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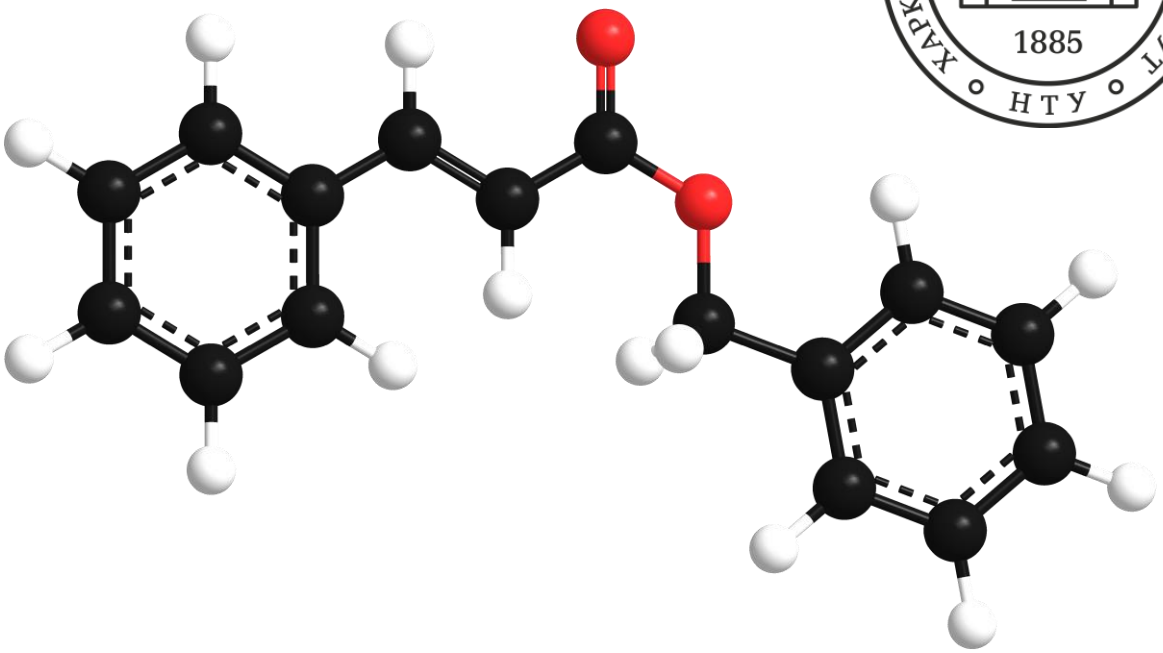

ХІМІЯ, БІО- І НАНОТЕХНОЛОГІЇ, ЕКОЛОГІЯ ТА ЕКОНОМІКА В ХАРЧОВІЙ ТА КОСМЕТИЧНІЙ ПРОМИСЛОВОСТІ

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phospholipids. This reduces the lipid order in cell membranes making them more rigid.

Surprisingly, in this study, semi-refined carrageenan was found to partially inhibit LPS-induced ROS generation and LPS-mediated changes in phospholipid bilayers of cell membranes *in vitro*. This is inconsistent with reports stating that carrageenans stimulate LPS-induced generation of TNF-α in leukocytes without affecting expression of this cytokine alone in the absence of LPS. However, *in vivo* studies demonstrate that carrageenan stimulates macrophages even in a more pronounced way compared with LPS. Moreover, experiments on murine models show that kappa-carrageenan magnifies LPS-induced inflammation via the bcl-10-NF-kB pathway. These inconsistencies between our findings and literature data may be explained by the direct interactions of LPS and carrageenans, since carrageenan has been shown to directly alter the morphology and, thus, functional activity of LPS suggesting, on the contrary, the protective and inhibitory role of carrageenans in LPS-induced inflammation.

Thus, our results don't exclude the synergistic pro-inflammatory effects of carrageenans and LPS *in vivo*. Therefore, future researches should aim at closing the gap in elucidating the crosstalks between the ingested carrageenan, gut microbiome and host immune system. This is especially important for individuals with the compromised intestinal barrier integrity and already existing intestinal inflammation.

Conclusion. E407a attenuates LPS-induced alterations of redox homeostasis in rat PBMCs and LPS-mediated modifications of cell membrane lipid order in leukocytes. Thus, semi-refined carrageenan (E 407a) reduces the LPS-mediated pro-inflammatory effects in leukocytes as a whole and PBMCs in particular.

THE STUDY OF THE SEMI-REFINED CARRAGEENAN (E407a) INFLUENCE ON REACTIVE OXYGEN SPECIES PRODUCTION IN PERIPHERAL BLOOD MONONUCLEAR CELLS AND ON LEUKOCYTE CELL MEMBRANE

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Natural additives have been gaining popularity for decades due to the awareness about their benefits both among industrial companies and consumers. However, even legally sanctioned food additives originated from naturally occurring sources don't meet high safety standards. Based on these premises, the major European regulatory body the European Food Safety Authority (EFSA) has been carrying out the re-evaluation of currently recognized as safe food additives since 2012. Among multiple food additives whose safety for consumers is controversial, carrageenans (registered as E407 and E407a) are of great concern. Carrageenans, which are also referred to as Irish moss, are highly sulfated polyanionic marine polysaccharides with no nutritional value. These polymers are made up of alternating 3-O-substituted β-D-galactopyranosyl rings and 4-O-substituted α-D-galactopyranosyl monomers. It is important to note that only high-molecular-weight carrageenans (200-800 kDa) are officially permitted to be used in the food industry as thickeners, gelling agents, emulsifiers and texture improvers (E407 – food-grade carrageenan or E407a – semi-refined carrageenan). At the same time, there is compelling evidence that their low-molecular-weight counterparts called degraded carrageenans and poligeenans are toxic and, thus, their use in foodstuffs is officially prohibited.

Accumulating evidence on food-grade carrageenan toxicity has established a basis for setting up a programme by the EFSA for the risk assessment of carrageenan consumption. In 2018, the EFSA released a call for technical and toxicological data on carrageenan (E 407) for uses in foods for all population groups including infants below 16 weeks of age (EFSA-Q-number: EFSA-Q-2018-00771).

In contrast to the studies outlined above, some researchers have claimed that carrageenans exert no cytotoxic effects towards different types of cells. In particular, a study performed on human intestinal and hepatic cell lines revealed that food-grade carrageenans neither upregulated pro-inflammatory cytokines via the nuclear factor

kappa-light-chain-enhancer of activated B cells (NF-kB) pathway, nor affected the intracellular redox homeostasis. Semi-refined carrageenan has been found to be incapable of triggering apoptosis in leukocytes, but slightly upregulated anti-apoptotic bcl-2 protein in lymphocytes. Moreover, it has been experimentally shown that carrageenans don't stimulate TLR4 signaling.

Aim of the study was to assess the effects of semi-refined carrageenan (E407a) on reactive oxygen species generation in peripheral blood mononuclear cells and on cell membranes of leukocytes.

Methods. Blood samples collected from 8 intact rats were incubated with E407a (10 mg/ml), E407a (50 mg/ml), E407a and without this compound (controls) for 24 h in RPMI 1640 medium enriched with 5% fetal bovine serum. reactive oxygen species generation in peripheral blood mononuclear cells obtained from the incubated samples was estimated by flow cytometry using 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA) staining. The impact of E407a on leukocyte cell membranes was evaluated spectrofluorimetrically using the fluorescent probe 2-(2′-hydroxy-phenyl)-5-phenyl-1,3-oxazole.

Results. In this study, semi-refined carrageenan did not induce reactive oxygen species generation in leukocytes after incubation for 24 h, even at high concentrations. These data corroborate our earlier findings on the inability of E407a to alter redox homeostasis of rat leukocytes after a short-term incubation for 2 h, but, on the other hand, this contradicts other studies that support the presence of direct pro-oxidant effects of carrageenans on immune cells *in vitro*. However, the other experimental *in vivo* data provide evidence that carrageenans are known to induce oxidative stress in leukocytes, activate nitric oxide synthesis, cytokine production in macrophages and promote apoptosis of circulating leukocytes.

Conclusion. Semi-refined carrageenan (E407a) stimulated neither reactive oxygen species overproduction in peripheral blood mononuclear cells, nor changes in the physic-chemical properties of cell membranes in leukocytes directly exposed to this food additive for 24 h.