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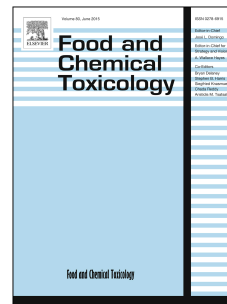

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Credit author statement

Guillermina Font conceptualized the work and acquire the funding. L. Manyes supervised and administered the Project. A. Maietti, P. Tedeschi and N. Marchetti were responsible of the carotenoids extraction and determination. Manuel Alonso-garrido designed and made the experiments, analyzed the data and its visualization, wrote the manuscript and followed the updates by editors and reviewers as corresponding author.

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Mitochondrial transcriptional study of the effect of aflatoxins, enniatins and carotenoids *in vitro* in a blood brain barrier model.

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Abstract

C. maxima (var. Delica), a variety of pumpkin, is well known for its high concentration on carotenoids, possessing dietary benefits and antioxidant properties. Aflatoxins and enniatins are common mycotoxins present in food and feed with an extended toxicity profile in humans and animals. Both types of substances reach a wide range of tissues and organs and have the capability to penetrate the blood brain barrier. Since carotenoids and mycotoxins have been reported to modify diverse mitochondrial processes individually, transcriptional *in vitro* studies on human epithelial cells ECV 304 were conducted to analyze the relative expression of 13 mitochondria related genes. ECV 304 cells were differentiated for 9 days and treated for 2h with: a) pumpkin (500 nM); b) aflatoxins (100 nM); c) enniatins (100 nM); d) aflatoxins (100 nM) and pumpkin (500 nM); e) enniatins (100 nM) and pumpkin (500 nM). Even at low concentrations, dietary carotenoids activity on mitochondrial genes expression reported a beneficial effect and, for most of the genes studied across the Electron Transport Chain (ETC), developed a protective effect when mixed with aflatoxins (AFs) or enniatins (ENs).

Keywords: qPCR, ECV 304, mycotoxicity, antioxidants, neurodegenerative diseases, alzheimer.

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Introduction

Due to globalization and long-term storage, mycotoxins are a great issue in food control and safety. They are common fungal compounds present in food and feed with a wide toxicity profile in humans and animals: hepatotoxic, cytotoxic, neurotoxic, genotoxic, estrogenic, nephrotoxic, immunosuppressive, mutagenic, teratogenic and/or carcinogenic effects (Eskola et al. 2018; Ostry et al. 2017). Many mycotoxins such as aflatoxins (AFs), deoxynivalenol (DON), enniatins (ENs), ochratoxin, cause oxidative stress (Del Regno et al. 2015; Prosperini et al. 2013; Jilani et al. 2012; Da Silva et al. 2018). Mycotoxins induce reactive oxygen species (ROS) causing DNA, proteins and lipid damage at a cellular level, while carotenoids act on oxidable substrates as ROS scavengers.

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β-carotene antioxidant activity has been well documented up to date, being one of the most potent dietary ROS scavengers. It has also been associated with protective effect against numerous neurodegenerative diseases (Hira et al. 2019; Guerra-Araiza et al. 2013). Studies performed using natural substances like anthocyanin, melatonin or minerals, have reported antioxidant ability to modulate the oxidative stress caused by mycotoxins with positive results (Sorrenti et al. 2012; Yenilmez et al. 2010; Shi et al. 2012). For example, a study on cellular bioavailability and cell proliferation performed by Strasser and colleagues (2013) in murine YAC-1 lymphoma cells confirmed a protective role of β-carotene against DON related oxidative stress activity.

Lutein is a xanthophyll with a certain polar solubility due to its oxygenated cycles acting as a direct ROS scavenger because of its many double bonds in its chemical structure. Several studies *in vivo* and *in vitro* have reported lutein protection against pathologies such as age-related macular and retina degeneration, osteoporosis, ischemia and chronic degenerative diseases of the brain (Li et al. 2018; Cheng et al. 2015; Kamoshita et al. 2016; Erdman et al. 2015; Brennan & Kantorow, 2009). There are many fruits and vegetables with high contents in carotenoids. One particular case is *C. maxima (var. Delica)*, a pumpkin cultivated in the South Po, Italy, which has been identified to possess high levels of β-carotene and lutein (Bergantin et al. 2018).

Focusing on the mitochondria, Complex I (CI) deficiency is the most common genetic abnormality in mitochondrial energy production, being responsible for approximately a third of the oxidative phosphorylation (OXPHOS) related disorders. Cysteine oxidation (S-oxidation, S-glutathion and S-nitrosylation) is also mediated by redox signals, which has also been reported to regulate CI activity. Therefore, even minimum defects in CI redox signals can generate OXPHOS disruption leading to oxidative stress and lately, disease. CI reduced activity has been reported in Central Nervous System (CNS) of patients with Parkinson Disease (PD), which could lead to ROS misbalance provoking mitochondrial DNA (mtDNA) damage. Mutations and polymorphisms in mtDNA have also been associated to PD development or increasing PD risk (Lin and Beal, 2006). Complex IV (CIV) binuclear center (CuB-heme a3) binds to the ubiquinol oxidases like the mitochondrially encoded cytochrome c oxidase I (MT-CO1). MT-CO1 is key to maintain proton pumping and dioxygen reduction. It has also been associated to diverse pathologies, including Alzheimer disease (AD) (Hu et al. 2017). Complex V (CV) activity falls with ageing, and supression of CV activity provokes oxidative damage to

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nuclear DNA, which could result in reduced gene expression with ageing. Neurodegenerative diseases are thought to be linked to decreased Adenosine Triphosphate (ATP) synthesis (Van Bulck et al. 2019).

Nuclear codified genes are also important for mitochondrial functionality and OXPHOS balance. Oxidative Stress Induced Growth Inhibitor 1 (OSGIN1) is regulated by p53 and activated by DNA damage. OSGIN1 codifies an oxidative stress response regulating cell death and apoptosis by causing cytochrome c release from mitochondria. Protein loss is linked with uncontrolled cell growth and tumor formation (Hu et al. 2015). AFB1 has been found to inhibit the Electron Transport Chain (ETC) at the cytochrome oxidase level, precisely between cytochrome b1 and c, inducing also the release of different ROS species (Theumer et al. 2018; Sharma 2018). Also, affinity of AFB1 for mitochondrial genes is 3 or 4 times higher than for nuclear DNA. ENs play an important role in the disruption of redox balance too by altering the ionophoric channels, which could provoke the activation of different cell pathways (Prosperini et al. 2017).

In this work, two different mycotoxin mixtures and its analogues were chosen for treatment due to their different mechanism of action and their consideration by the authorities: legislated aflatoxins (AFB1, AFB2, AFG1, AFG2) and non-legislated enniatins (EN A, EN A1, EN B, EN B1). This study contributes to a better understanding of the interaction at transcriptional level between mycotoxins and carotenoids and their possible role in the mitochondrial OXPHOS balance.

Material and methods

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Reagents

The reagent grade chemicals and cell culture components used, DMEM/F-12 and DMEM medium (Thermo Fisher, USA), penicillin/streptomycin, phosphate buffer saline (PBS), AFs (AF B1, AF B2, AF G1, AF G2) and ENs (EN A, EN A_1, EN B, EN B_1) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) and methanol were obtained from Fisher Scientific (Madrid, Spain). Deionised water (resistivity <18 MV cm) was obtained using a Milli-Q water purification system (Millipore, Bedford, MA, USA). Stock solution of mycotoxins at 1000 ppm was prepared in methanol and maintained at –20 °C. Evaporation of mycotoxins was performed using nitrogen flux and diluted in DMSO. Pumpkin extract (pumpkin) was obtained from *C. maxima* (*var. Delica*) thanks to N. Marchetti (Department of Chemistry and Pharmaceutical Sciences, University of Ferrara) and kept at -80 °C (Bergantin et al. 2018). pumpkin was also dissolved in DMSO for cell treatment. Final concentrations of mycotoxins (100 nM) and pumpkin (500 nM) in the assay were achieved by their dilution in the culture medium. The final DMSO concentration in the medium was 1% (v/v).

Cell culture

ECV 304 cells were initially thawed from liquid nitrogen, plated, grown to confluence, trypsinized and suspended in culture medium. They were maintained in DMEM medium supplemented with 100U/mL penicillin, 100 mg/mL streptomycin and 10% (v/v) FBS inactivated and amphotericin B 0,1% (Gibco^{TM}). Absence of mycoplasma

was checked routinely using the Mycoplasma Stain Kit (Sigma–Aldrich, St. Louis MO, USA). Culture medium was replaced daily every 2 days from day 4 and the co-culture was maintained at 37 °C, with a relative humidity of 90% and the atmosphere of 5% CO_2. ECV 304 has been described as an appropriate mono-culture model to assess permeability in the blood brain barrier (BBB), demonstrating tighter barrier function than other endothelial cell lines. In combination with chromatography-mass spectroscopy analysis, is a fast and successful technique to screen bioactive compounds crossing the BBB (Yang et al. 2018).

Primer design and Quantitative Real-Time PCR assays

Gene-specific primers were designed using Primer-BLAST (Ye et al. 2012) using default criterion of the software with amplified products ranging from 75 to 150 bp and Tm at 59 °C. Primer sequences from Escrivá et al. (2018) were used in qPCR analyses. Standard curve by qPCR was performed for all primer pairs and a single amplification product for each gene was obtained by the melting curve assay. Primer amplification efficiency was determined from standard curve generated by serial dilution of cDNA (5 fold each) for each gene in triplicate. Correlation coefficients (R^2 values) and amplification efficiencies (E) for each primer pairs were calculated from slope of regression line by plotting mean Cq values against the log cDNA dilution factor in StepOne software. Realtime amplification reactions were performed in 96 well plates using SYBR Green detection chemistry and run in triplicate on 96-wells plates with the StepOne Plus Real-time PCR machine (Applied Biosystems). Reactions were prepared in a total volume of 10 µL containing: 3 µL of 1:5 diluted template, 1 µL of each amplification primer (5 µM) and 5 µL of 2x Fast SYBR Green (Applied Biosystems).

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Non-template controls (NTC) were also included for each primer pair, replacing the template by water DNAse and RNAse free from the RNA extraction kit (ReliaPrep™ RNA Cell Miniprep System, Promega). The cycling conditions were set as default: initial denaturation step of 95 °C for 5 min to activate the Taq DNA polymerase, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 59 °C for 15 s and elongation at 72 °C for 30 s. The melting curve was generated by heating the amplicon from 60 to 90 °C. Baseline, threshold cycles (Ct) and graphs were automatically determined using the StepOne Plus Software version 2.3 (Applied Biosystems). Three technical replicates were performed for each condition. Every experiment was performed according to MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) guidelines (Bustin et al. 2009).

Table 1. Primers used for qPCR analysis.

Gene	*Sequence 5'-3'*	*Amplicon(bp)*	*Efficiency (%)*	*Linearity (R^2)*
MT-ND2	F:CGTAAGCCTTCTCCTCACTC R:CAACTGCCTGCTATGATGGA	51	141.3	0.997
MT-ND3	F:CCCTCCTTTTACCCCTACCA R:GCCAGACTTAGGGCTAGGAT	100	82.0	0.996
MT-ND4	F:CACACGAGAAAACACCCTCA R:AAACCCGGTAATGATGTCGG	82	151.9	0.992
MT-ND4L	F:CCCACTCCCTCTTAGCCAAT R:GGCGGCAAAGACTAGTATGG	53	121.0	0.993
MT-ND5	F:CATCCCCCTTCCAAACAACA R:GTCCTAGGAAAGTGACAGCG	69	125.2	0.991
MT-CO1	F:TCATAATCGGAGGCTTTGGC R:GTTGTTTATGCGGGGAAACG	80	121.1	0.992
MT-CO3	F:CTTCCACTCCATAACGCTCC R:GTTACATCGCGCCATCATTG	78	129.6	0.991
MT-ATP6	F:CTAGAAATCGCTGTCGCCTT R:ATGTGTTGTCGTGCAGGTAG	76	76.4	0.985
MT-ATP8	F:CCCTGAGAACCAAAATGAACGA R:GATTGTGGGGGCAATGAATGA	56	112.9	0.996
MT-RNR2	F:GTAAATCGGAATGGACCCCC R:CTGCTGGGGGATTTTCTTGT	93	85.5	0.991
MRPL12	F:GATGGGTGGTGTGATGTCTG R:TGTCCGTTCTTTCGCTATGG	88	123.8	0.992

OSGIN1	F:TCTTTGATGCCCTTCTACGC R:CGACTTCATGTTTCCCCCAA	53	142.9	0.980
SRXN1	F:GGTCTAGGGGAAGAGGTGTT R:CTTGGTTTTCAGAAGCCCCT	141	137.5	0.992
TXNIP	F:GTGAAGGTGATGAGATTTCC R:CTCTGACTGATGACAACTTC	146	125	0.985
*S18**	F:CGGCTACCACATCCAAGGAA R:GCTGGAATTACCGCGGCT	100	101.5	0.994

Gene expression analysis

In order to assess the statistical analysis, ΔCt (experimental Ct –housekeeping Ct mean) obtained by qPCR was used. Levene's test was applied to evaluate the equality of group variances and all the group variances were equal. T-student was used to evaluate differences between groups. Statistical analysis was performed with SPSS 24.0 (IBM Corp., Armonk, NY, USA). $p \leq 0.05$ was considered to indicate statistically significant differences. Pathway assignments were carried out using PathVisio software with Hs_Derby_Ensembl_85 bridge gene dataset (Kutmon et al. 2015). Adjusted $p \leq 0.05$ was used as the threshold to identify the statistically significant pathways.

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<u>Results</u>

Differential Expression of Genes (DEG) analysis of several genes belonging to the different complexes of the ETC was performed throughout qPCR reporting statistically significant results for most of the compared treatments in the genes studied.

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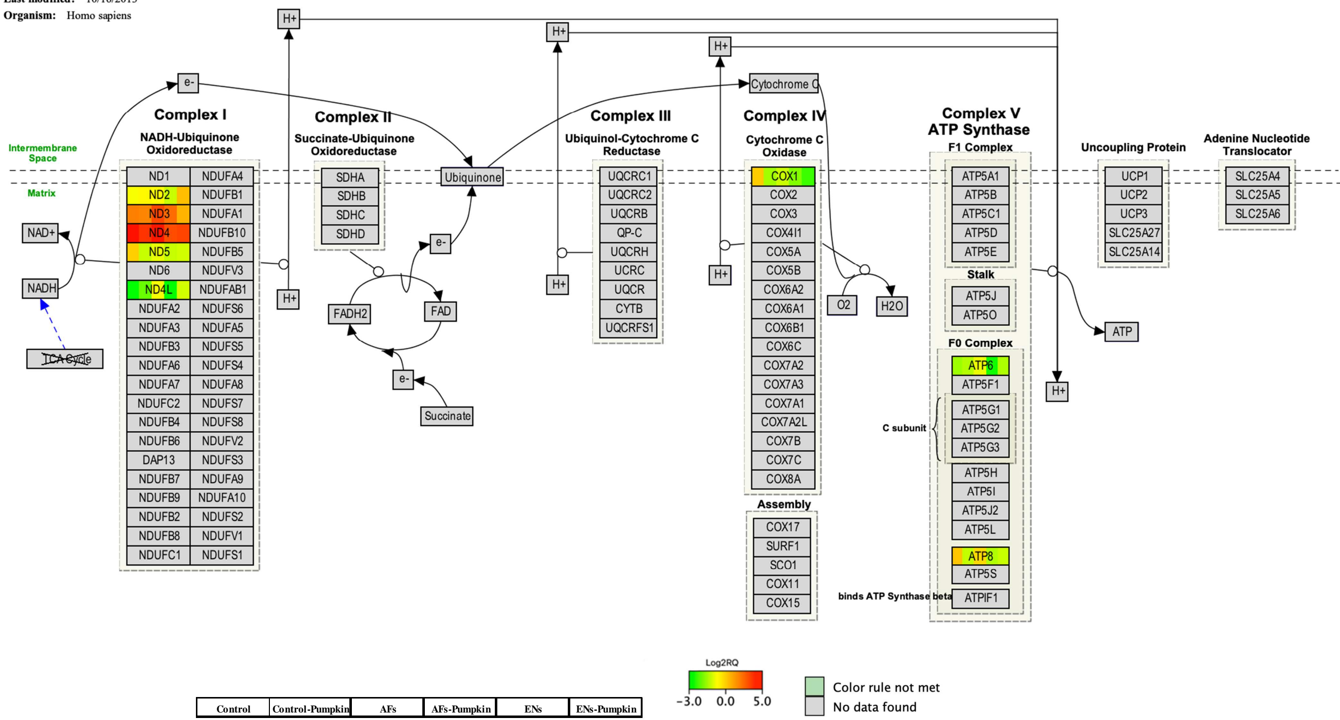
a)



b)

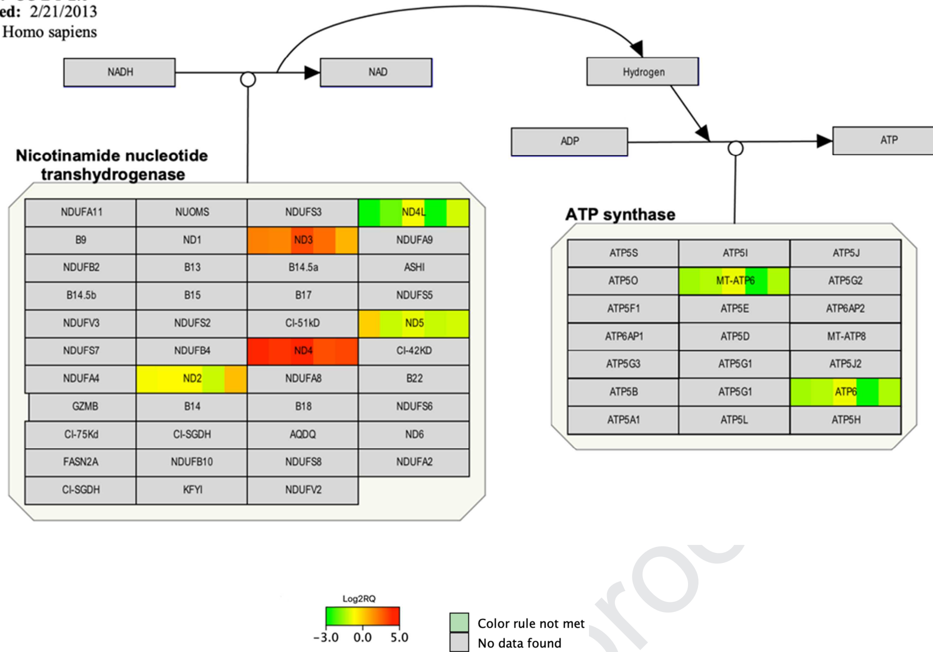


Figure 1. a) Genes involved in the ETC pathway for Homo sapiens are shown: in green the down-regulated genes and in red the up-regulated for all conditions tested. b) Genes involved in the OXPHOS pathway for Homo sapiens are shown: in green the down-regulated genes and in red the up-regulated for all conditions tested.

Complex I

In this study, MT-ND2 was down-regulated for every condition, but for AFs-pumpkin, which reported a similar expression as the control. ENs followed the same trend with more accused differences, finding that ENs-pumpkin significantly reverted ENs down-regulation ($p \leq 0.05$, data not shown). MT-ND3 and MT-ND4 were up-regulated for every condition, but ENs for MT-ND4. MT-ND4L was slightly up-regulated for every condition, but ENs. MT-ND5 was slightly down-regulated for every condition, except for pumpkin (Fig. 1).

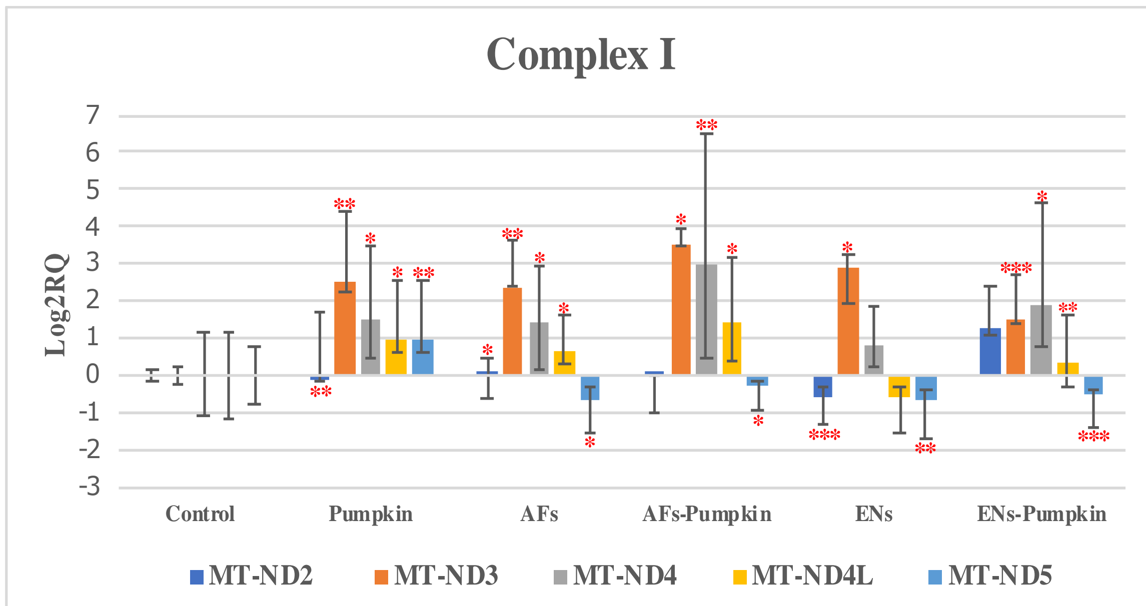
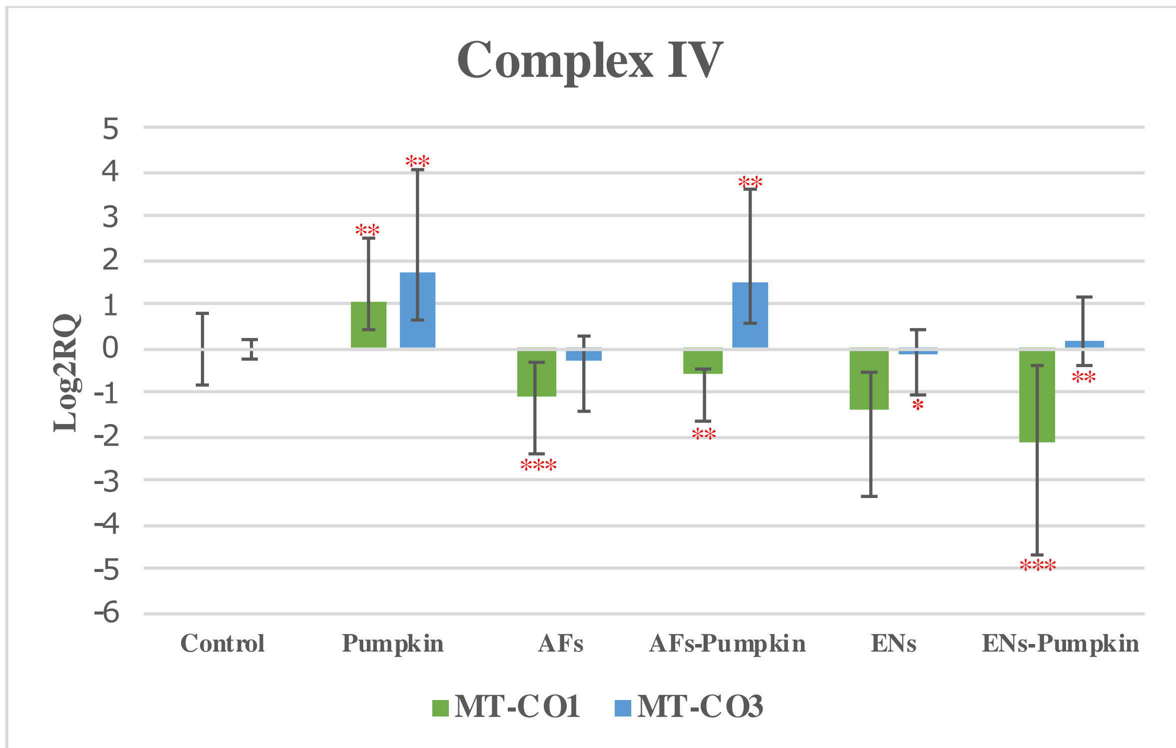


*Figure 2. Bar plot showing CI genes relative expression when compared to control (log2RQ = 0) after 2h-exposure to the different treatments by qPCR. RQ, relative quantification. Error bars, log2RQmin and log2RQmax. *p < 0.05; **p < 0.01; ***p < 0.001.*

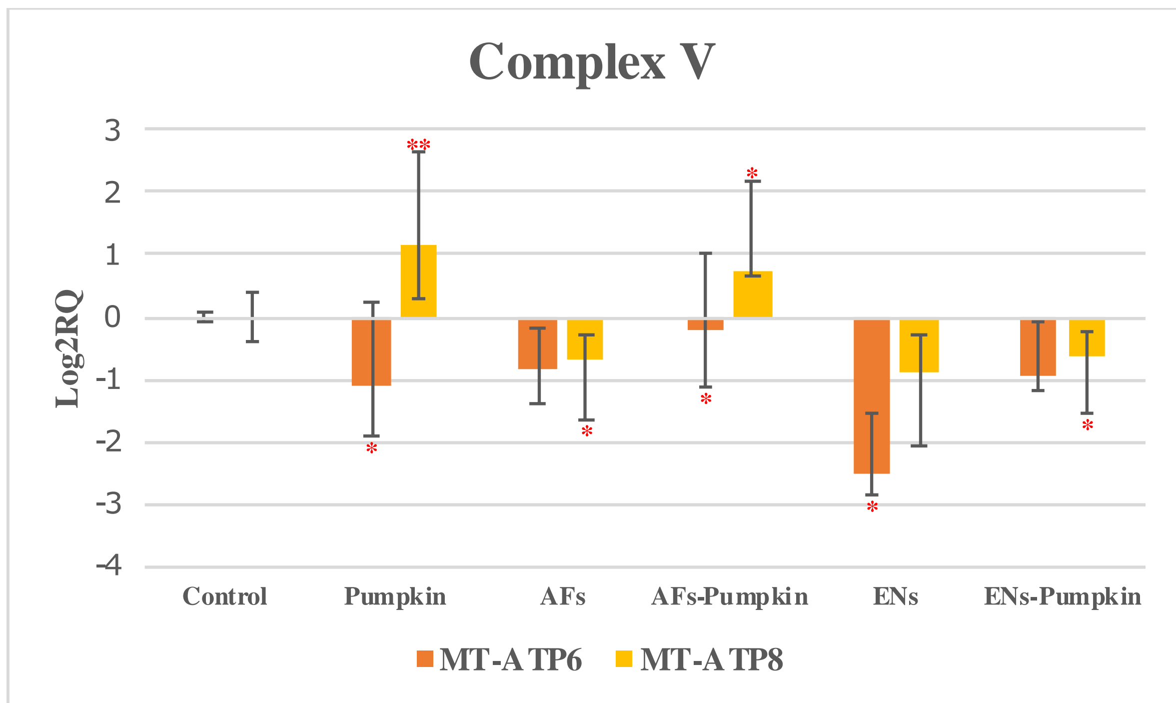
Complex IV

Cytochrome *c* oxidase (CIV) is a proton-pump form by heme-cooper oxidases representing the final step of the energy transfer enzymes of the ETC both in mammals and prokaryotes. MT-CO1 was slightly down-regulated for all treatments, but pumpkin and ENs, when compared to the Control. Interestingly, both AFs and ENs, mixed with pumpkin, reported significant results compared to AFs and ENs individually (Fig. 3, a).

a)



b)



c)

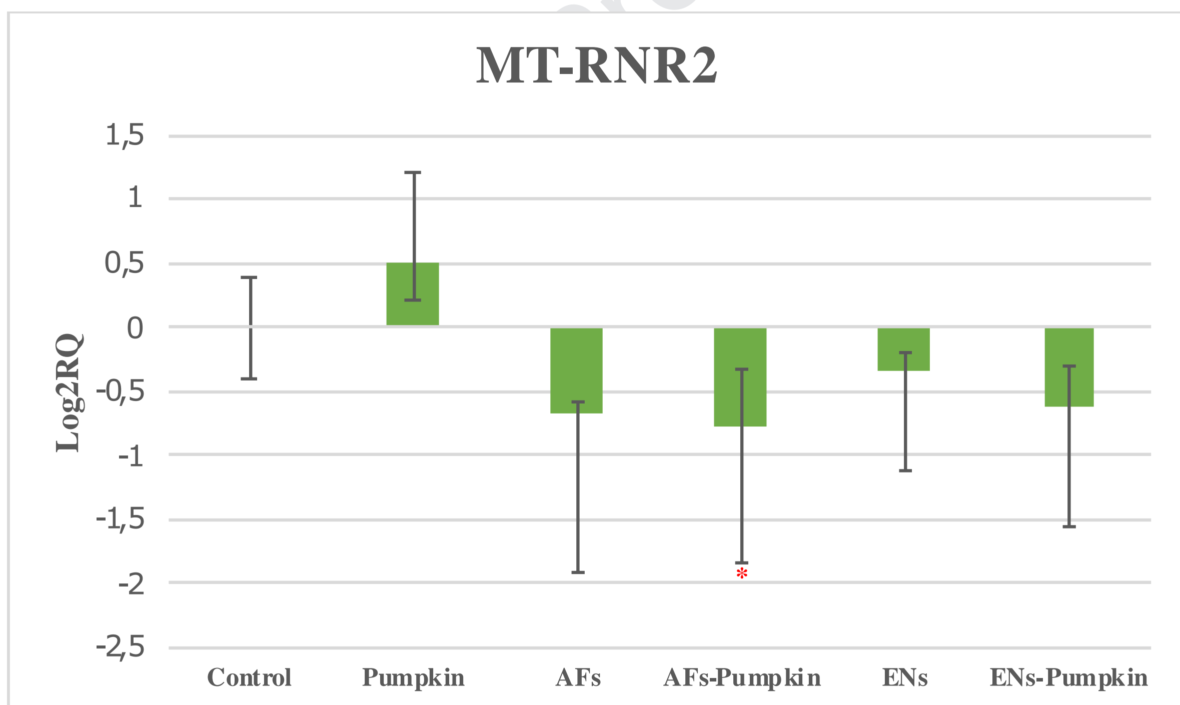


*Figure 3. A. Bar plot showing MT-CO1 and MT-CO3 relative expression when compared to control (log2RQ = 0) after 2h-exposure to the different treatments by qPCR. B. Bar plot showing CV genes relative expression when compared to control. C. Bar plot showing MT-RNR2 relative expression when compared to control. RQ, relative quantification. Error bars, log2RQmin and log2RQmax. *p < 0.05; **p < 0.01; ***p < 0.001.*

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Complex V

Mitochondrially encoded ATP synthase membrane subunit 6 (MT-ATP6) and Mitochondrially encoded ATP synthase membrane subunit 8 (MT-ATP8) are involved in the synthesis of $F_{0,}$ a main subunit of CV. DEG analysis for MT-ATP6 showed down-regulation for pumpkin, AFs-pumpkin and ENs and no significative alteration for AFs and ENs-pumpkin. MT-ATP8 behave differently, being significantly altered for every condition, except ENs (Fig. 3, b).

Mitochondrially Encoded 16S rRNA

Mitochondrially Encoded 16S rRNA (MT-RNR2) is a non-coding mitochondrial DNA related to apoptosis and biogenesis of ribosomes in eukaryotes. DEG analysis of MT-RNR2 showed no alteration for any of the treatments, but AFs-pumpkin, suggesting it is not a target for any of the mycotoxins or carotenoids tested, at least at these concentrations (Fig. 3, c).

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Nuclear encoded mitochondrial genes

In eukaryotes, mitochondria function and structure are also mediated by genes belonging to the nuclear DNA. For the current project, 4 important genes were selected: 3 coding antioxidant proteins and 1 coding for ribosome formation.

OSGIN1 was altered for every treatment, but ENs. Sulfiredoxin-1 (SRXN1) only was significantly down-regulated for AFs-pumpkin and ENs treatments. Mitochondrial Ribosomal Protein L12 (MRLP12) was repressed for every treatment, but ENs.

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Thioredoxin Interacting Protein (TXNIP) was significantly up-regulated for pumpkin and ENs-pumpkin and down-regulated for AFs-pumpkin (Fig. 4).

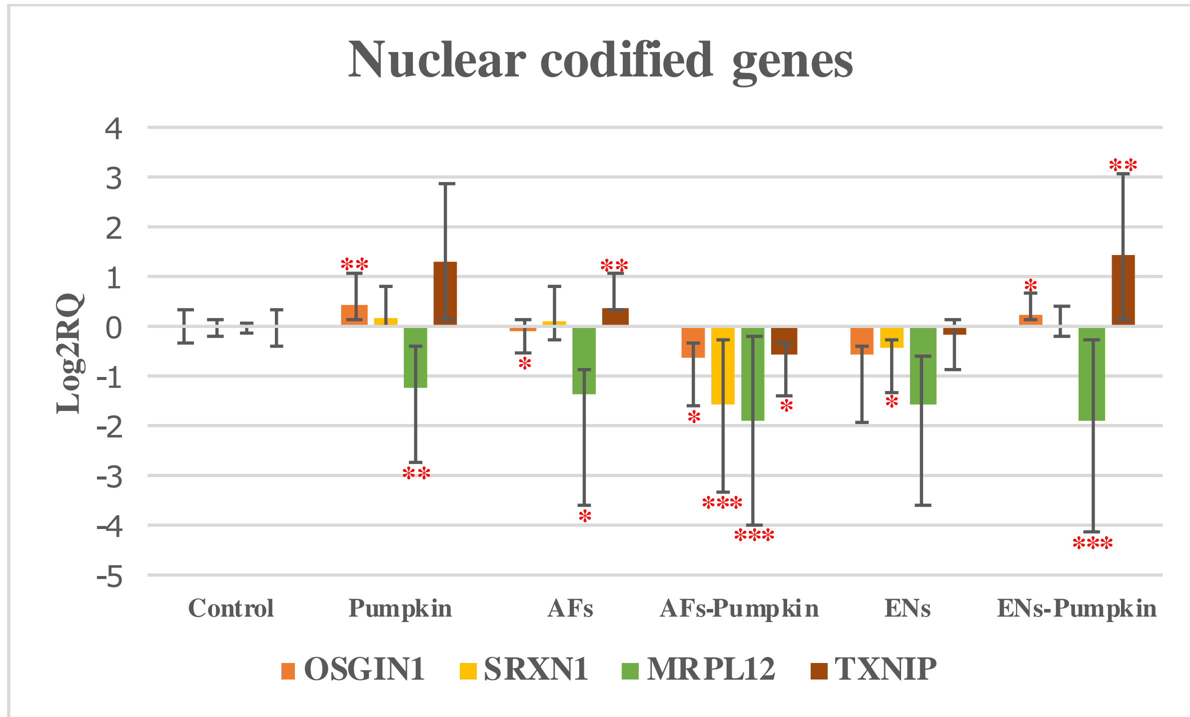

*Figure 4. Bar plot showing nuclear genes relative expression when compared to control (log2RQ = 0) after 2h-exposure to the different treatments by qPCR. RQ, relative quantification. Error bars, log2RQmin and log2RQmax. *p < 0.05; **p < 0.01; ***p < 0.001.*

Pathvisio analysis

Due to the specificity of the assay, 2 data points meeting criterion (r) were overlapped for all treatments and two pathways were statistically significant for the 5 studied treatments were the OXPHOS and the ETC, with 28,57% and 25% affected genes, were the most altered pathways. Individual analysis of every treatment also reported two altered pathways common to every condition: Effects of Nitric Oxide pathway and quercetin and Nf-kB/ AP-1 Induced Cell Apoptosis (Table 2).

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Tabla 2. Pathways overlapped in all conditions by PathVisio (p < 0.05).

Pathway	positive (r)	measured (n)	total	%	Z Score	p-value
Electron Transport Chain	8	8	118	100	2	0,014
Oxidative phosphorylation	7	7	68	100	1,53	0,003
Effects of Nitric Oxide	1	1	16	100	0,33	0,004
Quercetin and Nf-kB/ AP-1 Induced Cell Apoptosis	1	1	27	100	0,33	0,003

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Discussion

In vitro experiments on human cells have shown protective effects of carotenoids in a wide spectrum of pathologies due to its antioxidant capacity. Gong et al. (2017) used different concentrations of lutein, lycopene and β-carotene (0.5–2.0 µM) in retinal human epithelial cells to test the possible effect of macular carotenoids on the retina protection, finding that lutein and lycopene inhibited the growth of human retinal pigment epithelial cells and protected them against cell death induced by oxidative stress. Nishide et al. (2015) tested the protective effect of soy isoflavones and β-carotene (0.1–10 µM) on osteoblast differentiation, resulting in early differentiation induced by β-carotene, which could lead to a positive balance of bone turnover. Lutein and oxidized lutein has been found to induce a promising double effect, in hyperglycemic ARPE-19 cells and rat retina, stimulating mitochondrial activity by up-regulating MT-ND4 on one side and also reducing the associated ROS increment by up-regulating SOD1 and SOD2 (Nanjaiah et al. 2019). Furthermore, astaxanthin (3-14 µM) has also been found to increase *C. elegans* lifespan over 20% by disassembly of CIII and most likely supercomplex I+III and reducing ROS species, confirming also the same effect across species using mitochondria from mice, rat, plants and humans

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(Hoffman et al. 2019). In our case, pumpkin (500 nM) induced significant alteration for every gen tested, but MT-RNR2, SRXN1 and TXNIP. MT-ND4 and MT-ND3 were the most overexpressed genes, which suggests an increase of the activity of these two genes by carotenoids even at low concentrations and a possible protective effect.

Complex I

CI deficiency is the most common genetic abnormality in mitochondrial energy production, being responsible for approximately a third of the OXPHOS related disorders. Therefore, even minimum defects in CI redox signals can generate OXPHOS disruption leading to oxidative stress and lately, disease (Lin and Beal, 2006). Fumonisin B_1 has been found to inhibit CI, increasing also ROS production in cell cultures of human neuroblastoma (SH-SY5Y) and rat primary astrocytes, but no cell death after 24h exposure was described (Domijan et al. 2011). Rat renal cortical mitochondria phosphorylation rate was diminished by citrinin (1 mM) inhibiting enzymes like NADH oxidase and NADH cytochrome c reductase and increasing the activity of succinate oxidase, glutamate dehydrogenases, malate and succinate cytochrome c reductase (Chagas et al. 1992a; Chagas et al. 1992b). EN B was also found to be involved in CI disruption and ATP decrease by microarray analysis, altering CI by downregulating three members of the Ndufs family (Ndufs1, Ndufs4 and Ndufs8), although only Ndufs4 was statistically significant (Jonsson et al. 2016). Also, CI has been reported as the main target for verrucosidin, inhibiting energy production in the mitochondria in *in vitro* experiments focused on finding therapies for Triple negative breast cancer (Thomas et al. 2013). Furthermore, previous reports using RNA-seq and data analysis by ConsensusPathDB and Pathvisio showed the mitochondria and its inner mitochondrial membrane protein complex, as the most affected pathways by

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EN B which could affect the Oxidative Phosphorylation (OXPHOS) in the mitochondria (Alonso-garrido et al. 2018). This study shows a general up-regulation for the CI genes studied. Interestingly, AFs-pumpkin behave as the control, which could mean that a balance between these possible dietary compounds is necessary to maintain MT-ND2 normal expression. For the rest of the genes, AFs-pumpkin increased the solely mycotoxin effect, causing more expression that the pumpkin alone. ENs-pumpkin followed the same trend, but for MT-ND3 (Fig. 2). Both effects suggest that an appropriate combination of mycotoxins and dietary carotenoids could be more effective, in case of CI deficiency, than carotenoids alone.

Complex IV

AFB1 mechanism of action implies inhibition of the ETC between CIII and CIV with a wide spectrum causing mutations and other DNA damage (Sharma et al. 2018). T-2 mycotoxin was found to reduce activity of mitochondrial complexes III, IV and V. Also, T-2 activated apoptosis pathway caspase-9 and 3 in chondrocytes and mitochondrial cytochrome c release (Liu et al. 2014). Moreover, previous studies developed by our group reported that treatment with EN B triggered slight up-regulation of MT-CO1 on Jurkat T cells (Alonso-Garrido et al. 2018). ECV 304 cells, when treated with ENs, showed no significative result, although a slight down-regulation can be observed. Differences between Jurkat and ECV 304 cells could be due to tissue specificities, doses tested or other intrinsic factors. On the other side, MT-CO3 showed up-regulation for pumpkin treatment and a reversion of the mycotoxins effect when mixed with pumpkin. Results suggest independent behavior of both genes at the doses tested, with a protective effect of carotenoids on both mycotoxin treatments for MT-CO3 (Fig. 3a).

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Complex V

Neurodegenerative diseases are thought to be linked to ATP synthesis decreased (Van Bulck et al. 2019). Interestingly, changes of expression induced by pumpkin, slightly down-regulated MT-ATP6 and up-regulated MT-ATP8, while AFs and ENs down-regulated both genes expression. Interestingly, both mycotoxins effect was mitigated by pumpkin, inducing a protective effect (Fig. 4). This last result is consistent with the reported results on the ETC by other authors, which have shown protective effect of these antioxidants by increasing mitochondrial activity and therefore, ATP production but not the ROS amount. Nevertheless, this hypothesis should be confirmed by further studies.

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Nuclear codified genes

Nuclear codified genes are key for mitochondrial structure, functionality and OXPHOS balance. All antioxidant related genes studied (OSGIN1, SRXN1 and TXNIP) reported slight up-regulation when treated with pumpkin, although only OSGIN1 was statistically significant. pumpkin treatment for OSGIN1 reported up-regulation, which could lead to an increase in cell death and apoptosis by oxidative stress response (OSGIN1). AFs treatment showed slight fold change for OSGIN1, SRXN1 and TXNIP and surprisingly, its down-regulation was increased in the case of AFs-pumpkin, suggesting a more acute toxic effect than using AFs alone. On the contrary,–ENs treatment down-regulated all three genes expression and mixed with pumpkin reverted ENs effect ($p \leq 0.05$, data not shown), showing a possible protective effect of pumpkin against ENs toxicity (Fig. 4). MRPL12 was slightly down-regulated for all treatments,

being more accused for mycotoxin mixtures and pumpkin, which could lead to a lack of expression and synthesis of unit 39S and therefore mitoribosomes, ultimately associated to development of neurodegenerative disorders (Surmeier et al. 2017).

Conclusion

These results contribute to a better understanding of the underlying molecular mechanisms triggered by mycotoxins and dietary carotenoids in the BBB. While pumpkin increased the expression of one or more genes in the three mitochondrial complexes checked, both mycotoxin mixtures assayed increased the ETC CI expression, but decreased the one of CIV and CV. Dietary carotenoids activity on mitochondrial genes expression of ECV 304 differentiated cells reported a beneficial effect even at low concentrations. Moreover, dietary carotenoids offered a protective effect when mixed with AFs or ENs for most of the genes studied across the ETC.

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Highlights

- Dietary carotenoids activiy reported beneficial effects even at low concentrations.
- Aflatoxins and enniatins alteration was mitigated by dietary carotenoids.
- Mycotoxins increased Complex I and decreased Complex IV and Complex V expression.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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