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Effect of agro-industrial by-products on inflammation and oxidative stress using an in vitro cell model

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ABSTRACT

Agro-industrial by-products can play an important role in the animal feeding, being an alternative to conventional raw materials. These two wastes can be considered as important sources of bioactive compounds that can be used in animal feed with multiple benefits for animal health. This study has investigated the anti-inflammatory and anti-oxidant in vitro capacity of grape seed and sea buckthorn meals, as waste resulted from the oil industry in order to be used in swine nutrition. The *in vitro* study was realised on swine peripheral blood mononuclear cells challenged with LPS and treated or not with grape seed meal and sea buckthorn meal extracts. Our results show that both extracts have the capacity to attenuate the inflammation and oxidative stress induced by LPS. The effect induced by the grape seed meal extract was in majority of cases more important than of the sea buckthorn, this effect might be due to a different composition in bioactive compounds (polyphenols, PUFA etc) of the two extracts.

Keywords: porcine blood mononuclear cells, swine, grape seed meal, sea buckthorn meal

INTRODUCTION

In the last years the utilization of natural waste/ by-products gained a lot of interest, taking into account the increase of consumer demands for the use of more natural/organic compounds associated with the necessity of agroindustry waste reutilization (Cheng et al., 2010; Rockenbach et al., 2011).

Of a great interest are the waste from the oil industry and especially those resulted after the use of the cold-pressing technique. This method doesn't use heat or chemical treatments for oil extraction and the oil resulted

contain more bioactive compounds than the oil traditionally extracted using organic solvent or mechanical techniques (Parry et al., 2006). The yield is usually lower than that with conventional extraction but the quality of oil is higher and the waste contain more bioactive compounds and can be used successfully in the animal nutrition (Ominski et al., 2021).

Grape seed meal (GSM), the residue left after oil extraction from grape seeds, has a high content of polyphenols, as catechins and procyanidins and tannins (Maman and Yu, 2019). GSM contain also high quantity of fatty acids as stearic acid, palmitic acid, oleic acid and linoleic acid (Kapcsándi et al., 2021).

Sea buckthorn (SB) was used traditionally for its medical and nutritional qualities (Vilas-Franquesa et al., 2020). SB as well as the sea buckthorn meal (SBM) are rich in nutraceuticals as: vitamins A, B1, B2, B6, C, fatty acids, minerals and polyphenols with important anti-inflammatory and antioxidant properties (Shah et al., 2021). SB oil has a unique composition of fatty acids, the seeds being rich in polyunsaturated fatty acids (ω-3 and ω-6) ~~fatty acids~~, while in the pulp there are mainly omega-7 fatty acids (Teleszko et al., 2015). The fatty acids from sea buckthorn oil may play an important role in cardiovascular disorders, improving also the immune response and promoting cognitive functions and bone health (Olas, 2018).

Grape products as well as sea buckthorn products were used as dietary supplements against many diseases as inflammation, cardiovascular disease, diabetes, hypertension, cancer etc due to their high content in bioactive compounds (Beveridge et al., 1999; Gupta et al., 2020).

Agro-industrial byproducts can play an important role in the animal feeding, being an alternative to conventional raw materials (cereals and oilseeds) (Ajila et al., 2012). Their use can reduce the cost of animal feed and consequently improve the profitability of livestock farms and the quality of the final product (Negi, 1985). On the other hand, the recycling of the high-polluting waste reduces the negative impact of the ago-industry on the environment (Singh et al., 2021). Even the agro-industrial waste are biodegradable, they represent a potential source of pollutants to the environment because it requires time to be mineralized.

This study has investigated the anti-inflammatory and anti-oxidant in vitro capacity of grape seed and sea buckthorn meals, as waste resulted from the oil industry used in swine nutrition on swine peripheral blood mononucelar cells (PBMC).

MATERIALS AND METHODS

Waste extracts. Grape seed meal and sea buckthorn meal (SBM) used in this study were purchased from S.C. OLEOMET S.R.L, Bucharest, Romania. The

extraction of polyphenols and the assessment of total polyphenol concentration was already described (Pistol et al., 2020)

Cell culture. PBMC were isolated from blood (Marin and Pistol, 2021) and cultivated in complete culture medium. PBMC were treated for 48h with LPS (10μg/mL) and grape seed or sea buckthorn meal extracts (5mg/mL gallic acid Eq).

Cell viability assessment. Cell viability was assessed using MTT assay as already described (Marin and Pistol, 2021).

Total antioxidant status. The antioxidant capacity assay was performed in cellular lysates as previously described (Marin and Pistol, 2021) and inhibition percentages were expressed as μmol trolox/mL.

Nitric oxide. Nitric oxide (NO) level was measured by Griess assay as already described (Taranu et al., 2014). Nitrite absorbance was measured at 550 nm and the concentration was expressed as μmole/L.

Cytokine synthesis. The concentration of IL-6, IL-8 and TNF-α, in the supernatants of PBMC cell culture were analysed using ELISA kits (R&D Systems, USA) as indicated and as already described (Marin et al., 2020)

Statistical analysis. One way ANOVA, followed by Bonferroni test was used to perform statistical analyses. Values of P lower than 0.05 were considered significant.

RESULTS AND DISCUSSION

Effect of grape seed meal and sea buckthorn extracts on PBMC viability. As it can be seen in the Figure 1, LPS does not significantly affect cell proliferation ($P<0.05$). Other studies have shown that LPS stimulation has a very low effect on cell proliferation, with a low percentage of cells in the S phase of cell cycle by contrast to concanavalin A stimulated PBMC (Amadori et al., 2018).

When the LPS stimulated cells were treated with GSM extract there was a significant increase of cell proliferation with 63% as compared with LPS stimulated cells. The treatment of LPS challenged cells with SBM extract induces a non-significant increase of cell viability by 57% as compared with control and by 18% as compared with LPS cells. Other studies have also shown that grape seed extract are able to increase cell viability in MDPC-23 cell culture after 10 days of treatment (Coelho et al., 2019). However, in cancer cell lines, GSM was responsible for an inhibition of cell viability and a corresponding increase of apoptosis (Laurent et al., 2004; Yen et al., 2015).

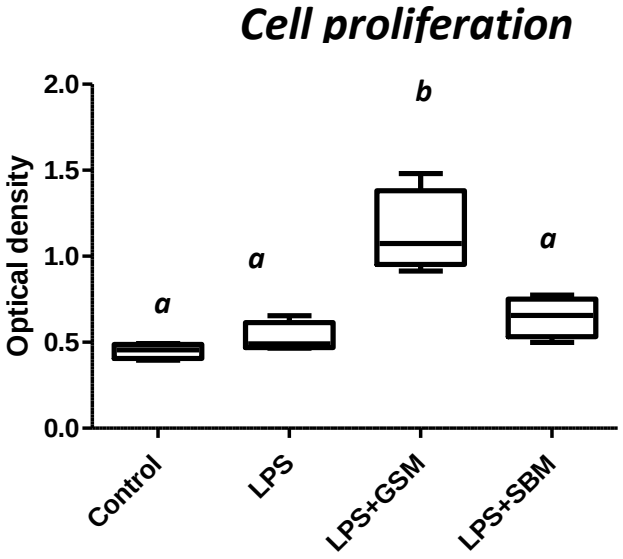

Figure 1. *Effect the extracts on viability of PBMC cells stimulated with LPS*

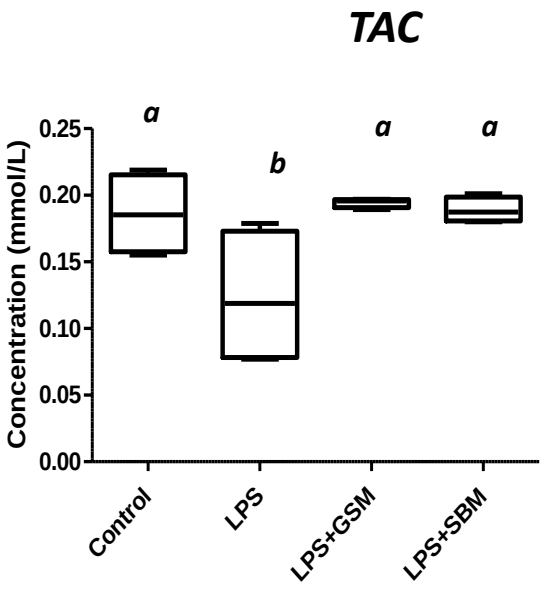

Figure 2. *Effect of the extracts on total antioxidant status of PBMC cells stimulated with LPS*

Effect of grape seed meal and sea buckthorn extracts on the antioxidant status. Bacterial lipopolysaccharide (LPS) triggers the expression of ROS through the induction of NADPH oxidase and the inhibition of the anti-oxidative enzymes as catalase, superoxide dismutase, glutathione peroxidase (Li et al., 2010). In our experiment, LPS induced a significant decrease of total antioxidant status by 33.7% (Figure 2). Other studies have shown that LPS was able to decrease the total antioxidant status both *in vitro* (Yin et al., 2021) and *in vivo* (Halawa et al., 2018). The treatments with both GSM and SBM extracts were able to counteract the LPS effect, by increasing the antioxidant status by 57% (GSM) and by 53% (SBM) as compared with LPS, near to the control level. Recent studies have shown that some polyphenols from the grape seed (oligomeric pro- anthocyanidins – GSOPs) might be used in the prevention and treatment of gastrointestinal infections in swine (Kovács et al., 2020). These authors have shown that GSOPs possess potent in vitro antioxidant activity in LPS-treated IPEC-J2 porcine epithelial intestinal cells. Sea buckthorn paste can offer a protection against lung injury induced by LPS through Nrf2 translocation and activation, due also to its high content in polyphenols (Du et al., 2017).

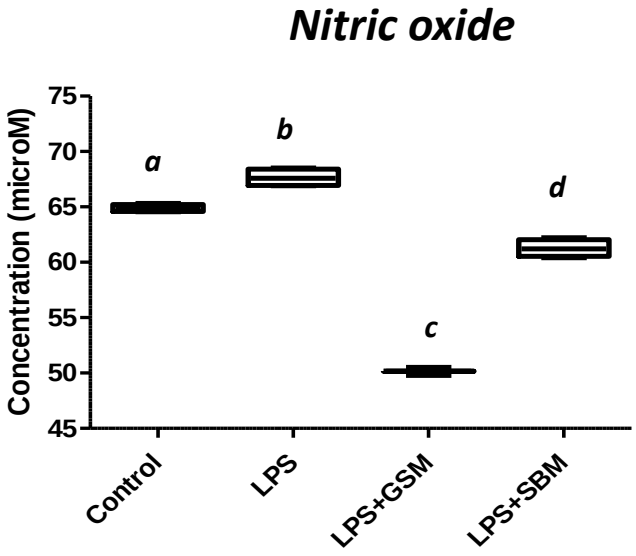

Figure 3. *Effect of Effect of GSM and SB extracts on nitric oxide concentration after LPS stimulation*

As it can be observed in Figure 3, LPS induced an increase of nitric oxide concentration by 4.3% as compared with the control. Nitric oxide (NO) acts as

a host defense molecule against different pathogens and its concentration increases after exposure to bacteria, viruses, fungi, and parasites (Joo et al., 2014). After LPS challenge, nitric oxide increases rapidly, by the activation of inducible nitric oxide synthase (Pallarès et al., 2013). Both GSM and SBM extracts decreased nitric oxide concentration by 29% and 5%, respectively; GSM decreased dramatically the nitric concentration under the control level.

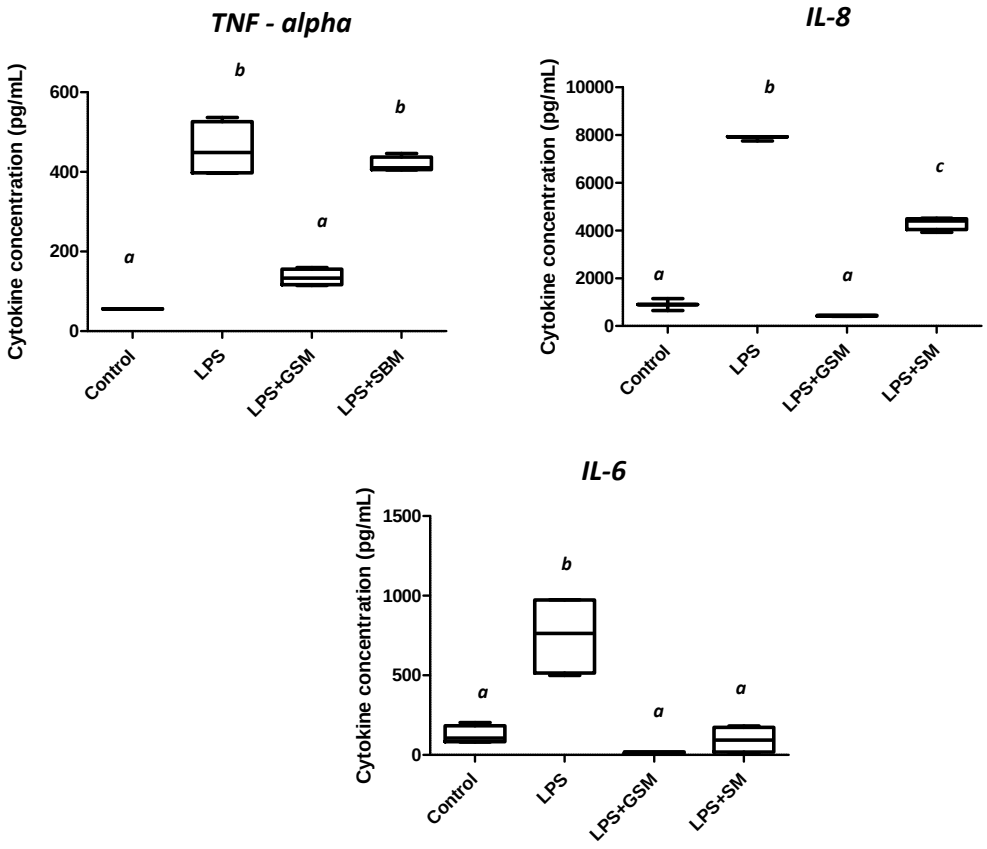

Figure 4. *Effect of GSM and SB extracts on inflammatory cytokine synthesis*

Similarly, in a rat model of septic shock induced by the intraperitoneal injection of LPS, grape seed procyanidin extract downregulated the expression

of genes IL-6 and iNos and decreased proinflammatory marker NO in the plasma, liver, and spleen (Pallarès et al., 2013).

LPS is responsible for an inflammatory status characterized by the secretion of pro-inflammatory cytokines (IL-6, IL-1β, TNF-a, IL-12), chemokines (IL-8) and anti-inflammatory cytokines (IL-10) (Ngkelo et al., 2012). Indeed, our results have shown that LPS significantly increases the pro-inflammatory cytokine synthesis by 4.6 times as reported to the control.

The treatment with GSM dramatically decreases the inflammatory cytokine synthesis, below the control level, by 48.8% (IL-6), 18% (IL-8), 3.6% (TNF-a). A moderate decrease of inflammation was induced by SBM: 7.9% (IL-6), 1.8% (IL-8), 1.2% (TNF-a).

Indeed, previous studies have shown that bioactive compounds derived from plants derived from plants have anti-inflammatory effects in endotoxin-treated rats (Liu et al., 2019). In particular, grape seed demonstrated a high potential to reduce inflammation. Thus, an extract of grape seed rich in pro-anthocyanidin reduces inflammation and oxidative stress in LPS treated Caco2 cells by scavenging intestinal reactive oxygen species. Our results are similar with other studies which show that sea buckthorn is able to reduce inflammation by decreasing the concentration of protein C reactive, a marker of inflammation (Larmo et al., 2008). In general, in human medicine, it was shown that sea buckthorn have been shown to possess hypotensive, hypocholesteromic and anti-inflammatory properties (Wani et al., 2016).

Conclusion

In conclusion, our results show that both extracts have the capacity to attenuate the inflammation and oxidative stress induced by LPS. The effect induced by the grape seed meal extract was in majority of cases more important than of the sea buckthorn, this effect can maybe be due to a different bioactive compounds composition of the two extracts. However, the decrease of cytokine IL-6 and IL-8 as well as of NO under the control levels means that other extract concentration have to be investigated. These two wastes can be considered as important sources of bioactive compounds that can be used in animal feed with multiple benefits for animal health.

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