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Investigation of oxidative stress status and apoptotic markers of juvenile trout exposed to arsenic toxicity

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Arsenic is one of the most abundant elements on earth. Arsenic, also called metalloid, is used as a raw material in many industries. Arsenic causes the acceleration of free radical production in the body and the resulting oxidative stress. In juvenile trout, the interactions of arsenic with metabolism, biochemical analysis, and apoptosis stimuli were investigated. Results were demonstrated by several marker applications, including oxidative stress parameters, proinflammatory cytokine expressions, DNA damage, and apoptosis markers. In our study, arsenic was applied to juvenile trout (*Oncorhynchus mykiss*) at concentrations of 25, 50, and 75 mg/L for 96 h. After exposure, the brain tissues of the fish were collected and homogenized. SOD, the GSH-Px, CAT, and MDA levels were determined by spectrophotometric methods in the supernatants from the brain tissues of the juvenile trout. Levels of NF- κ B, TNF- α , IL-6, Nrf-2, GSH, caspase-3, AChE, and 8-OHdG were determined with an ELISA kit. When the brain tissues of the fish were examined after the study, it was found that the levels of NF- κ B, TNF- α , IL-6, Nrf-2, Caspase-3, MDA, and 8-OHdG increased, and the levels of GSH, CAT, SOD, AChE, and GSH-Px decreased. It was found that oxidative stress occurred as a result of the effect of the heavy metal arsenic in the brain tissues of the fish after application.

Key words: arsenic; cytokines; NF- κ B; Nrf-2; rainbow trout.

Introduction

Arsenic is one of the most abundant elements on earth. Arsenic, also called metalloid, is used as a raw material in many industries. It has negative effects on many living organisms, especially humans. In particular, arsenic mixed with groundwater can cause serious health problems.^{1–3} Arsenic causes the acceleration of free radical production in the body and the resulting oxidative stress. Arsenic-induced oxidative stress has been found to cause DNA damage through the production of superoxide and hydrogen peroxide. At the same time, it is known that apoptosis is observed in NB4 cells with the production of hydrogen peroxide.^{4,5}

In studies conducted on aquatic organisms exposed to heavy metals, it was observed that the levels of some antioxidant enzymes and apoptosis markers changed significantly in the tissue cells of living organisms. Among cytokines, TNF- α (tumor necrosis factor- α) and interleukins may be secreted in large amounts by the cell to coordinate intercellular communication and respond to inflammation in response to oxidative stress caused by heavy metal exposure.⁶ Cytokine groups such as IL-1 (interleukin), IL-6, and TNF act by activating the immune system to accelerate the destruction of various pathogens and eliminate the inflammatory state by acting on inflammation. When studying the tissues of fish exposed to heavy metals, it is found that the levels of some cytokines in the tissue cells change.^{7,8}

NF- κ B (Nuclear Factor Kappa-B) is a dimeric transcription factor involved in the regulation of genes during apoptosis and inflammation. NF- κ B plays an important role as a regulator of many genes, including immune and inflammatory responses. For this reason, it is suggested that NF- κ B may be a very important regulatory factor in innate and acquired immune responses.⁹

NF- κ B is present in many living organisms and in most cell types and has been recognized as an essential type of executive protein in the regulation of many gene expressions. In some studies, it has been observed that complete or continuous suppression of NF- κ B in tumor prevents the increase of cell number, suppresses intracellular cycling, and causes cell death by apoptosis.¹⁰

Nrf-2 (Nuclear Factor Erythroid-2) is a cellular protective shield that is activated by xenobiotics, electrophiles, radiation, and especially oxidative stress and is extremely active against stress conditions during this stress.¹¹ Similar to cytokines, the activity of Nrf-2 also changes significantly in cells under oxidative stress. Nrf-2 is found in the cytoplasm of the cell, bound to the Keap1 molecule. When the reactive oxygen species formed under oxidative stress rise above the antioxidant capacity, the Keap1 molecule releases Nrf-2. When Nrf-2 is released, it passes into the nucleus with the help of some transcription factors. Passage of Nrf2 to the nucleus causes some antioxidants and cytoprotective proteins and enzymes responsible for glutathione (GSH) formation to be activated within the cell.¹² Glutathione, a tripeptide synthesized from glycine, also reacts with free radicals and peroxides and protects cells from oxidative damage.¹³

Caspases, which provide cell death during apoptosis, are initially synthesized inactively. They are then activated by various pathways, recognizing tetrapeptide motifs in the cell and cleaving some cell proteins from aspartic acid residues. In this way, they lead the cell to death in a controlled manner. Caspase-3, one of these enzymes, is one of the very important effector caspases that performs apoptosis and prevents cell damage.¹⁴ Measurement of the amount of malondialdehyde, one of the reactive carbon compounds formed as a result of lipid peroxidation in living