

## ABSTRACT

During the course of my Ph.D., I focused my studies on two main topics: the cytotoxic effects induced by 4-nonylphenol (4-NP) and 4-octylphenol (4-OP) and the anti-cancer properties of natural cardenolides extracted from the aerial parts of the plant *Pergularia tomentosa*. For both topics, human cell cultures were used as a suitable study model.

4-NP and 4-OP are widely distributed environmental pollutants in different compartments such as water, soils and sediments. They are mainly derived from the degradation of polyethoxylated alkylphenols, which due to their chemical characteristics are widely used in many industrial applications. Humans are continuously exposed to these substances mainly through ingestion of contaminated water and food, but also through inhalation and dermal absorption. After their introduction into the human body, they accumulate in various organs and biological fluids, particularly in the liver and intestine. They can act as endocrine disrupting chemicals and exert toxic and harmful effects on many organs and tissues. Therefore, my aim was to understand the molecular and biochemical mechanisms by which they induce cytotoxicity in human cell lines. The results of my research revealed that alkylphenols (APs) profoundly alter human cell physiology by inducing viability reduction linked to a cell cycle arrest and to the triggering of apoptosis. Moreover, they are responsible for inducing organelles stress conditions and damage, such as ER-stress, altered calcium homeostasis, mitochondrial dysfunction, oxidative stress and induction of autophagic flux.

The natural compounds, investigated in my research, have been extracted, from the first time, from the leaves of *P. tomentosa*, a member of the Asclepiadaceae family. They belong to the group of cardiac glycosides. Structurally, they have a steroid nucleus, a glycosidic portion and a lactone ring. However, cardenolides extracted from *P. tomentosa* have peculiar structural features, i.e. transfused A/B rings and sugar moiety linked by a double bond, generating the dioxanoid attachment. Precisely, the compounds under investigation are calactin, calotropin and their derivatives. Over time, it has been shown that cardiac glycosides, in addition to their classic cardiotonic action, are able to exert effects that counteract cancer cells. Therefore, my aim, here, was to understand the molecular mechanisms by which these compounds exerted their anti-cancer action. I realized, firstly, that the compounds are not selective for tumour cells, however, calactin strongly reduces the viability of HepG2 tumour cells on which, consequently, I decided to focus my attention. I showed that *P. tomentosa* compounds reduced the viability, proliferation and migration of cancer cells. Furthermore, they were able to trigger autophagy and apoptosis in the same cell line. In light of these results, they represent good candidates for developing anti-cancer drugs or adjuvants to current therapies.

In conclusion, by using the same *in vitro* models represented by human cell cultures, and similar methodological approaches, I obtained findings useful to elucidate the biochemical and molecular mechanisms by which APs induced cytotoxicity and those underlying the anti-tumour properties of *P. tomentosa* compounds.