Fungi are among the most valuable sources of biologically active substances that exhibit strong anti-inflammatory, antioxidant, and immunomodulatory properties. In recent years, special attention has been drawn to exosome-like nanovesicles (ELNs) secreted by mushrooms, such as button mushroom (*Agaricus bisporus*), oyster mushroom (*Pleurotus ostreatus*), shiitake (*Lentinus edodes*), and honey fungus (*Armillaria ostoyae*). Nanovesicles are tiny particles that play an important role in communication between cells, transporting biologically active substances like proteins, lipids, and RNA. While nanovesicles derived from animal and plant cells have been extensively studied, their role in fungi is still not fully understood.

The aim of this project is to investigate the properties of nanovesicles derived from various species of mushrooms and their potential to improve intestinal barrier function and modulate inflammatory responses. The intestinal barrier is a crucial protective mechanism that prevents harmful substances, such as pathogens, toxins, and allergens, from entering the bloodstream. Unfortunately, various factors can lead to its weakening, resulting in a phenomenon known as "leaky gut," which is associated with autoimmune diseases, such as inflammatory bowel diseases (IBD), celiac disease, and even neurodegenerative disorders.

The project will focus on investigating the physicochemical properties of the nanovesicles, such as their size, structure, surface charge, and protein composition. Techniques such as UV-VIS spectroscopy, electron microscopy (TEM), and proteomics will be used to analyze the composition and activity of these nanovesicles. The project will also investigate their potential to improve the integrity of the intestinal barrier, particularly focusing on proteins responsible for barrier tightness, such as occludin, claudin, and zonulin. Studies have shown that changes in the levels of these proteins can lead to intestinal barrier damage, increasing the risk of inflammation and autoimmune diseases.

The research team will investigate how nanovesicles from mushrooms affect the integrity of the intestinal barrier. In vitro studies using intestinal epithelial cells (Caco-2 and HT-29) and macrophages (THP-1) will help understand the mechanisms by which nanovesicles support the regeneration of the intestinal barrier and enhance its protective function. One of the key aspects of the project will also be investigating how nanovesicles can modulate the inflammatory response, particularly cytokine production, which regulates the body's inflammatory reactions.

Additionally, the project will involve animal model studies to assess how nanovesicles behave in more complex biological systems. Laboratory mice will be used in experiments involving two models of intestinal inflammation – a small intestine permeability model induced by castor oil and a colitis model induced by dextran sulfate sodium (DSS). These tests will help determine how nanovesicles influence the intestinal barrier, cytokine levels, and protein expression related to barrier function.

The project will also use advanced artificial intelligence techniques, including machine learning, to integrate data from various sources, such as proteomics, imaging, intestinal barrier function tests, and inflammatory activity analysis. These tools will allow researchers to identify hidden patterns and correlations between different types of data, enabling more precise predictions about the effects of nanovesicles and their potential applications in therapy.

The expected results of this project could have wide implications for the treatment of inflammatory bowel diseases, particularly in the case of leaky gut. The use of nanovesicles from fungi could open up new therapeutic possibilities, enabling the development of innovative drug delivery systems based on natural nanocarriers. Additionally, the project has the potential to contribute to the development of modern regenerative therapies that could support the repair of damaged intestinal barriers and enhance their protective functions.