UNIVERSITI TEKNOLOGI MARA

MEDIATION OF BISPHENOL ANALOGS-EXPOSED DEVELOPMENTAL TOXICITY AND MALFORMATIONS WITH PROCYANIDIN-C1 IN ZEBRAFISH EMBRYOS

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ABSTRACT

Recent research has raised concerns about widespread exposure to bisphenol analogues with structural or functional similarities to bisphenol A (BPA). Due to strict regulations on BPA production and use, various bisphenol analogues such as bisphenol S (BPS), bisphenol F (BPF), and bisphenol AF (BPAF) are increasingly being used to replace BPA in a wide range of applications and industries. Unfortunately, some analogues have similar disruptive physiological and endocrine effects on humans and animals, particularly during the embryonic period, due to their potential toxicity and endocrine disrupting properties. These persistent contaminant activities have the potential to disrupt normal biological functions, rattle developmental patterns, and influence hormonal activity, primarily by altering mechanisms and causing genome mutations. Little is known about its analogues and how they affect embryonic morphology, developmental defects, mitochondrial activity, and related genes. This study sought to determine the effects of Procyanidin-C1 (PCY1) intervention on developmental toxicity and cytoskeletal malformations in zebrafish embryos exposed to bisphenol analogues. Studies have shown that PCY1 neutralises free radicals, protects cells from DNA damage, and reduces the effects of environmental toxins. This study included 300 zebrafish embryos. The zebrafish embryo acute toxicity test (ZFET) was used to assess embryotoxicity. Toxicological endpoints were monitored every 24 hours until 120 hours of exposure. The embryos' heartbeats, morphologies, developmental defects, survivability, and hatchability were all studied. Heartbeats were detected as early as 48 hours after conception. Total RNA was isolated, converted to cDNA, preamplified, and then run through a microfluidic quantitative real-time polymerase chain reaction (qRT-PCR). BioMark Real-Time PCR Analysis Software was used to analyse the genes. The effects of PCY1 intervention in bisphenols-exposed embryos on the expression of apoptotic genes (*baxa, bcl2a, casp3a*, and *tnfa*), oxidative stress genes (*gpx1a, hif1aa, nrf1,* and *sod1*), mitochondrial energy metabolism genes (*etfa, mfn2, opa3,* and *pink1*), cytoskeletal organisation genes (*grip2a, klf4, midip1l,* and *nanog*), and growth-related genes (*fbp1a, foxn3, hk1,* and *pepd*) were determined. Confocal Laser Scanning Microscopy was used to determine the pixel intensities of mitochondrial activities and ROS levels. This study confirmed that a concentration of 20 nM of BPA, BPS, BPF, and BPAF can cause embryotoxicity and developmental defects as shown by the upregulation and downregulation trends in the expression of selected genes. This study also demonstrated that PCY1 at a concentration of 100 nM can inhibit the toxicity of the investigated bisphenols. However, the efficacy of PCY1 in mitigating the negative effects of bisphenol exposure on embryos varied across different bisphenol types, modes of action, treatment durations, regions of interest, targeted genes, and treatment settings. The findings also showed that the presence of BPA, BPS, BPF, and BPAF reduced mitochondrial distribution and activity while increasing ROS levels in zebrafish embryos. The level of ROS decreased across all PCY1 intervention groups. This finding implies that PCY1 intervention can reduce ROS levels while also protecting embryos from the harmful effects of bisphenols.

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INTRODUCTION

1.1 Introduction

Currently, the production of large quantities of industrial chemicals releases pollutants that provoke our ecosystem to end up causing dangerous imbalances. These imbalances give rise to numerous adverse health effects such as promoting obesity, impairing male fertility, developing cancer, cardiovascular problems, and metabolic diseases (Caserta et al., 2008; Mok-Lin et al., 2010; Kinch et al., 2015; Feroe et al., 2017; Rubin et al., 2017; Encarnação et al., 2019; Mentor, Brunström, et al., 2020). Bisphenol A is one of the listed chemicals concerned. It is the most familiar endocrine-disrupting chemicals (EDCs) worldwide used in plastic and epoxy manufacturing. These plastic materials are used to line food containers and cans made of metal (İyigündoğdu et al., 2020).

EDCs are emitted into the environment from a multitude of sources, including sewage treatment plant effluent, agricultural farm releases, livestock feedlot flushes, waste burning, and manufacturing direct discharges. They have been shown to affect endogenous hormone biosynthesis, secretion, transport, binding action, and metabolism, as well as the activity of some genes. As a result, EDCs have the potential to disrupt organ system regulation, negatively impacting the developmental process, homeostasis, reproduction, growth, and metabolism (Gore et al., 2018; Wee & Aris, 2019; Kassotis et al., 2020; Marlatt et al., 2022).

Consumers worldwide are unwittingly exposed to BPA every day through food and beverage containers, personal care products, medical supplies, and other products. Studies have suggested that BPA might be harmful to human health because of its ability to disrupt normal biological processes, impact endocrine functions, interrupt normal behavioural states, cause DNA damage and alter epigenome profiling (Chioccarelli et al., 2020; Meli et al., 2020). According to recent estimates by Costa