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(RESEARCH ARTICLE)

Prospective hepatotoxic effect of UV-328 and its affirmable rescue by Dimethoxy curcumin in Zebrafish

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Abstract

The unprecedented usage of BUV-328 (Benzotriazole Ultraviolet Stabilizer) in many biological and environmental matrices is of acute environmental importance because of its toxicity even at low concentrations. To better understand the protective function of DiMC on the liver tissues of zebrafish exposed to sublethal concentration of BUV-328 was assessed in the present investigation. Adult zebrafish were exposed to BUV-328 at sublethal concentrations of 55µg/l. The responses were assessed in the liver tissues at 28 days and another group was supplemented with DiMC to investigate its ameliorative potential against BUV-328 induced hepatotoxicity. Biochemical markers of oxidative stress, histopathological changes, and antioxidant enzymes were measured in the exposed groups. The outcomes of our present study revealed that BUV-328 exposure upregulated the oxidative stress markers and diminished the activities of the antioxidant enzymes, altered the biochemical constituents in liver. Histopathological abrasions such as hypertrophy, cellular and nuclear enlargement, cytoplasmic and nuclear degeneration, necrosis with pyknotic nuclei, lipid and cytoplasmic vacuolization and nuclear displacement to the periphery were found to be increased in BUV-328 exposure group. The oxidative, biochemical and histological alterations induced by BUV-328 were almost recuperated in DiMC supplemented group which signifies its protective influence against BUV-328 incited hepatotoxicity.

Keywords: Oxidative stress; Antioxidants; Liver; Histopathology; UV-328; DiMC; Zebrafish

1. Introduction

Sunscreen organic UV filters, especially benzotriazoles, are the common environmental contaminants posing a mounting serious health concern due to their increasing presence in all parts and parcels of the ecosystems. These are the most commonly used filters for their ability to protect from sunburn by absorbing a broad spectrum of ultraviolet radiation. Benzotriazole ultraviolet stabilizers (BUV's) are projecting chemicals used to protect against ultraviolet radiation, which could absorb the spectrum of both UV-A and UV-B. BUVSs have also been widely used in assorted range of industrial and commercial products, such as plastics, building materials, cosmetic products, paints, coatings, etc [1]. Due to their high-volume of production and usage, BUV's have been found to be ubiquitously disseminated in various biotic and abiotic samples, including air, soil and water as well as in human samples (including breast milk, adipose, urine and serum). Because of the the widespread and high concentrations in many biotic and abiotic interfaces, BUV's have up-stretched pressing apprehensions of the adverse effects on environmental and human health [2,3].

Many nuclear receptors including PPARs, CAR and AHR that are activated by the exposure of BUV's and play crucial role in disrupting the usual functions in liver cells. In addition, albumin, one of the most abundant transporter proteins in serum, that mainly produced by the hepatocytes and showed potential high binding affinity with benzotriazoles. These reported data indicated that liver is a potential target organ of BUV's. In fact, studies with fishes have found that liver is a major target organ for accumulation of BUV's and it could cause organ toxicity in exposed fishes [3-6]. However, the toxicological evaluation of BUV's especially UV-328 on zebrafish liver and its underlying toxic mechanisms have still not

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been well-studied. Despite the knowledge about its widespread contamination, the toxicological effects and mechanisms of its toxicity on various organisms yet remain under explored. Studies identified that UV-328, is the potential endocrine disrupting chemical [7], dysregulated thyroid hormone system in zebrafish [8], modulated the nuclear receptors and disrupted the endocrine function [9]. Environmental contaminants like UV-328 elicited its toxicity associated with oxidative stress. Oxygen toxicity is a very pernicious consequence caused by cytotoxic reactive oxygen species (ROS), produced during metabolic transformation of xenobiotics in organisms. Under normal conditions, antioxidant defense system of an organism can remove ROS and protect complex biological macromolecules from ROS attack. However, when ROS levels induced by pollutants exceeds the scavenging capability of antioxidant defense system, the balance will be destroyed, therefore weakening the activity of antioxidant enzymes. Organisms will suffer oxidative stress, resulting in lipid peroxidation (indicated by the significantly enhanced level of malondialdehyde (MDA)), chain breakage, enzyme-protein gluing, and even cell damage, death or canceration. Antioxidant defense systems consist of a variety of enzymatic (e.g., catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione S-transferase (GST)) and non-enzymatic antioxidants such as reduced glutathione (GSH) [10, 11].

Owing to the complexity of organic UV filters in aquatic environment, the induction of oxidative stress with the significantly affected antioxidant defenses can be used as a potential marker to evaluate the quality of the aquatic ecosystem and to reflect the comprehensive pollution impact of PCPs in aquatic environment and to evaluate the environmental risks of polluted water. Zebrafish are discerning model organism with a concrete history of purpose for harmfulness testing and assessment in ecotoxicological studies. The zebrafish can be utilized as a potential model fish in ecotoxicology examinations and its genome has been fully sequenced and has a general closeness to the human genome is around 80% in qualities connected with sicknesses making zebrafish, a helpful biomedical model organism [12]. Studies with antioxidants supplementation have potentially been proved to be beneficial against xenobiotics induced oxidative stress mediated organ dysfunction in animals [13]. Dimethoxycurcumin (DiMC) is a structural equivalent of curcumin in which methoxy groups replaced with the phenolic-OH groups. It has a symmetric design and is synthetically steadier than curcumin with expanded cell reinforcement, apoptotic viability as well as less harmfulness in ordinary cells and expanded metabolic firmness (bioavailability). Plasma levels for dimethoxycurcumin were generally reach higher (3-times) in comparision with curcumin at same infused portion of 5 mg/kg bw in mice. In scrutiny with curcumin, digestion of DiMC is less comprehensive subsequently it is conceivable that DiMC displays expanded antioxidant potential over curcumin.[14]

Our previous explorations confirmed that constant exposure of organic UV filters significantly provoked several biochemical and pathological alterations in the zebra fish model [15-17]. Thus, we speculated that persevering exposure to UV-328 at sub lethal level to cause oxidative hepatotoxicity in zebrafish and its recuperation through the supplementation of DiMC. It has already been reported to exhibit renoprotecction against UV-328 in zebrafish model [18] To test this conjecture, we treated zebrafish to 55µg/L of UV-328 and supplemented with 50mg/kg, BW of DiMC through diet for four weeks and to analysis the hepatic destructiveness of UV-328 and its possible convalescence through DiMC supplementation. Specifically, markers related with oxidative insult and antioxidative pathways were evaluated along with histological variables to uncover the possible furtive toxic impact of UV-328 and the protective effect of DiMC in UV-328 impelled hepatic deficiencies in zebrafish.

2. Materials and methods

2.1. Ethics statement

All analyses, maintenance, and treatment dealing with zebrafish were completed according to the rules of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Mass biotech,Chennai, Tamil Nadu. India. Endorsement number: MB/IAECCC/2022/03/06.

2.2. Chemicals

Benzotriazole UV stabilizer - 328 (BUV-328; 98% purity) was obtained from Sigma-Aldrich, USA. Dimethoxy curcumin (DiMC) was supplied by Biosynth Ltd, UK as a gift sample. Dimethyl sulphoxide (DMSO) supplied by Sigma-Aldrich (USA) was utilized to prepare the stock solutions of BUV-328. The other chemicals employed in the present investigation were of analytical grade and used without further purification.

2.3. Experimental set-up

Adult wild-type (AB strain) Zebrafish (*Danio rerio*) with a body length of 2.46 ± 0.04 cm and a mean body weight of 0.28± 0.04 g were obtained from Mass biotech zebrafish facility, Chennai. The fishes were adjusted to the research

facility conditions for a week time in glass aquarium going before to the trials as per the rules of the Organization for Economic Co-operation and Development (OECD, 1996). The fishes were raised in re-circling circulated air through freshwater kept up with at 26 ± 1 °C, with a photo-period time of 12:12 h (light/dark) routine. During the acclimatization time frame, they were taken care of with fish food at not obligatory and water reestablishment was done one time each day. After acclimation, fish (450 numbers) were arbitrarily isolated into three trial gatherings, for example, water control group, UV-328 treated group at centralization of 55µg/L. Furthermore, UV-328 with DiMC treated group were treated with a similar grouping of UV-328 alongside DiMC 50mg/kg BW through the feed regimen. Each group was kept up with in three duplicates and each replicate contains 50 fish in 25 L test arrangement. Stock arrangements of UV-328 were arranged newly in DMSO. In order to maintain the aquarium's water quality and the appropriate concentrations of UV-328 and DiMC, the test solutions were changed every 24 hours. Prior to the experiment, they were starved for 24 hours. On day 28, fish were haphazardly chosen from openness and control and treated tanks (n = 15/replicate) and liver samples were gathered and utilized promptly for biochemical examination. One more provision of liver tissues were fixed in 10% formalin for histopathological perception.

2.4. Biochemical analysis

The liver tissues were flushed, homogenized with 50 mM super cold potassium phosphate cradle (pH 7.0), centrifuged for 10 min (10,000 rpm) and the clear supernatant was gathered to gauge the protein content, chemical exercises (SOD, CAT, GPx GR and GST) GSH and MDA level. Each examine was acted in sets of three. GSH level was examined by the strategy for Moron et al. [19] and expressed at a density of g/mg protein. SOD movement was assessed by Marklund and Marklund [20] by estimating the hindrance of pyrogallol autooxidation at 420 nm, and the catalyst action was communicated as Units/mg protein. CAT activity was assessed by estimating the absorbance of hydrogen peroxide at 590 nm and communicated as µmol H_2O_2 consumed/min/mg protein [21]. GST assayed in liver sample after the complexation of glutathione (GSH) with 1-chloro-2, 4-dinitrobenzene CDNB at 340 nm, and the outcome was given in µmol of CDNB form shaped/min/mg protein [22]. GPx activity was assessed after the oxidation of glutathione (GSH) within the sight of H_2O_2 at 412 nm and the information was communicated as µg GSH shaped/min/mg protein [23]. The catalytic activity of glutathione reductase was determined spectrophotometrically at 340 nm by catalytic conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) for the consumption of NADPH. The specific activity was expressed as the nmol of NADPH consumption per min per mg of protein [24]. MDA content was assessed by Devasagayam et al. [25] at 532 nm, which depends on 2-thiobarbituric corrosive (4,6-dihydroxypyrimidine-2-thiol; TBA) reactivity, and the outcome was expressed as nmol/mg protein. Lowry et al. [26] method was used to determine the protein concentration with bovine serum albumin as the standard reference.

2.5. Histopathological investigation

Liver tissues were at first fixed in 10% unbiased cradled formalin. The decent tissues were dried out in a progression of reviewed ethanol, implanted in paraffin wax, segmented at 5µm thickness, and stained with hematoxylin and eosin (H&E) for histopathological examination [27-29]. The segments were inspected and shot utilizing a light microscope (OLYMPUS CX 31) with 200x magnification.

2.6. Statistical analysis

Statistical investigation was completed by utilizing GraphPad Crystal 5.0 programming bundle (GraphPad Programming Inc., San Diego, CA). The outcomes acquired from each exploratory groups were exposed to one-way investigation of difference (ANOVA), trailed by Dunnett's post-hoc correlation. Values differed with $P < 0.05$ were viewed as genuinely significant between the compared groups.

3. Results

No mortality was seen during the acclimatization and exposure period, and there was no tremendous distinction between the blank (water) and dissolvable control (DMSO) for any of the biomarkers during the exposure. Thus, the water in control group was kept up with as the reference group.

3.1. Impact of UV-328 on cellular antioxidant and lipid peroxidation

The activity of SOD, CAT, GPx, GR and GST and the degree of GSH in the liver of zebrafish presented to UV-328 are portrayed in the Table.1 and in the Fig. 1 separately. It is clear that when contrasted with the control, the activities of antioxidant enzymes and the GSH level were altogether diminished when exposed to UV-328 for 28 days. These adjusted variables were essentially recovered in DiMC treated group means the expected defensive antioxidant role of DiMC against UV-328 prompted hepatic oxidative stress and keeps up with attuned antioxidant status. The fact that the MDA

level was significantly higher in UV-328-treated liver tissue and significantly lower in DiMC-supplemented liver tissue (Fig. 1) demonstrates DiMC's antilipoperoxidative properties.

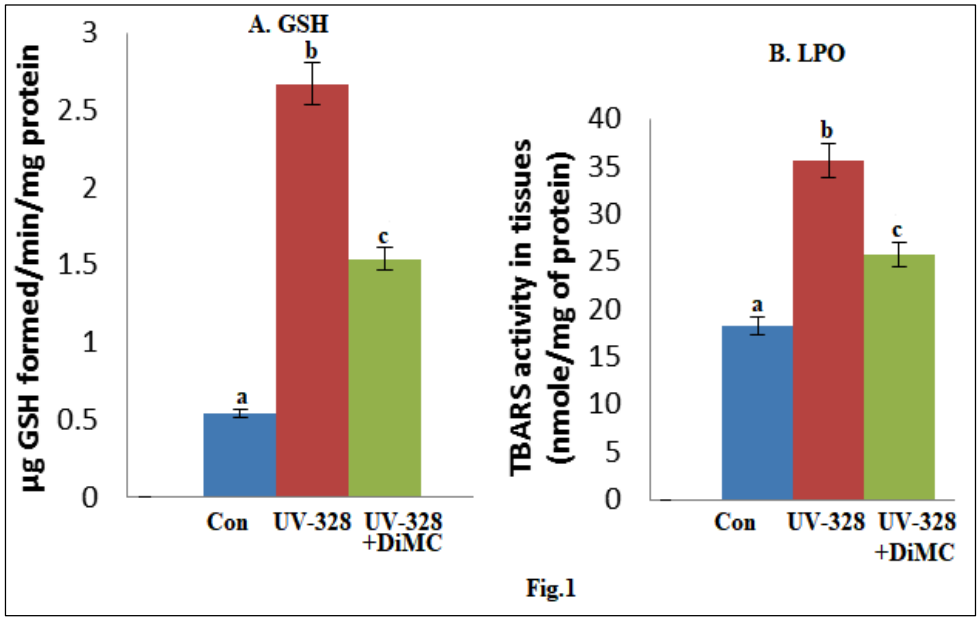

Figure 1 Effect of UV-328 and DiMC on the LPO and GSH content in the liver of zebra fish.

Values are expressed as mean ± SE. The letters (a, b and c) indicate significant differences from the control and experimental groups determined by one way analysis of variance followed by Dunnett's post-hoc comparison, $p < 0.05$ (DMRT).

Table 1 Effect of DiMC on UV-328 induced changes in the hepatic enzymatic antioxidants of Zebra fish

Experimental Groups	**Liver**
SOD (Units/mg protein)	
Control	34.75 ± 1.59[a]
UV-328	21.33 ± 1.74[b]
UV-328+ DiMC	30.17 ± 1.87[c]
CAT(µmol of H_2O_2 consumed /min/mg protein)	
Control	24.23 ± 0.61[a]
UV-328	15.72 ± 1.37[b]
UV-328+ DiMC	21.03 ± 0.72[c]
GPx (µmol of GSH oxidized/min/mg protein)	
Control	12.69 ± 0.31[a]
UV-328	7.34 ± 0.62[b]
UV-328+ DiMC	9.72 ± 0.59[c]
GST (µmol of CDNB conjugate formed/min/mg protein)	
Control	0.072 ± 0.009[a]
UV-328	0.034 ± 0.003[b]
UV-328+ DiMC	0.058 ± 0.005[c]

GR(µmol GSH utilized/min/mg protein)	
Control	12.72 ± 0.31[a]
UV-328	43 ± 0.51[b]
UV-328+ DiMC	9.81 ± 0.67[c]

Values are expressed as mean ± SE. The letters (a, b and c) indicate significant differences from the control and experimental groups determined by one way analysis of variance followed by Dunnett's post-hoc comparison, $p < 0.05$ (DMRT).

3.2. Histology & Histopathology

To understand the toxicity of UV-328 in a better way, the histological photomicrographs of liver sections from adult zebrafish from control and treated groups have been shown in Fig. 2. The liver section from the control group appeared to be normal. The hepatocytes and nuclei were uniform in size and shape (Fig. 2A). Fishes exposed to a sublethal concentration of UV-328 for 4 weeks showed severe histolopathological lesions such as liver sinusoidal dilations, with a squat degree of hepatocyte vacuolation, necrosis, inflammation and nuclear enlargement (Fig. 2B). These changes were observed throughout the liver tissue. Furthermore, the sections from the DiMC supplemented UV-328 group displayed a better recuperation of hepatic histoarchitecture with normal veins, hepatocytes and regular sinusoidal spaces (Fig. 2C), which clearly indicates the potential beneficial impact of DiMC against UV-328 induced oxidative hepatic injury.

Samples were stained with hematoxylin and eosin and photomicrographs were taken using 200x magnification. (A). Control group showing the rigid morphological features of hepatic tissue with hepatocytes and regular sinusoids. (B). UV-328 exposed hepatic tissue showing the altered pathological features like necrosis, desquamation, inflammation vacuolization of hepatic structure. (C). UV-328 + DiMC treated renal tissue of Zebra fish showing almost normal features of the hepatocytes with mild dilation of sinusoids.

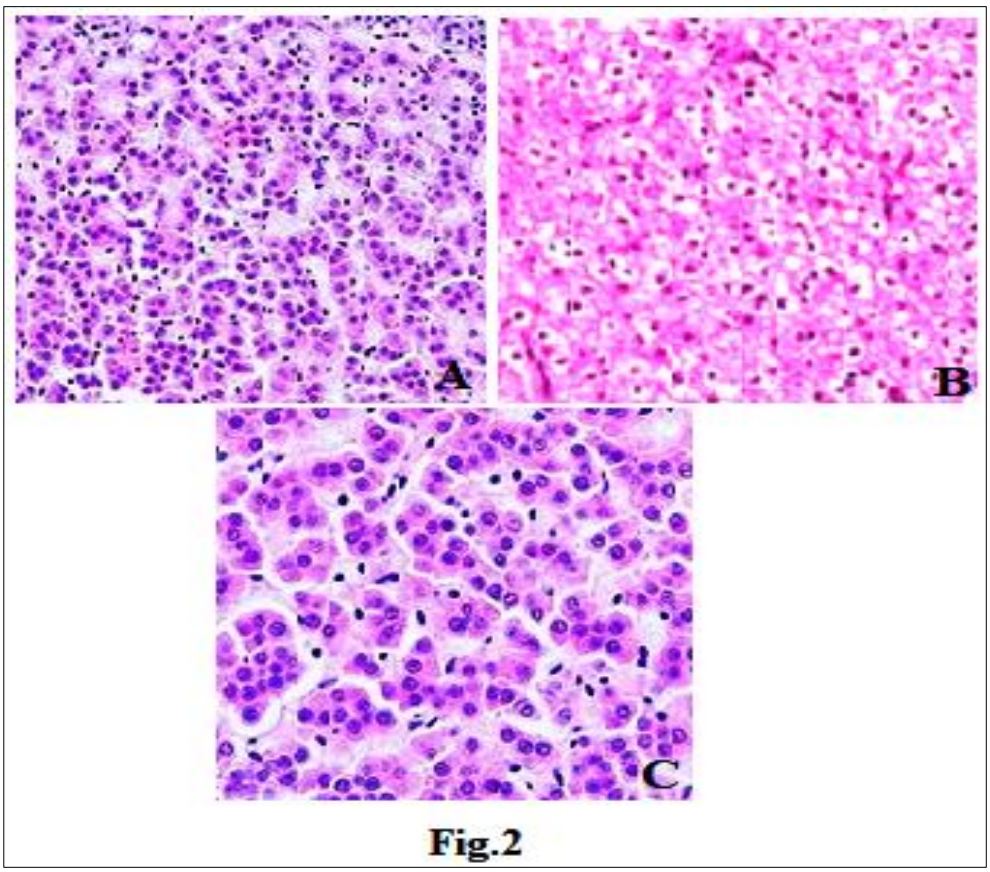

Figure 2 Light micrographs of sections through liver of zebrafish showing histological structure of the control and experimental groups (H&E x 200).

4. Discussion

It has been well documented that organic UV filters could be released into the aquatic environment, accumulated by organisms in the food chain, consequently causing latent hazards on fertility and reproduction of aquatic organisms [30]. According to the data obtained from the present study, UV-328 cause oxidative damage to fish liver under chronic sublethal exposure of UV-328 and it has been effectively recuperated with the DiMC supplementation.

4.1. Antioxidative Responses

Estimating the impact of xenobiotic contaminants on living beings by the progression of key chemicals in unambiguous responses is a broadly utilized technique to concentrate on the situation with oxidative insult. SOD, the first line of defense against oxidative stress, is able to catalyze the transformation of super oxide anions into water and hydrogen peroxide. CAT, is a key enzyme which uses hydrogen peroxide, a nonradical ROS, as its substrate. This enzyme is responsible for neutralization through decomposition of hydrogen peroxide, thereby maintaining an optimum level of the molecule in the cell which is also essential for cell signaling processes. GST, a stage II detoxification metabolic enzyme, can catalyze the limiting of electrophilic groups of xenobiotics with sulfydryls of GSH to build its hydrophobicity. GST likewise has GPx action, which can hinder lipid peroxidation [31].

In this study, compared to the control group, the antioxidative defense enzymes diminished severely in the liver of zebrafish following 4weeks of exposure to UV-328. Extreme ROS formation in fish after UV-328 exposure, which surpasses the capacity of antioxidant enzymes to eliminate the overwhelming release of ROS by UV-328, was a reason for the outcomes [11]. Initially their activities were reported to be increased significantly in tissues of UV-328 exposed zebrafish, indicating that they were involved in the detoxification of this UV filter and its mediated ROS. But prolonged exposure of UV-328 probably resulted in a failure of antioxidant reinforcement system lead to oxidative injury in the liver of zebrafish [16]. Following 28 days of exposure to UV-328, hepatic GST activity was exhausted notably, which may be because of the continual buildup of its metabolites. These outcomes demonstrated that GST was associated with the biotransformation of UV-328, and the complex of GST and UV-328 was formed to detoxify the UV-328 in zebrafish liver. GSH can detoxify by binding directly to ROS and electrophilic compounds, as well as serving as a substrate for GPx and GST. Glutathione reductase is responsible for maintaining the supply of reduced glutathione; one of the most abundant reducing thiols in the majority of cells. In its reduced form, glutathione plays key roles in the cellular control of reactive oxygen species [32].

Previous studies have apparently revealed that exposure to natural contaminants brought about the increment or reduction of GSH levels in living beings relying upon the species, dose and duration [15-18]. In our assessment, tremendous depletion in GSH levels was found in the liver of UV-328 treated zebrafish. Initially expanded GSH levels in the liver of UV-328 treated zebrafish was reported most likely come about because of the upgraded hepatic take-up of aminoacid substrates and the use of biosynthetic chemicals to safeguard the animals against oxidative harm. The utilization of GSH because of the direct attenuation of ROS or as a co-factor for GST/GPx exercises may also essentially diminish the hepatic GSH level [33]. This suggests that GSH and its associated enzymes may be better suited biomarkers for evaluating the antioxidant status of animals exposed to organic UV filters like UV-328 at low levels and may be more sensitive to low doses.

4.2. Lipid Peroxidation

In biological frameworks, oxidative insult is defined as a irregularity between free radical formation and cellular reinforcement defenses, which can prompt the exhaustion of cell reinforcement stores and the annihilation of cell macromolecules. In this notoriety, free radicals lead to lipid peroxidation (LPO) and produce reactive aldehydes, for example, malondialdehyde which further damages biomembranes. These by-products of LPO are utilized as effective indicative biomarkers to evaluate the seriousness of oxidative harm by xenobiotics [34].

In the current study, the exposure of UV-328 notably expanded the LPO level and diminished the cellular antioxidant defense of the liver tissue of zebrafish, affirmed the reports of Miltonprabu et al [17]. It has proactively been accounted for UV-328 exposure caused abundant release of reactive oxygen free radicals. This high oxidative pressure in the liver tissue caused the peroxidation of membrane lipids, protein carbonylation and nuclear DNA damage through antioxidant depletion [17]. The supplementation of DiMC had the efficacy to prevent the expansion in LPO and decline in antioxidant defense through its antioxidative capability in the liver tissue of zebrafish exposed to UV-328. It appears that DiMC through its cell reinforcement properties limits the toxic manifestations of UV-328 mediated hepatic oxidative stress. DiMC is chemically similar to curcumin, while the methylene group among the β-diketone structure provides them with remarkable antioxidant properties. Meanwhile, the methylation of both hydroxyl groups makes DiMC more stable and lipophilic than curcumin, further providing it with a significantly reduced degradation rate and a much improved drug

delivery system. It was also found that DiMC could respond to normal healthy cells in a way that is similar to curcumin but exerted more potent antioxidant properties [35].

4.3. Histology and Histopathology

In the present study, the ultrastructure of liver in the control group was normalwith regular hepatocytes, sinusoids and veins . However, UV-328 treated zebrafish liver displayed severe pathological structural changes like hypertrophy, cellular and nuclear enlargement, cytoplasmic and nuclear degeneration, necrosis with pyknotic nuclei, lipid accumulation, cytoplasmic vacuolization and inflammed sinusoid. The observed hepatocyte vacuolization of BUV-328 exposure suggests the interference in the lipid metabolism, inhibition of protein synthesis and energy depletion in the liver of zebrafish under oxidative stress. Increased level of lipid peroxidation upon UV-328 exposure probably the primary reason for these altered morphological changes in the liver tissue. Furthermore, the occurrence of necrotic areas in the liver was severe in case of fish treated with UV-328, which reflects the failure of cellular protective mechanisms in the presence of oxidative stress and altered the normal morphological structure of zebrafish. Intervention with DiMC significantly reversed all these pathological alterations of hepatic tissue through its potential antioxidant action and aids in the recuperation of normal hepatic architecture in zebrafish.

5. Conclusion

The present findings opens up a lot of new perspectives about the mechanism by which the UV filters affect the zebrafish liver. Organic UV filters have been used a lot all over the world, but there are still a lot of them being manufactured, marketed, and exploited in various industrial applications. From our current findings, It is articulated that when fishes are in continuous direct or indirect contact with UV filters, face an immense loss of its diversity. Numerous biochemical and histological changes were additionally seen in zebrafish when exposed to even sub-lethal concentration of UV-328 and these huge numbers of alterations were obviously ameliorated with the supplementation of DiMC. With the help of these findings, we can better understand the way by which the UV-328 filter could harm the zebrafish and the efficacy of the phytoantioxidant i.e., DiMC mitigated the hepatotoxic effects through its enhanced antioxidative potential.

Compliance with ethical standards

Acknowledgement

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Disclosure of conflict of interest

The authors declared that there is no conflict of interest.

Statement of ethical approval

All analyses, maintenance, and treatment dealing with zebrafish were completed according to the rules of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Mass biotech, Chennai, Tamil Nadu. India. Endorsement number: MB/IAECCC/2022/03/06.

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