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MULTI-MARKER APPROACH FOR THE EVALUATION OF NEUROLEPTIC CHLORPROMAZINE ENVIRONMENTAL TOXICITY UTILIZING BIVALVE MOLLUSCS AS BIOINDICATORS

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Due to the extensive use, pharmaceuticals belong to the micropollutants of emerging concern in the surface waters [5]. They enter water bodies through inadequately purified wastewater from various sources, particularly hospitals. Among them, antipsychotic drugs have garnered special attention. The first-generation or "typical" neuroleptics that are used to treat symptoms of plural psychiatric disorders are known as a 'dirty drugs' [2], because, according to complex pharmacological profile, they realize the effect on receptors common to various phyla. Chlorpromazine (Cpz) is one of the most popular drugs in this group. Moreover, its utilizing has a tendency to increase due to its potential as an anti-cancer agent and antiviral activity against SARS-CoV-2. The scant known investigations detected its adverse effects on the aquatic species and accumulation in their organisms [1].

Bivalve molluscs are recognized as bioindicators of surface

water pollution. Their sessile filter-feeding lifestyle enables them to concentrate xenobiotics. However, the bioindication of pharmaceuticals is mainly focused on the oxidative stress effects, which may occur as non-specific side effects following exposure independently on the substance. Therefore, more information is needed on the specific effects of Cpz, in bivalve molluscs, especially considering the particularities of freshwater and marine indicative species.

The aim of this study was to compare of the biochemical effects of Cpz on the bivalve molluscs *Mytilus galloprovincialis* and *Unio tumidus*, a valuable bioindicators of marine and freshwater quality correspondingly. Two low (pM and nM) Cpz concentrations (Cpz 1: 12 ng L^{-1}; Cpz 2: 12 or 18 μg L^{-1}) were administered to mussels for 14 days. The set of studied parameters included the cytotoxicity indexes (enzymes of apoptosis and lysosomal membrane stability), oxidative/reductive stress responses, low weight thiolome functionality, and biotransformation-related enzymes in the digestive gland.

The lysosomal membrane stability as a most verified index of cytotoxicity decreased prominently in both exposures and both species. However, the enzymes of apoptosis demonstrated the species-dependent responses. In the *M. galloprovincialis*, both exposures caused the prominent increase of cathepsin D activity within lysosomes and its efflux. Caspase-3 was up-regulated in Cpz II group of *M. galloprovincialis.* In opposite, in the *U. tumidus*, the total and extra lysosomal activity of cathepsin D was not changed or decreased (efflux in the Cpz 1-group), and caspase-3 activity was not changed or decreased (in Cpz 1-group). The activity in phase I of biotransformation, as exemplified by CYP450-dependent EROD, increased exclusively in the Cpz I group of *M. galloprovincialis* and Cpz 2 group *of U. tumidus,* whereas glutathione *S*-transferase activity increased in both exposures of *M. galloprovincialis* and decreased in both groups *of U. tumidus*. These particularities reflected the different sensitivity or even different adverse outcome pathways in two organisms subjected to the same stress.

The evaluation of stress response have shown that in both applied concentrations and in both species, Cpz provoked the misbalance of superoxide dismutase (SOD) and catalase (CAT)

activities, when SOD activity increased or did not change comparing to control value, while CAT decreased, provoking the increased production of H_2O_2. Correspondingly, it was detected an elevated lipid peroxidation (TBARS) and increased level of protein carbonyls (PC), particularly in the Cpz 2 groups in both species of molluscs. On the other hand, the level of reduced glutathione (GSH) increased in both exposures and in both species. The level of GSSG increased only in the Cpz 1 group in the *M. galloprovincialis*. Consequently, GSH/GSSG ratio was elevated in the Cpz 2 group of both species and in the Cpz 1 group of *U. tumidus*, indicating the elevated redox-potential of thiols. It also was indicated the increase of the concentration of low weight stress-related-proteins metallothioneins (both total and metalated forms). Importantly, the typical response of these stress-related proteins is the loss of Zn with the increase of the part of apoform [3]. However, in the present study, the metal-binding capacity of metallothioneins increased due to the high redox potential within the cells.

A comparison of the magnitudes of anti- and pro-oxidative manifestations (SOD+CAT+GSH)/(TBARS+PC+GSSG) accomplished for marine mussel indicated a pro-oxidative shift in both exposures. Consequently, in this study we confirmed the oxidative injury as an typical sign of adverse effect independently of the nature of stressor. In these circumstances, low weight cellular thiols, that have rather high concentration, can provide the antioxidant support in the tissue. Importantly, the ratio of $NADH/NAD^+$, indicated in the *U. tumidus*, increased substantially. This manifestation accompanied by the reductive strengthening of thiols was specific for the Cpz effect and probably can be explained by its inhibitory effect on the electron transfer activity at respiratory complex I [4].

These findings show that Cpz induces similar non-specific symptoms of stress in the marine and freshwater bivalves, whereas the adverse outcome pathways related to the enzymes of apoptosis and biotransformation were specific for each species. The higher Cpz concentration caused the exhausting of the responses of detoxification system, particularly in the *U. tumidus*.

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