Research Article

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Sublethal effects of acrylamide on thyroid hormones, complete blood count and micronucleus frequency of vertebrate model organism (*Cyprinus carpio*)

Subletal akrilamidin omurgali model organizma *Cyprinus carpio*'da tiroid hormonları, tam kan sayımı ve mikronükleus frekansı üzerine etkileri

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Abstract

Objectives: Acrylamide, a widely used chemical in industry, clinical laboratory and waste treatment plants, is considered a carcinogen in humans. The present study examined the hormonal, hematologic, and genotoxic responses in the invertebrate model common carp *Cyprinus carpio* after exposure to sublethal acrylamide.

Methods: Fish were exposed to acrylamide at 10 and 50 mg/L for 96 h, along with the respective control group. Serum levels of cortisol and thyroid hormones were measured using diagnostic ELISA direct immunoenzymatic kits. For micronucleus (MN) frequency assay, thin smears of the peripheral blood of fish were prepared.

Results: Serum levels of cortisol in both treatment groups considerably increased, which proposed that acrylamide caused a stress reaction of acrylamide exposed fish ($p<0.05$). Fish demonstrated significant decreases in triiodothyronine (T_3), free thyroxine (FT_4), and free triiodothyronine (FT_3) concentrations in a dose-dependent manner after acrylamide exposure ($p<0.05$). However, serum thyroxine (T_4) concentrations did not alter significantly in the treatment groups. Mean MN frequencies of fish erythrocytes increased significantly in acrylamide exposed groups suggesting that acrylamide is genotoxic in common carp ($p<0.05$). The hematocrit, hemoglobin, and erythrocyte numbers of carp increased significantly in exposure groups ($p<0.05$).

Conclusions: These results suggested that acrylamide can significantly affect the hemopoietic system. Furthermore, this study confirmed that the widespread use of acrylamide, even in sublethal concentrations, could affect the survival of non-target organisms, especially fish, in aquatic environments.

Keywords: acrylamide; cortisol; *Cyprinus carpio*; micronuclei; thyroid hormones.

ÖZ

Amaç: Sanayi, klinik laboratuvar ve atık arıtma tesislerinde yaygın olarak kullanılan kimyasal olan akrilamid,

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insanlarda kanserojen olarak Kabul edilmektedir. Bu çalışmada, akrilamidin subletal konsantrasyonlarına maruz bırakılan sazan balıklarında (*C. carpio*) hormonal, hematolojik ve genotoksik etkiler araştırılmıştır.

Gereç ve Yöntem: Balıklar, ilgili kontrol grubu ile birlikte 96 saat boyunca 10 ve 50 mg/L'de akrilamide maruz bırakılmıştır. Kortizol ve tiroid hormonlarının serum konsantrasyonları, tanısal ELISA direkt immünoenzimatik kitleri kullanılarak ölçülmüştür. Mikronükleus (MN) tayini için, balıkların periferik kanından ince yayma preparatlar hazırlanmıştır.

Bulgular: Her iki uygulama grubunda yer Alan balıkların serum kortizol konsantrasyonlarının önemli ölçüde artış göstermesi, akrilamidin uygulama grubu balıklarda stres tepkisine neden olduğunu göstermektedir ($p<0.05$). Akrilamide maruz kalan balıklar, doza bağlı bir şekilde triiodotironin (T_3), serbest tiroksin (FT_4) ve serbest triiodotironin (FT_3) seviyelerinde önemli düşüşler göstermiştir ($p<0.05$). Ancak serum tiroksin (T_4) konsantrasyonları uygulama gruplarında önemli ölçüde değişmemiştir. Akrilamide maruz kalan gruplarda sazan eritrositlerinin ortalama MN frekanslarının önemli ölçüde artması, akrilamidin sazanlarda genotoksik olduğunu ve bireylerde kansere yol açtığını düşündürmektedir ($p<0.05$). Hematokrit, hemoglobin ve eritrosit sayıları, subletal akrilamide maruz kalan sazan balıklarında önemli ölçüde artmıştır ($p<0.05$).

Sonuç: Bu sonuçlar, toksik kimyasal akrilamidin balıkların hemopoietik sistemini önemli ölçüde etkileyebileceğini göstermektedir. Ayrıca, bu çalışma, akrilamidin öldürücü olmayan konsantrasyonlarda bile yaygın olarak kullanılmasının, su ortamlarında hedef olmayan organizmaların, özellikle balıkların hayatta kalmasını etkileyebileceğini göstermektedir.

Anahtar kelimeler: *Cyprinus carpio*; kortizol; mikronükleus; tiroit hormonları.

Introduction

Acrylamide (C_3H_5NO; CAS No: 79-06-1) is a high-volume compound used in many research and clinical diagnostic laboratories for electrophoresis gels preparation [1]. In addition, acrylamide, known as one of the heat-induced toxic substances, is an industrially produced chemical used as a monomer for producing polyacrylamides widely used for flocculant for the clarification of the drinking and the treatment of the wastewater as waterproofing agents in dam and tunnel constructions, agricultural processes, and textile manufacturing [1, 2]. Besides the industrial and laboratory uses, humans are exposed to acrylamide via the diet. The acrylamide formation in the foods is primarily linked with the Maillard reaction between amino acids & reducing sugars [3]. Acrylamide was classified as a "probable human and rodent carcinogen" by International Agency for Research on Cancer (IARC) in 2002, following reports of effects in several animal models [1, 4]. These findings increased concerns about possible risks to human health.

Acrylamide can easily contaminate the aquatic systems by releasing from the manufacturing processes dyes, organic chemicals, pesticides, and plastics [2]. Acrylamide cannot degrade rapidly in water with high contamination risks on the surface and ground for high solubility and mobility in water systems. The residues were noticed between 0.5 and 600 mg/L in the flocculants applied by the water treatment activities [2, 5]. Despite the contamination risk of acrylamide in water systems due to high usage and solubility, there is a lack of information about non-target organisms in aquatic environments. Also, there is no information to link acrylamide-caused thyroid endocrine system changes to deterioration in fish health in the field.

Laboratory study results in different species showed that acrylamide might induce cancers and genetic damages [6]. Studies have revealed that acrylamide is moderately toxic to aquatic organisms. United States Environmental Protection Agency (U.S. EPA) [7] has summarised the 24 and 96-h median lrthal concentation (LC_{50}) values for goldfish (*Carassius auratus*) as 460 and 160 mg/L, respectively. The 7 days LC_{50} value for the guppy (*Poecilia reticulata*) is approximately 35 mg/L. The 24 h LC_{50} for *Daphnia magna* (water flea, first instar) is 230 mg/L [8], while the 48 h LC_{50} for *D. magna* is determined as 160 mg/L by Krautter et al. [9]. The 48 h LC_{50} values were calculated for rainbow trout (*Oncoryhchus mykiss*) as 110 mg/L; fathead minnows (*Pimephales promelas*) as 120 mg/L; bluegill (*Lepomis macrochirus*) as 100 mg/L [9]. Exposure to acrylamide caused neurotoxic and genotoxic effects as well as biochemical and histopathological alterations in some fish species [10–14].

Impairment of the thyroid endocrine system by environmental pollutants has attracted much attention due to thyroid hormones (THs) being especially significant in maintaining many physiological functions involved in the normal development and reproduction of vertebrates. In fish, the hypothalamus secretes corticotropin-releasing factor (CRF) to induce thyroid-stimulating hormone (TSH) secretion from the pituitary and the regulation of TH synthesis and release within the hypothalamic-pituitary-thyroid (HPT) axis [15]. Thyroxine (T_4), the predominant hormone of thyrocytes, is primarily converted to the biologically active hormone tri-iodothyronine (T_3) [16]. Alteration of thyroid hormone regulation may cause an endocrine response in which many physiological instabilities coexist, affecting reduced individual health. Therefore, it is pivotal to recognize thyroid-disrupting

chemicals that alter thyroid hormonal homeostasis and evaluate their risks to wildlife [17].

Many different groups of emerging compounds may interrupt thyroid hormone function, such as perfluorooctane sulfonate (PFOS) [15], pentachlorophenol [18], and synthetic pyrethroids [19]. Previous studies proposed that the effect of environmental substances on the hypothalamic-pituitary-thyroid (HPT) axis, involving changes in regulatory enzyme activities and hormone levels, could be carried out to examine the thyroid endocrine disruption [20].

As for cortisol, a predominant circulating corticosteroid hormone secreted from the hypothalamic-pituitary-interrenal axis (HPI-axis) is involved in the physiological reaction to stress conditions and in osmoregulatory processes [21]. Cortisol is a sensitive stress indicator in fish due to its rapid elevation in response to various stressors. Furthermore, the HPI-axis has been shown to modulate the thyroid axis in fish and other vertebrates [22].

The common carp, *C. carpio*, was chosen as the sentinel organism in this study because of its easy adaptability to laboratory conditions, tolerance to various environmental conditions, high ecological and economic importance, and wide distribution in the freshwater environment representing a well-suited species for toxicity studies. In addition, the common carp provides one of the most promising alternatives, the harmonized and cost-effective vertebrate models for predicting human health risks from acrylamide exposure.

Fish are commonly used to monitor environmental pollution due to wide distribution worldwide and bioaccumulate xenobiotics in response to even low concentrations of environmental contaminants. The response of fish to xenobiotics is similar to the higher vertebrates like humans. Therefore, the trend for improvements in *in vivo* toxicological bioassays with fish as vertebrate models is increasing. Fish in the upper levels of the food chain in aquatic ecosystems are in direct and/or indirect contact with environmental pollutants. In this case, chemicals found in aquatic ecosystems, especially at sublethal levels, affect fish the most. The accumulation of environmental pollutants in various organs and tissues of fish and their biological transfer to humans through the food chain adds a serious ecotoxicological dimension and importance to the phenomenon. Furthermore, the main structure and function of the HPT axis across all vertebrates tend to be well conserved. Hence, the results of fish researches with environmental toxicants can potentially serve as the basis for practical cross-species extrapolation of potential effects.

Peripheral erythrocyte micronuclei assay (MN), a validated and reliable test for determining genotoxicity, is standardised by the guidelines of OECD [23]. Hematological parameters are useful indicators to assess the toxicological effects and the status of fish health [24].

Although there are some studies on acrylamide toxicity and accumulation in the tissues of some fish species, there is a lack of data on the effects of acrylamide on common carp, *C. carpio*. The present investigation was carried out to determine the hormonal, hematologic, and genotoxic responses of common carp, *C. carpio*, the most produced and widespread freshwater species, to reflect the stress response and health status of fish as a vertebrate model after exposure to sublethal acrylamide.

Materials and methods

Fish

Freshwater fish *C. carpio* is recommended as a test species and useful vertebrate model for toxicity studies (OECD 1992, 2000). Juvenile common carp (*C. carpio)* were obtained from a local fish farm and used for the experiment with an average weight and standard length of 60.72±2.66 g and 16.91±0.23 cm, respectively. Fish were allowed to acclimate for 15 days in lab conditions in glass tanks containing 300 L of water, including 10 fish each. The composition of test water (pH 7.2, dissolved oxygen 7 mg/L, water temperature 18–20 °C) was measured daily following the standard procedures described in APHA [25]. Carp were fed once daily with commercial fish food dry pellets at a rate of their 2% of body weights. The ethical protocol for using *C. carpio* in the experiments was approved by the Gazi University Research Ethical Commission (G.Ü.ET-14.034 26.05.2014–06).

Experimental design

Following the acclimation period, experimental fish were transferred to 300 L volume aquariums in groups of 10 fish per tank. One tank is the control, and the other two are acrylamide sublethal exposure groups. Groups of experimental fish were selected randomly and exposed to the selected concentration of acrylamide in aerated aquaria. The experimental concentrations were selected according to literature and pre-test before the experiment. The acrylamide exposure concentrations were selected from the information of LC_{50} values of other aquatic vertebrates, reporting the threshold over 100 mg/L [4, 9]. The LC_{50} (96 h) determined for freshwater fish was from 100 mg/L (bluegill), 110 mg/L (rainbow trout) 119.5 mg/L (goldfish) and 120 mg/L (fathead minnow) [4, 9]. The sublethal concentrations were selected as 1/2 and 1/10 of 96 h LC_{50} concentration and decided as 10 mg/L and 50 mg/L acrylamide. Carp were treated with acrylamide for 96 h, and the concentrations were selected as 10 and 50 mg/L. The control group was kept the same way without acrylamide. During the experimental period, carps' behavioral changes and survival were monitored daily.

Blood sampling

At the end of the 96 h exposure period, the fish were immediately anesthetized (benzocaine, 60 mg/L; Sigma), and the total length and

the weight of the fish were measured. Blood samples were obtained from the caudal vein. They were put into the non-heparinized microtubes, and then the microtubes were centrifuged at 3,000 *g* for 15 min at 4 °C. The supernatant (serum) was preserved at −80 °C until the analysis of the hormone assays.

Hormone assay

Serum levels of cortisol, total thyroxine (TT_4), total triiodothyronine (TT_3), free thyroxine (FT_4), and free triiodothyronine (FT_3) were analyzed using diagnostic ELISA direct immunoenzymatic kits (Diemetra, Italy). The assays were done according to the instructions given along with the kits. All samples were analyzed in duplicate.

Hematological assay

The blood samples were put into EDTA tubes by the "direct tail cutting method". Haematological variables "(WBC=White blood cells; Lym=lymphocyte; Mon=monocyte; Gra=granulocyte; RBC=Red blood cells; Hb=hemoglobin; Hct=hematocrit; MCV=Hct*10/RBC; MCH=Hb*10/RBC; MCHC=Hb/Hct)" were measured within 30 min by using an automated veterinary hematology analyzer MS 4 e automated cell counter (Melet Schloesing Laboratories, France).

Micronucleus (MN) frequency assay

For MN assay, thin smears of the peripheral blood of fish were prepared on pre-cleaned slides. After air drying, they were fixed with 96% ethanol for 20 min, re-air-dried, and then stained with 5% Giemsa solution for 20 min. Samples were scored from each slide under 1,000 × magnification from 1,000 cells of two slides per fish. Scoring criteria for MN and other nuclear abnormalities (NAs) except for micronuclei in erythrocytes were done according to Kirsh-Volders et al. [26]. Nuclear abnormality data were expressed as total NA calculated by the sum of all nuclear abnormalities (binucleated, blebbed nuclei, lobed nuclei, notched nuclei) [27]. A light microscope (Zeiss Primostar) was used to score the MN preparations.

Statistical analysis

The statistical analyses of micronucleus assay were carried out using a commercially available statistical software package (GraphPad Insta3). Data were tested for normality and homogeneity of variances using the Kolmogorov-Smirnov test. Statistical differences were tested using the non-parametric Kruskal Wallis test. Dunn's test was used for the multiple comparisons of the groups. For serum hormone and hematology assays, statistical analysis was performed using the SPSS 24.0 statistical program. One-way ANOVA followed by Duncan's multiple range tests were used to analyze the experimental parameters. Data were tested for normality and homogeneity of variances using the Shapiro-Wilks and Levene test. A value of $p < 0.05$ was considered statistically significant. All data were expressed as arithmetic mean ± standard error (SEM).

Results

Mortality and abnormal behavior were not determined in control and acrylamide exposed fish groups during the 96 h exposure period.

Serum thyroid hormones

The results of serum levels of TT_4, TT_3, FT_4, and FT_3 are shown in Table 1. As seen in Table 1, serum TT_4 levels did not change significantly in the control and sublethal acrylamide exposed groups. For TT_3, the concentration levels in both treatment groups significantly decreased compared to the control group ($p<0.05$). Regarding FT_4 and FT_3, the concentration levels decreased significantly in both treatment groups compared to the control group ($p<0.05$).

Serum cortisol

Serum cortisol levels are presented in Figure 1. There was a dose-dependent elevation in serum cortisol concentrations in treatment groups with considerably higher levels after 96 h exposure when compared with the control group ($p<0.05$).

Hematology

The hematological results of the experiment are presented in Table 2. As seen in Table 2, exposure to sublethal acrylamide of carp resulted in a significant increase in Hct

Table 1: Serum total thyroxine (TT_4), total triiodothyronine (TT_3), free thyroxine (FT_4), free triiodothyronine (FT_3) levels of *Cyprinus carpio*, control and treatment groups exposed to 10 and 50 mg/L acrylamide (AA). (mean ± S.E.M.; n=10).

Groups	FreeT_3, pg/ml	Free T_4(pg/ml)	Total T_3(ng/ml)	Total T_4, nmol/L
Control	4.6 ± 0.05^a	0.72 ± 0.03^a	1.01 ± 0.02^a	21.0 ± 0.52
10 mg/L AA	4.2 ± 0.42^a	0.67 ± 0.06^b	0.97 ± 0.02^b	20.1 ± 0.45
50 mg/L AA	1.9 ± 0.15^b	0.56 ± 0.03^b	0.96 ± 0.01^b	20.0 ± 0.31

Significant differences between control fish and treated fish are indicated (small letters in the column refers significance a, b; $p<0.05$) for each exposure groups.

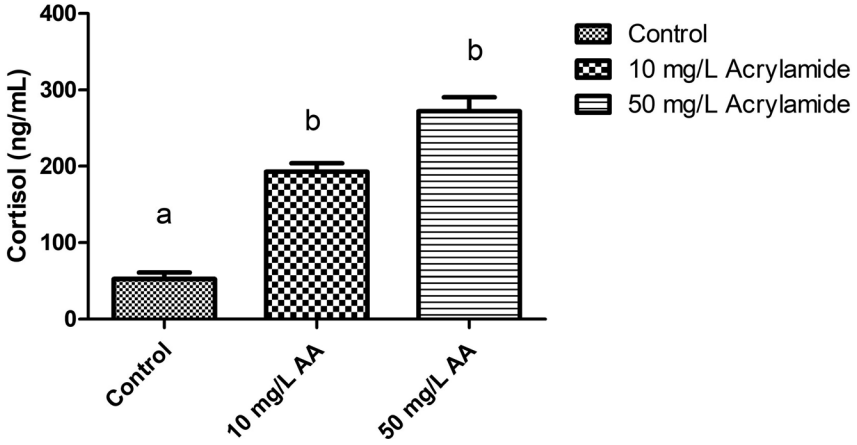


Figure 1: Serum cortisol levels of common carp, *Cyprinus carpio*, control and treatment groups exposed to 10 and 50 mg/L acrylamide. (Significant differences between control fish and treated fish are indicated (a, b $p<0.05$) for each exposure groups.

Table 2: The hematology parameters of carp (*Cyprinus carpio)* after exposed to acrylamide for 96 h). (mean ± S.E.M.; n=10).

Parameters	Control	10 mg/L	50 mg/L	p-Values
WBC, m/mm^3	9.08 ± 0.57	10.08 ± 0.36	10.13 ± 0.45	0.32
Lym, %	76.63 ± 2.30	74.97 ± 1.77	71.18 ± 2.28	0.27
Mon, %	2.28 ± 0.45	2.44 ± 0.24	2.71 ± 0.39	0.11
Gra, %	21.08 ± 2.15	22.59 ± 1.69	26.1 ± 2.16	0.24
RBC, M/mm^3	$1.43 \pm 0.03^{a*}$	1.54 ± 0.11^{a}	1.66 ± 0.07^{b}	0.01
Hb, g/dl	12.10 ± 0.56^{a}	12.70 ± 0.33^{a}	13.57 ± 0.67^{b}	0.03
Hct, %	27.90 ± 1.80^{a}	34.80 ± 1.90^{b}	35.50 ± 1.90^{b}	0.02
MCV, fl	195.10 ± 8.96	225.90 ± 17.07	213.80 ± 13.87	0.17
MCH, pg	84.60 ± 4.66	81.10 ± 4.71	81.69 ± 3.23	0.79
MCHC, g/dl	43.30 ± 1.53	35.89 ± 6.69	38.20 ± 2.18	0.08
THR, m/mm^3	265.43 ± 55.02	214.29 ± 23.12	273.57 ± 41.05	0.54

(Small letters in the row refers significance a, b; $p<0.05$) for each exposure groups. (WBC, White blood cells; Lym, lymphocyte; Mon, monocyte; Gra, granulocyte; RBC, Red blood cells; Hb, hemoglobin; Hct, hematocrit; MCV, Hct*10/RBC; MCH, Hb*10/RBC; MCHC, Hb/Hct).

(%), Hb (g/dL), and RBC (M/mm^3) ($p<0.05$). On the other-hand, the parameters of WBC (m/mm^3), Lym (%), Mon (%), Gra (%), MCV (fl), MCH (pg), and MCHC (g/dl), and were not affected significantly by sublethal acrylamide concentrations ($p>0.05$).

Micronucleus (MN) (‰) and nuclear abnormality (NA) (‰) frequency

MN and NA analyses of carp exposed to 10 and 50 mg/L acrylamide are shown in Table 3. Mean MN frequencies of carp erythrocytes increased significantly after exposure to 10 and 50 mg/L acrylamide for 96 h ($p<0.05$). Both acrylamide exposed and ethyl methanesulphonate (EMS) control group MN frequencies increased significantly compared to the control group. The NA frequencies of acrylamide exposed groups did not significantly change compared to control. Only EMS control group NA frequencies increased significantly compared to the control and the acrylamide exposed groups.

Discussion

The contamination of aquatic ecosystems with several environmental chemicals has attracted increasing attention due to their toxicity and bioaccumulation in the food chain resulting in sublethal toxic effects or death in fish populations. These chemicals can also inhibit endocrine system function in aquatic organisms like fish [28, 29]. Although the common carp is the most consumed fish species globally, the acrylamide toxicity effects related to serum cortisol and thyroid hormone concentrations, hematological parameters, and genotoxicological effects have not been studied in the open literature. Thus, we had to discuss our results with other teleost species.

Table 3: Frequency of micronucleated cells (‰, MN) and nuclear abnormalities (‰, NA) in carp exposed to sublethal acrylamide for 96 h (mean ± S.E.M.; n=10).

Groups	Erythrocytes analysed	MN (frequency/1,000 cells)	Confidence interval (95%)	NA (frequency/1,000 cells)	Confidence interval (95%)
Control	2000	1.86 ± 0.51^a	0.61–3.10	0.83 ± 0.31^a	0.04–1.62
EMS	2000	7.83 ± 0.95^b	5.4–10.27	6.20 ± 0.86^b	3.81–8.59
10 mg/L AA	2000	7.0 ± 0.79^b	5.01–8.92	3.33 ± 0.33^a	2.48–4.19
50 mg/L AA	2000	8.86 ± 0.91^b	6.62–11.09	4.00 ± 1.07^a	1.26–6.74

The small letters (a, b) in the column considered significant; $p<0.05$; EMS, ethyl methanesulphonate; AA, acrylamide.

In the current study, common carp exposed to sublethal concentrations of acrylamide indicated a dose-dependent increase in serum cortisol levels. An elevated serum cortisol level indicates significant functional changes in the HPI axis. These changes have been used as the primary biomarker of a stress reaction in fish in environmental monitoring [30]. The outcomes of this study propose that common carp subjected to acrylamide at sublethal concentrations promote physiological stress. Exposure to acrylamide activates the HPI axis, thus increasing serum cortisol concentrations. Our finding agrees with several previous studies indicating that different waterborne chemical exposures increase serum cortisol levels in fish [31, 32]. Tryptophan exposed fish exhibited elevated serum cortisol above control levels at 10 and 15 days [33]. In tilapia *Oreochromis niloticus*, exposure to cadmium ($CdCl_2$) during 24 and 48 h induced a significant increase in plasma cortisol levels, whereas at 96 h, no differences were found [31]. Similar results were obtained by [32] in crucian carp exposed to extracted microcystins at sublethal and lethal doses. In that study, cortisol levels in both treatment groups significantly increased and remained high throughout the experiment.

Environmental xenobiotics that interfere endocrine system could affect the normal thyroid function in fish. In the current study, we evaluated the effects of acrylamide on the serum thyroid hormone levels in common carp. Serum TT_4 levels did not change in the treatment groups. A significant decrease in the serum TT_3, FT_4, and FT_3 levels were observed following the acrylamide exposure, and the decrease in concentrations seemed to be dose-dependent during the experiment. Disruption of the HPT axis by acrylamide has also been reported in rats [34]. In that study, oral exposure to acrylamide at concentrations of 25, 100, and 500 µg/L showed no significant changes in serum TSH or T_3 levels. T_4 decreased at a high dose only. Our results demonstrated that exposure to acrylamide considerably affected the thyroid hormone levels in the HPT axis. These findings also indicate that acrylamide may directly impact the synthesis or secretion of the circulating thyroid hormones and the conversion of T_4 to T_3 in acrylamide exposed fish, showing its endocrine-disrupting activity on the HPT axis. In teleost fish, the biologically active form of thyroid hormone is T_3, which is from peripheral deiodination of T_4 principally due to D1 deiodinase enzymes [20]. In our study, significant decreases in TT_3 concentrations paralleled the unchanged serum T_4 concentrations in acrylamide exposed fish. The drop in T_3 levels was due to a reduction in hepatic T_4 monodeiodination. Stress could decline circulating T_3 concentrations by decreasing T_4 monodeiodination activity. Environmental chemicals have been reported in both lab and field to interact with the HPT axis in fish [32, 33]. Similar results to our study were obtained in tilapia, *O. niloticus* exposed to 25 mg/L $CdCl_2$ during 24, 48, and 98 h [31]. In that study, plasma T_3 levels decreased despite the unchanged T_4 levels [32]. demonstrated that crucian carp *C. auratus* exposed to extracted microcystin caused significant declines in T_3, FT_4, and FT_3 concentrations in a dose-dependent manner.

Free hormone levels are biomarkers of thyroidal status, and they are preferred clinically since total hormone levels may be mistakable under certain conditions [35]. In our study, the concentration levels decreased significantly in both treatment groups in a dose-dependent manner, when compared to the control group. Reduced concentrations indicate that the dose of thyroid hormones is inadequate for target tissues. Similar to present results decreased concentrations in a dose dependent-manner have been reported in crucian carp *C. auratus* exposed to extracted microcystins [32]. Considering our results, decreased thyroid hormone levels might have led to a disturbance of numerous physiological periods related to development, growth, metabolism, and reproduction.

The MN assay is a sensitive and rapid genotoxicity test system that assesses the effects of chemicals and provides an early warning of genotoxic threats [36]. Previous studies indicated that acrylamide is metabolized into reactive metabolite glycidamide in hepatocytes [36]. This reactive metabolite, when in contact with DNA, induces DNA damage [37]. Based on these experimental findings in vertebrates, acrylamide has been classified as a

genotoxicant, reproductive, and developmental toxicant [36]. Many studies have revealed that the peripheral fish erythrocytes have increased frequencies of MN after treatment with different waterborne pollutants such as pesticides, heavy metals, and the mixture of these contaminants [36]. Our study results showed that exposure to acrylamide increased MN frequency in common carp *C. carpio* peripheral erythrocytes. This finding indicates that acrylamide induces genotoxicity in fish. These results are consistent with the other studies that reported genotoxic effects of acrylamide in other fish species. The frequencies of micronuclei were significantly higher in the 96 h acrylamide (5, 10, 20 mg/L) exposed groups of fish *C. auratus* compared to controls [11]. In another study [4], golden fish *Carassius auratus* were exposed to several acrylamide concentrations for genotoxic damage. The results showed a dose-dependent increase in total DNA strand breakage and the formation of erythrocytic nuclear abnormalities.

The hematocrit, hemoglobin, and erythrocyte numbers of carp increased significantly after exposure to sublethal acrylamide. These results suggested that the toxic chemical acrylamide can significantly affect the hemopoietic system of the fish. Hematological parameters provide essential data about the physiological aspects of fish welfare assessments [38]. Similar hematological findings were also determined by authors of toxicological studies from different chemicals and stresses on carp [39, 40].

In conclusion, the results of the present study showed that widespread and regular use of acrylamide could threaten the survival and the health of the organisms, especially fish, in aquatic environments. Also, the results of the current study demonstrated that studied parameters supply essential biomonitoring tools for the evaluation of pollutant effects in aquatic ecosystems. Due to the wide usage of acrylamide, precautions should be taken for both human and ecosystem health. Further investigation on different toxicological endpoints of acrylamide using new molecular techniques is required for the adverse effects of acrylamide.

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