Effect of sea buckthorn meal extract in alleviating the toxic effect of ochratoxin A and zearalenone in porcine peripheral blood mononuclear cells

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ABSTRACT

The mycotoxins ochratoxin A (OTA) and zearalenone (ZEA) are frequent contaminants of cereals responsible for important toxic effects in human and animals. The aim of this study was to investigate the in vitro effect of the sea buckthorn meal extract in alleviating the toxic effect of ochratoxin A and zearalenone on oxidative stress and inflammation using porcine peripheral blood mononuclear cells. Our results have shown that both zearalenone and ochratoxin causes oxidative damage and alter the inflammatory response. The use of the sea buckthorn meal extract can improve some markers of the oxidative stress (total antioxidant status) and inflammation (pro-inflammatory cytokines IL-1 β , TNF- α , IL-8, IL-6) altered by exposure to the mycotoxins ZEA and OTA and our results indicate that this waste represents a promising biological method that can be used for the alleviation of the mycotoxins negative effects.

Keywords: porcine blood mononuclear cells, swine, ochratoxin, zearalenone

INTRODUCTION

Mycotoxins are secondary metabolites produced mainly by fungus of *Fusarium*, *Aspergillus*, *Penicillium*, *Alternaria* and *Claviceps* genera, responsible for important toxic effects in animals and humans (Abrunhosa et al., 2016). Among them, ochratoxin A (OTA) and zearalenone (ZEA) are frequent contaminants of cereals (Lioi et al., 2004). Kidney is the main target of OTA, the exposure to the toxin lead to nephropathy in pigs and poultry (Stoev and Denev, 2013), while reproductive system is the main target of ZEA, the toxin having important estrogenic effects (Hueza et al., 2014). Both OTA and ZEA has also other toxic effects as genotoxic, hepatotoxic and immunotoxic (Sorrenti et al., 2013; Zinedine et al., 2007).

Farm animals and in particular pigs are sensitive to mycotoxins due to the consumption of contaminated feed and important economic problems arise from the negative effect of the toxins on the animal health and reproduction capacity (Marin et al., 2013).

In comparison with physical and chemical methods for reducing the negative effects of mycotoxin, the use of the biological approaches is more recommended as they don't bound minerals or vitamins in the food/feed, are less toxic and could improve the health of the intoxicated animals through their rich content in bioactive substances (Varga et al., 2010). Recent studies have shown the efficacy of using agro-industrial wastes rich in bioactive compounds as counteracting solutions for alleviating the mycotoxins effect (Taranu et al., 2019).

Sea buckthorn (*Hippophae rhamnoides*) is a plant used in different fields as food, medicine and cosmetic industries (Ji et al., 2020a). The plant is rich in bioactive compounds as minerals, vitamins, unsaturated fatty acid, terpenoids, polyphenolic compounds, flavonoids, which have antioxidant and anti-inflammatory effects (Zadernowski et al., 2005).

The aim of this study was to investigate the in vitro effect of the sea buckthorn meal extract in alleviating the toxic effect of ochratoxin A and zearalenone on oxidative stress and inflammation using porcine peripheral blood mononuclear cells.

MATERIALS AND METHODS

Sea buckthorn meal extract. Sea buckthorn meal extract (SM) used in this study was obtained from S.C. OLEOMET S.R.L., Romania. The extraction of polyphenols from SM was performed as described by Pistol et al (Pistol et al., 2020) and Folin-Ciocalteu method was used for the assessment of the total polyphenol concentration expressed as mg of gallic acid equivalents (mg GAE)/100 g sea buckthorn meal.

Cell culture. Blood samples were obtained from jugular vein of commercially healthy piglets using tubes with anticoagulant (Vacutest, Italy). PBMC were isolated from blood as already described (Marin et al., 2011) and cultivated in complete medium: RPMI-1640 supplemented with 2mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin and 5 % foetal calf serum (FCS) in cell culture plates. Cells were stimulated with 10mg/mL LPS and treated with the following treatments 10mM toxin (OTA or ZEA) and/or sea buckthorn meal (SM) extract (5mg/mL gallic acid equivalents) for 48h at 37°C.

Cell viability assessment. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay was used for assessing cell viability as already described (Marin et al., 2010).

Determination of total antioxidant status. Total antioxidant capacity (TAC) assay was performed as already described (Marin et al., 2020) in cellular lysates and inhibition percentages were converted into trolox equivalent antioxidant capacity (TEAC), expressed as $\mu\text{mol TEAC/g tissue}$.

Cytokine synthesis. ELISA kits (R&D Systems, USA) were used for the quantification of the pro-inflammatory cytokine synthesis TNF- α , IL-1 β , IL-6 and IL-8 in the cell culture supernatants following the instruction of the manufacturer and as already described in our previous papers (Marin et al., 2019).

Statistical analysis. ANOVA tests were used to analyse the differences between treatments as compared to the control. The P values lower than 0.05 were considered significant.

RESULTS AND DISCUSSION

Effect of mycotoxins, sea buckthorn extract and their combination on PBMC viability. As it can be seen in the Figure 1, SM significantly increased the PBMC proliferation by 123%. Treatment with 10mM ZEA or the combination of 10mM ZEA with SM extract has no significant effect on PBMC proliferation. By contrast, 10mM OTA induced a significant decrease of cell viability (by 38%), while the concomitant administration of OTA and SM restore the PBMC proliferation near to the control. Previous studies have shown that ZEA has cytotoxic effect in PBMC isolated from different species (Morlett et al., 2015; Yang et al., 2016). In particular, zearalenone decrease the PBMC viability in swine (Marin et al., 2011; Yang et al., 2016) and it was shown that a concentration of 22.7 mM of ZEA induce a decrease of PBMC viability with 50% (Marin et al, 2011). However, the concentration of ZEA used in our study (10mM) was too low to induce a significant decrease of PBMC viability.

Figure 1. Effect of mycotoxins, sea buckthorn extract and their combination on PBMC viability

Similarly, other studies have shown that OTA decreased cell viability of human PBMC (Liu et al., 2012; Periasamy et al., 2016; Stoev et al., 2009) and porcine PBMC (Mwanza et al., 2009), porcine PBMC being more sensitive to OTA toxicity as compared with human cells (Mwanza et al., 2009).

Administration of sea buckthorn extract improved OTA-induced cytotoxicity. Comparable results were obtained for other bioactive compounds, as resveratrol, that was able to improve cell proliferation decreased after ZEA exposure (Sang et al., 2016).

Effect of mycotoxins, sea buckthorn extract and their combination on antioxidant status. Both zearalenone and ochratoxin enhances ROS formation and causes oxidative damage (Qin et al., 2015; Tao et al., 2018).

Figure 2. Effect of mycotoxins, sea buckthorn extract and their combination on oxidative status

However, in our experiment, neither ZEA or OTA were able to influence the total antioxidant capacity when they were administered to the cells in the presence or not of the SC extract (Figure 2). By contrast, SM extract alone significantly increase by 1.5 times the total antioxidant capacity (TAC) as compared with the control. Indeed, other biological wastes rich in bioactive compounds as grape seed meal waste increase the capacity of Caco2 cells to respond to the oxidative stress (Nallathambi et al., 2020) and prevents oxidative DNA damage in rats PBMC (Aguiar et al., 2011).

Effect of mycotoxins, sea buckthorn extract and their combination on inflammation. Oxidative stress is correlated with inflammatory status as reactive oxygen species (ROS) play a major role in the inflammation progression (Mittal et al., 2014). Mycotoxins as well as bioactive extracts are known as modulators of different markers of inflammation. In our

study, sea buckthorn meal extract reduced the concentration of pro-inflammatory cytokines TNF- α , IL-6 and IL-8 by 20.5%; 87.4% and 45.2% respectively (Fig. 3).

Figure 3. Effect of zearalenone, sea buckthorn extract and their combination on inflammation

Figure 4. Effect of ochratoxin, sea buckthorn extract and their combination on inflammation

Our results are sustained by other studies showing that administration of sea buckthorn leaf extract in arthritis rat model, significantly reduced joint inflammation as compared to control (Ganju et al., 2005). By contrast, SM was

not able to significantly decrease the concentration of IL-1b as compared with control.

The concentrations of the pro-inflammatory cytokines were not altered by the exposure of the PBMC to ZEA, with the exception of TNF- α concentration that was significantly decreased ($p < 0.05$) after 48h exposure to the toxin. Literature data concerning inflammatory effect of ZEA are controversial as *in vitro* or *in vivo* studies have shown an increase or a decline of inflammatory response induced by ZEA depending on the cell type or organ (Bulgaru et al., 2021). In PBMC cells treated with both toxin and sea buckthorn meal extract, SM was able to decrease the synthesis of IL-6 (by 7.8 times) and IL-8 (by 1.3 times), but not of TNF- α and IL-1 β .

Previous studies have shown that OTA induces kidney, liver and intestinal toxicity, characterized by inflammation and cell death (Klahr and Morrissey, 2003). OTA has a more powerful inflammatory capacity than ZEA and significantly increase the synthesis of proinflammatory cytokines IL-6 (by 3.16 times), TNF- α (by 2.97 times), IL-1 β (by 2.55 times) by PBMC, while having no effect on IL-8 synthesis ($p > 0.05$) - Fig. 4.

Concomitant exposure to both OTA and SM induced a high significant decrease ($p < 0.0001$) of pro-inflammatory cytokine synthesis IL-6, TNF- α , IL-1 β and IL-8 by 95%, 94%, 97% and 94%, respectively, as compared with the OTA treatment.

CONCLUSION

In conclusion, our results have shown that the toxic effects of OTA were more important than that induced by ZEA on swine PBMC. Indeed, according to the UE recommendations concerning the guidance levels that can be accepted in feed for swine, the maximum accepted level for OTA is 50 ppm while the level for ZEA is 100ppm, that clearly reflect a higher toxicity of OTA as compared with ZEA. In this paper, we have demonstrated that the use of the sea buckthorn meal extract can improve some markers of the oxidative stress and inflammation altered by exposure to the mycotoxins ZEA and OTA. Sea buckthorn contains high concentrations of bioactive compounds as: vitamin C, carotenoids, tocopherols and polyphenols (flavonoids, phenolic acids and tannins) that are responsible for the antioxidant activity and anti-inflammatory effect of the plant extract (Ji et al., 2020b) that are responsible for the SM beneficial activity observed in our study. This waste represents a promising biological method that can be used for the alleviation of the mycotoxins negative effects in farm animals.

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