – blood brain barrier; LC-ESI-QTOF-MS – liquid chromatography-electrospray ionisation quantitative time of flight-mass spectrometry

compared to control. Among these features, 74 were found in the samples not treated with the pumpkin extract and 90 in samples with this extract. Twenty-five features were reported in both the organic and aqueous phase in the same treatment group. Figure 2 shows the overlapping of components between the groups.

The total number of metabolites after removing feature repetitions in both the aqueous and organic phases and in positive and negative ionisation modes was 46. Of these, 14 were found in samples with and without pumpkin extract (Table 1). In samples treated with pumpkin extract, only 9-docosene and dihydromorelloflavone were identified regardless of the mycotoxin used, but only dihydromorelloflavone was significantly less abundant in these samples than in positive control (control treated with pumpkin extract), while it was not found in samples not exposed to the extract.

Our findings of antifungal and insecticide compounds including dimethylmaleic acid anhydride, dodemorph, hexazinone, *n*-cyclopropylammeline, and nigrifactin (in samples not treated with pumpkin extract) and spiromesifen, streptonigrin, tioconazole, chloralose, and octadeca-1(14),15,17-trien-13-one in samples treated with the extract point to a prospect of a new line of research and industrial application.

Reactome findings

Some of the above 46 metabolites are relevant for human metabolic pathways. One of them is leukotriene 14,15-LTE4, whose abundance was higher than control in ZEA and BEA+OTA+ZEA samples not treated with the pumpkin extract. It was also identified in BEA samples treated with the extract.

Another metabolite is prostaglandin D2-glycerol, found in supernatant samples treated with OTA+ZEA and BEA+OTA+ZEA combinations but not with the pumpkin extract.

Among the fatty acids relevant for human metabolism, we found oleic acid in BEA and pumpkin extract samples and oleamide in BEA+OTA+ZEA samples (Table 1). Phospho-glycerol (PG)(16:0/0:0) was only identified in control and ZEA samples. Higher xestoaminol C was found in ZEA samples (compared to control). 12,13-DiHOME-d4 was more abundant in BEA, OTA+ZEA, and BEA+OTA+ZEA samples. 9-docosene was more abundant than in control in all samples save for those treated with BEA and OTA (BEA alone, OTA alone, and BEA+OTA+ZEA combination).

Mercaptoethanol was found in ZEA and OTA+ZEA samples without the pumpkin extract and in OTA+ZEA samples with pumpkin extract (Table 1).

Table 1 Log2FC values of metabolites with significantly changed quantity ($p<0.05$) compared to respective controls (control containing vehicle or vehicle + pumpkin extract) found in the basal media after 2-hour exposure to mycotoxins (100 nmol/L each) with or without pumpkin extract (500 nmol/L beta-carotene equivalent)

Compound	BEA	BEA+E	OTA	OTA+E	ZEA	ZEA+E	OTA+ZEA	OTA+ZEA +E	BEA+OTA +ZEA	BEA+OTA +ZEA+E
(2R,3S,7S,10S)-2,3,7,10-tetramethyl dodecahydrocyclo penta[b]quinolizin-2-ol	15.57									
(5E)-(3S)-1alpha,25-dihydroxy-3-deoxy-3-thiavitamin D3 3-oxide / (5E)-(3S)-1alpha,25-dihydroxy-3-deoxy-3-thiacholecalciferol 3-oxide				15.68						
12-epi leukotriene B4-d4	18.12									
12,13-DiHOME-d4	18.35						18.49		18.34	
14,15-LTE4		15.56			15.56				15.56	
2-(3-phenylpropyl)pyridine			20.60		19.28			17.84		
2-butyl-5-methyl-4-propyloxazole	15.70			15.49		15.79		15.98	15.88	
2-ethyl-5-methylpyridine			19.36							
2-propenal, 3-(1,3-benzodioxol-5-yl)-	-19.15	18.18	-19.15	16.59		17.06		1.96	-19.15	1.87
2-protocatechoylphloro glucinolcarboxylate		-16.29						-16.29		-16.29
2,6-dihydroxy-4-methoxytoluene									2.06	
3-methylfluoranthene							16.09			
3-methylxanthine								16.28		
3,4-dichlorophenol	-17.93		-17.93				-17.93		-17.93	
4-(3-hydroxy-7-phenyl-6-heptenyl)-1,2-benzenediol			17.37							
6-hydroxy-4-decanolide				16.77	16.37					
6-hydroxykaempferol 7-rutinoside		-16.32		17.04				17.18		-16.32
6'-hydroxy-2,3,4,5,2',3',4'-heptamethoxychalcone	17.06									
9-docosene		16.35		16.94	16.63	16.65	16.58	16.33		16.38
Asp-Met-OH			16.18						16.80	
(7R)-7,15,17-trihydroxy-11-methyl-12-oxabicyclo[12.4.0] octadeca-1(14),15,17-trien-13-one		-17.71		-17.71		-17.71		-17.71		-1.68
Dihydromorelloflavone		-16.68		-16.18		-16.18		-16.18		-16.18
Dimethylmaleic acid anhydride		17.61	18.04	16.06	17.80		17.12	2.05	17.75	1.92
Dodemorph		-16.98	18.23	-16.98	-16.82	-16.98		16.30		16.16
Herbacetin 8-(2",3",4"-triacetylxyloside)				15.76						
Hexazinone				-18.83	-16.76		-16.76			-18.83
Ketophenylbutazone								17.33		17.17
Mercaptoethanol							16.46	19.32	-19.14	
Methylmethionine sulfonium									0.73	
N-(2'-(4-benzenesulfonamide)-ethyl) arachidonoyl amine	21.56	21.28	19.63		16.46	17.65			21.39	
N-(2'-(4-benzenesulfonamide)-ethyl) arachidonoyl amine					17.29	17.69		18.54		

Compound	BEA	BEA+E	OTA	OTA+E	ZEA	ZEA+E	OTA+ZEA	OTA+ZEA +E	BEA+OTA +ZEA	BEA+OTA +ZEA+E
Nigrifactin								18.22	18.52	
Oleamide									16.96	
Oleic acid(d2)		15.34								
Peroxyoctanoic acid					1.53					
PG(16:0/0:0)	-17.39	-17.11	-17.39	-17.11		-17.11	-17.39	-17.11	-17.39	-17.11
Pregna-4,16-diene-3,11,20-trione								17.12		
Propyl levulinate			17.22							
Prostaglandin D2-1-glyceryl ester							17.60		18.09	
Prostaglandin D2-1-glyceryl ester										
Rhamnetin 3-rhamnoside			16.60					17.31		16.07
Spiromesifen				16.13				16.45		
Streptonigrin								16.70		16.51
Tioconazole								19.47		
Vestitone 7-glucoside							17.07		17.05	
Xestoaminol C	-16.08	-16.70	-16.08		16.50			-16.72		-16.70

BEA – beauvericin; E – pumpkin extract; OTA – ochratoxin A; ZEA – zearalenone. Positive values indicate higher and negative values lower expression compared to control

DISCUSSION

We identified several metabolites involved in inflammatory response, which confirmed the protective effect of carotenoids against BBB damage induced by BEA, OTA, and ZEA. Thanks to the untargeted metabolomics approach, our findings of *N*-(2'-(4-benzenesulfonamide)-ethyl) arachidonoyl amine, 12-epi LTB4-d4, 14,15-LTE4, and prostaglandin D2-1-glyceryl ester point to a possible involvement of the arachidonic acid pathway in human blood-brain barrier response following low mycotoxin exposure. These metabolites of arachidonic acid oxidation activate mediators of wound healing and inflammation (22–25) and increase BBB permeability (26), leukotriene in particular (27). We believe that cell exposure to ZEA, BEA, and OTA in our study, alone or combined, is related to lipid peroxidation, as reported elsewhere (28, 29).

Samples exposed to pumpkin extract in our study, in turn, had neither leukotriene B4 or prostaglandin D2-1-glyceryl ester, which points towards flavonoid protection against mycotoxin-induced damage (Table 1). The main protective mechanism of flavonoids is related to their potent free radical scavenging, which lowers the risk of oxidative damage and consequently of inflammation (30). This anti-inflammatory action of flavonoids is related to the inhibition of enzymes involved in arachidonic acid metabolism, such as cyclooxygenase and 5-lipoxygenase (31).

Interestingly, samples treated with BEA plus pumpkin extract still showed 14,15-LTE4 in the basal media but not the rest of the abovementioned metabolites. If an interaction between BEA's ionophoric activity, which increases ion permeability in biological membranes and reactive oxygen species levels (28, 32), and carotenoid instability is the reason for increased lipid peroxidation (33) we have not observed with other mycotoxins, this is something that deserves to be studied in the future. It is also worth mentioning that unlike OTA and ZEA, BEA has not been regulated in terms of limits and is often detected in cereal-based food around the world (34).

CONCLUSION

The BBB human *in vitro* model employed in our study points to the involvement of the arachidonic acid pathway in mycotoxin action through metabolites like N-(2'-(4-benzenesulfonamide)-ethyl) arachidonoyl amine, 12-epi LTB4-D4, 14,15-LTE4, and prostaglandin D2-1-glyceryl ester, which was confirmed by their drop under the influence of pumpkin extract. The only exception was concomitant exposure to BEA and the extract, in which this protective effect was absent.

The limitation of our study was that it yielded low metabolite concentrations in the basal samples, which means that some metabolites released into media in even smaller concentrations may have been overlooked. Future research may solve this issue with the use of larger wells that can take greater volumes of the media and the use of well inserts with a wider surface. Nevertheless, our experimental design and statistical analysis, including multiple testing corrections, yielded reliable results with the detected metabolites.

Future studies of molecular events triggering a toxicological pathway after exposure to BEA alone and in

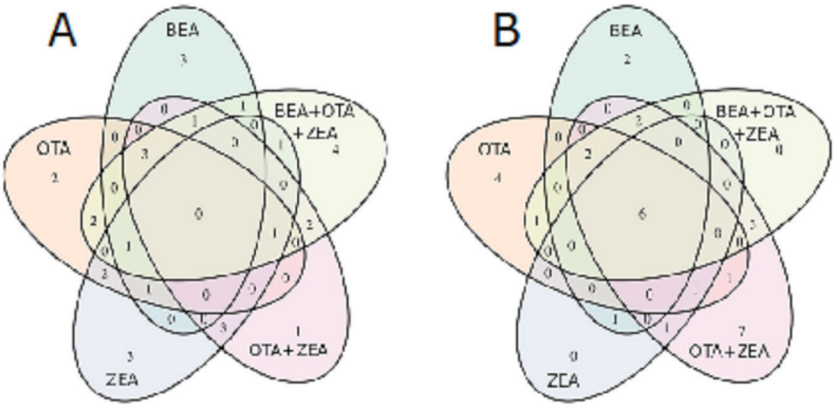

Figure 2 Venn diagrams of the number of significant metabolites ($p<0.05$) after 2-hour mycotoxin exposure (100 nmol/L per mycotoxin) without pumpkin extract (A) and with pumpkin extract (500 nmol/L) (B)

combination with other mycotoxins could reveal a possible synergy or antagonism with other mycotoxins or dietary components.

Acknowledgements

This work was supported by the Generalitat Valenciana (PROMETEO/2018/126) and the Spanish Ministry of Science and Innovation (PID2019-108070RB-I00-ALI). Co-authors Manuel Alonso-Garrido and Noelia Pallarés wish to thank the Spanish Ministry of Science and Innovation and the University of València for their respective PhD grants (BES-2017-081328and UV-INV-PREDOC16F1-384781).

Conflict of interests

None to declare.

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Uloga karotenoida iz ekstrakta mesa bundeve u zaštiti od oštećenja krvno-moždane barijere uzrokovane mikotoksinima *in vitro*

Pojedini mikotoksini poput bovericina (BEA), okratoksina A (OTA) i zearalenona (ZEA) prelaze krvno-moždanu barijeru, a to je i razlog zbog kojega smo istražili djelovanje ekstrakta karotenoida iz mesa bundeve protiv upalnih procesa izazvanih ovim mikotoksinima i njihovim kombinacijama (OTA+ZEA i OTA+ZEA+BEA) na modelu krvno-moždane barijere koji se sastojao od kultura stanica ECV304 i C6, oslanjajući se pritom na neciljani metabolomički pristup. Stanice su tretirane mikotoksinima u koncentraciji od 100 nmol/L po mikotoksinu odnosno ekstraktom karotenoida u koncentraciji od 500 nmol/L. Za kontrolu smo upotrijebili samo otapalo (stanična kontrola) odnosno otapalo s bundevinim ekstraktom (ekstraktna kontrola). Nakon dva sata tretmana uzorci su analizirani metodom tekućinske kromatografije / masene spektrometrije (HPLC-ESI-QTOF-MS), a dobiveni metaboliti identificirani su usporedbom s bazom podataka *Metlin*. Primjena ekstrakta značajno je smanjila količinu metabolita proupalne arahidonske kiseline eoksina (14,15-LTE4) u kulturama tretiranima samo zearalenonom odnosno kombinacijom BEA+OTA+ZEA. Drugi upalni biljeg, prostaglandin D2-glicerol ester, otkriven je samo u kulturama tretiranima kombinacijama OTA+ZEA odnosno BEA+OTA+ZEA, ali ne i u onima koje su usto tretirane bundevinim ekstraktom. Osim toga, u prisutnosti mikotoksina značajno je porasla koncentracija metabolita dihidromoreloflavona iz bundevina ekstrakta. Time je taj ekstrakt iskazao zaštitno djelovanje protiv stanične upale uzrokovane mikotoksinima zahvaljujući svojstvima flavonoida koji se nalaze u njezinu mesu.

KLJUČNE RIJEČI: bovericin; ECV304; metabolomika; okratoksin A; zearalenon