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Research Article

Investigating the Effect of Aspirin on Mercury Toxicity

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The effect of aspirin on the toxicology of mercury was investigated by using fish. The variations between blood parameters of the fish, which were made exposed directly to mercury solutions prepared at certain concentrations (500 μg/L, 250 μg/L, 125 μg/L, 62.5 μg/L, and 31.25 μg/L), and blood parameters of the fish, which were made exposed to mercury at the same concentrations after they had been interacting with aspirin, were investigated. At the end of the study, increases in blood parameters were observed depending on the increases in mercury concentration. Statistically significant variations were observed in blood parameters of the fish, which were made exposed to mercury at the same concentrations after they had been interacting with aspirin, compared to blood parameters of the fish, which were made exposed directly to mercury ($P < 0.05$). It was found that aspirin has caused significant increases in especially the levels of serum aspartate aminotransferase and alanine aminotranspherase and significant decreases in cortisol and glucose levels among to blood parameters. It was concluded that aspirin alters the toxic effect of mercury.

1. Introduction

Aspirin is the popular name of a salicylate ester of acetic acid. Aspirin (acetyl salicylic acid) is an anti-inflammatory medicine, which has been in the use prevalently since 1899 for rheumatic diseases because it is very effective and inexpensive. Aspirin affects antioxidant system by shifting the equilibrium between oxidants and antioxidants in favor of oxidants causing hepatic and renal damages [1]. Although many nonsteroidal anti-inflammatory drugs (NSAID) have been added to the treatment in the recent 30 years, aspirin indisputably keeps its position during treatment of rheumatic diseases like RA and Kawasaki and new areas in which it can be used like antiphospholipid antibody syndrome and Alzheimer have emerged. Aspirin is absorbed rapidly after it is dissolved and hydrolyzed in the liver, and it can be seen in plasma after 10–20 minutes. Serum reaches the peak value in two hours. Aspirin is hydrolyzed into salicylic acid by nonspecific esterases in liver and bound to salicylate serum proteins, especially albumin. Cytoplasmic enzyme (LDH) released due to salicylates without depending on dose has an effect on hepatotoxicity [2].

Many plant and animal species act as a bioindicator to observe many pollutants in the ecosystem surrounding us [3–8]. It is becoming more important day by day to predict and describe the hidden and harmful effects, which come into sight when that fish is exposed to low toxic matter without showing any symptom of poisoning, in toxicological studies [9, 10]. Heavy metal deposition, which has been increasing every day, in fish has a toxic effect and furthermore, affects human health because fish represent a loop of the biological cycle and they are eaten as a significant protein resource [11]. Fish are the organisms having close relations with the environment in which they live. They can demonstrate stress resources, which might be present in the water surrounding them, ease in their bodies by a metabolic way due to the close relations between them and the water [12]. The heavy metal effect is another stress factor for fish in addition to famine, reproduction, and hypoxic conditions.

Mercury is one of the most toxic heavy metals and has been introduced into the environment in the form of inorganic and organic mercury compounds through paper industry, gold and silver mining, electricity industry, paints, fungicides, and drug industry [13]. Mercury may deposit in

plants and animals. Furthermore, it may cause damage in the central nervous system in case of intake by the human body. Metallic mercury and organic mercury determination has come under consideration in recent years due to their tendency to deposit in aquatic organisms and human body and due to its toxicological effect. According to WHO, the allowed limit for mercury in potable water is much lower than 1 μg/L [14].

When an organism perceives an adverse situation as a stress resource, it gives a response to it and as a result the level of certain hormones (adrenaline, cortisol, glucose, etc.) in the blood increases [15–17]. Thus, it is possible to investigate the effects of the stress on the organism by determining blood parameters in these hormones in case of stress states. Cortisol is released from cortex layer of the adrenal gland in stress responses given to many forms of environmental stress. Cortisol may cause many physiological variations if the time during which it is released extends [18]. Glucose is the essential high-energy compound in vertebrate, and stored in muscle and liver tissues in the form of glycogen. Therefore, it is very important to determine variations especially in blood parameters of aquatic organisms under the effect of pollutants in metabolic and physiological events because they indicate the pollution level in that environment [19].

The energy required by fish is supplied by lipids and proteins, in addition to carbohydrates, mediated by gluconeogenic enzymes like aspartate aminotranspherase (AST) and alanine aminotranspherase (ALT) [20]. Aspartate aminotranspherase and alanine aminotranspherase catalyze two of the most important aminotranspherase reactions [21]. Aminotranspherases are normally intracellular enzymes. High aminotranspherase levels in plasma indicate damage in cells, which are rich in such enzymes. For example, a physical trauma or any disease causes cell destruction and thus, intracellular enzymes find their way to the blood. Two of the aminotranspherase have a diagnostic value. These are AST and ALT. High AST and ALT levels occur in plasma in almost all liver diseases [21, 22].

Salicylates may react to produce a chelate complex. In other words, they may act as a chelate producer, which can collect certain metals in the body [23]. This indicates a need for a study on whether or not aspirin causes different effects on organisms containing heavy metals.

This study was conducted to investigate how much aspirin influences toxic effects of Mercury, which is one of the most toxic heavy metals, by using certain blood parameters of fish.

2. Material and Methods

2.1. Reagents. Chemicals with the use pure water, which were produced by Millipore's ultrapure water device, was used during the analyses. All chemicals, which were used, are pure at analytic grade and were purchased from Merck (Darmstadt, Germany). Stock mercury solution (μg/L) was prepared by using Mercury (II) chloride. Then, this stock solution was diluted to prepare standard solutions (500–31,25 μg/L). Aspirin was used in the form of a tablet. Each tablet contains 500 mg salicylic acid.

2.2. Investigation Media and the Processes Which Were Applied to Fish. All materials made of glass were treated with 10% HNO_3 (Merck) solution, maintained in 30 mM Na_4EDTA (Merck) solution for a night to remove metal ions, which might have been adsorbed on their surfaces, and rinsed with ultrapure water before being used in the laboratory. The fish, Carp (Cyprinus Carpio), which was used as the material of the study, was supplied by Gaziosmanpaşa University's Almus Vocational High School, Department of Fisheries. The fishes were placed in glass aquariums, each has a volume of 40 L (sizes 39 × 36 × 28.5 cm), fed regularly for a week to ensure their adaptation. The weight of the fish used in the study is 55 g while their length is 15 cm. Temperature, pH and dissolved oxygen level of the water inside the aquariums were kept constant during the experiment. In the experiment, the aquariums were divided into two separate groups. Solutions at concentrations of 500, 250, 125, 62.5, and 31.25 μg/L were added to the aquariums in the first group while one of them was specified as the control. Three fish were placed in each aquarium in this group. The fish were made exposed to mercury for 48 hrs in the aquariums. Aspirin was applied to the fish in the aquariums in the second group in the form of tablet at a dose of 1 tablet per day for 15 days (187.5 mg/L aspirin in total was applied to each aquarium) and then, the treatments to which the fish in the first group had been exposed were repeated for the second group.

2.3. Taking Blood Samples from the Fish Samples. Blood samples were collected from the heart of the fish, which had had an impact on the head, without applying anesthesia, through the puncture. For this purpose, a 5 mL sterile disposable syringe with a green end was inserted at an angle of 40–45 degrees at a distance of ones third from the bottom into the heart just before the pectoral arch formed by cleithrum bone after the gill cover of the fish resting on its side had been lifted and the drawn blood was transferred into suitable test tubes [24, 25]. Blood samples were collected from 36 fishes in total. These samples were brought to the lab under suitable storage conditions and immediately analyzed.

2.4. Analyses of Blood Parameters. Samples were placed into centrifuge tubes, which were free from any anticoagulant, and centrifuged at 3500 RPM (cycles/minute) for 5 minutes (NF 800 R) to move the serum to be used in determining glucose, aspartate aminotranspherase, alanine aminotranspherase, lactate dehydrogenase, and cortisol to the upper phase. Then, glucose, aspartate aminotranspherase, alanine aminotranspherase, lactate dehydrogenase, and cortisol levels in the blood samples were read in the Cobas 6000 device with the help of on autoanalyzer by using a commercial kit in Gaziosmanpaşa University's Medical School's Biochemistry Laboratory.

2.5. Statistical Analysis. The SPSS statistics program was used to find the variations that occurred depending on varying mercury concentrations and the variations between the groups treated and not treated with aspirin. Because the data do not show a normal distribution, they were analyzed

Table 1: Average values and standard deviations for blood parameters of the fish versus different mercury concentrations.

Hg (μg/L)	Glucose (mg/L)	ALT (U/L)	AST (U/L)	LDH (U/L)	Cortisol (ug/dL)
Standard	9,67 ± 0,57	74,67 ± 0,57	2465,00 ± 0,55	3731,67 ± 1,52	0,09 ± 0,01
500	779 ± 10	264,67 ± 0,57	14159,33 ± 1,15	18321,00 ± 3,60	30,37 ± 0,05
250	700 ± 00	219,67 ± 1,52	6951,00 ± 1,00	16050,00 ± 2,00	27,94 ± 0,03
125	54,00 ± 1,00	160,00 ± 1	6186,33 ± 1,15	9880,00 ± 2,00	17,30 ± 0,30
62,5	5,00 ± 00	135,00 ± 0	5504,00 ± 1,00	7729,00 ± 1,73	16,00 ± 1,00
31,25	10,33 ± 0,57	110,00 ± 1	4669,67 ± 0,57	6204,00 ± 1,15	7,65 ± 0,01

Table 2: Average values and standard deviations for blood parameters of the fish exposed to aspirin and mercury.

	Glucose (mg/L)	ALT (U/L)	AST (U/L)	LDH (U/L)	Cortisol (uL)
Standard	9,67 ± 0,57	74,67 ± 0,57	2465,00 ± 0,55	3731,67 ± 1,52	0,09 ± 0,01
Aspirin (μg/L)	4,66 ± 0,57	209,66 ± 1,52	5754,67 ± 0,57	5574,66 ± 0,57	5,10 ± 0,10
Aspirin + Hg (500 μg/L)	10,00 ± 0,00	425,33 ± 0,57	18345,00 ± 1,00	15171,00 ± 1,00	29,76 ± 0,05
Aspirin + Hg (250 μg/L)	9,33 ± 1,52	349,67 ± 0,58	12344,67 ± 0,58	10974,67 ± 0,57	26,7 ± 0,43
Aspirin + Hg (125 μg/L)	8,33 ± 0,58	340,33 ± 0,58	11754,67 ± 0,57	10520,33 ± 2,51	10,20 ± 0,60
Aspirin + Hg (62,5 μg/L)	5,00 ± 0,00	294,66 ± 0,58	10283,33 ± 3,78	10455,00 ± 1,00	7,20 ± 0,13
Aspirin + Hg (31,25 μg/L)	4,66 ± 0,57	283,33 ± 2,08	8294,66 ± 0,58	10270,00 ± 2,00	5,36 ± 0,06

by using the Kruskal-Wallis H nonparametric test as an alternative to ANOVA. Mann-Whitney U test was used to compare the relation between the two groups. Variations were considered as significant when the variations between the groups were $P < 0.05$.

3. Results

Table 1 shows average values and standard deviations for blood parameters of the fish versus different mercury concentrations.

Table 2 shows average values and standard deviations for blood parameters of the fish exposed to aspirin and mercury.

Regular increases were observed, compared with the standard, in blood parameters of the fish in the first group (I), which had been directly exposed to mercury, and those of the fish in the second group (II), which had been treated with aspirin at a total concentration of 187.5 mg/L for 15 days before being exposed to mercury (Tables 1 and 2). This increase was considered as statistically significant for almost all parameters ($P < 0.05$).

In comparison of the two groups, the difference between the blood parameters was considered as statistically significant (Table 3).

Glucose and cortisol values from blood parameters of the fish taking place in Group II have decreased significantly compared with the Group I while ALT and AST values increased significantly. LDH values increased significantly compared with the Group I except the fish exposed to mercury at concentrations of 500 μg/L and 250 μg/L (Tables 1, 2, and 3).

Table 3: P values between blood parameters of the fish taking place in [a](the group exposed to mrcury) and [b](the group exposed to aspirin and mercury).

Hg (μg/L)	Glucose	ALT	AST	LDH	Cortisol
500	0,037	0,043	0,046	0,05	0,05
250	0,037	0,046	0,046	0,046	0,05
125	0,046	0,046	0,043	0,05	0,05
62,5	1	0,034	0,05	0,046	0,05
31,25	0,43	0,05	0,043	0,043	0,046

[a]I. Grup: The Grup exposed to mercury.

[b]II. Grup: The Grup exposed to aspirin and mercury.

4. Discussion

Aspirin is prevalently used by people, who are healthy and suffer from cardiovascular disease risk, to decrease blood viscosity and due to its antithrombotic effect [26–29]. Like all other medicines, aspirin has side effects besides its healing effect. The dose, which will cause hematological side effect, is not known for aspirin. Aspirin inhibits thrombocyte aggregation and extends the bleeding time. Cytoplasmic enzyme (LDH) released due to salicylates without depending on dose has an effect on hepatotoxicity [2]. Such side effects of aspirin should be taken under consideration beside its acceptable benefits by everybody. Salicylates may react to produce a chelate complex. In other words, they may act as a chelate producer, which can collect certain metals in the body [23]. This indicates a need for a study on whether or not aspirin causes different effects on organisms containing heavy metals. This study was conducted to investigate how much aspirin influences toxic effects of mercury, which is one of the most toxic heavy metals by using certain blood parameters of fish. Former studies realized successfulIy on the determination of mercury in Marine Fish Species [30, 31]. Besides, various studies are available and were conducted on aspirin and blood parameters by using test animals. Nergiz (1985) [32] investigated alterations caused by aspirin in liver of white mice. Durgun et al., (1998) [26] studied effects of

aspirin on certain blood parameters in rabbits fed by high-cholesterol ration in their study. Çam (2007) [33] investigated blood parameter levels to examine the activity of phenethyl ester of caffeic acid in mice stimulated by aspirin in this study. In our study, effect of mercury in fish stimulated by aspirin was investigated by examining blood parameters. Metals freed in aquatic media cause certain disorders in fish and affect their blood parameters also [34]. Studies are available in the literature evidencing effects of certain metals on blood parameters of fish. It was determined that metal effect in the fish increases the release of stress hormones like cortisol, epinephrine, and catecholamine to cause changes in carbohydrate metabolism [35, 36]. Metals like copper, chromium, nickel, and cadmium as well as chelate complexes play a role in inducing carcinogenesis during and after lipid peroxidation [37]. Hilmy et al., (1985) [38] determined that serum AST and ALT levels increased under metallic effect at the beginning in Cyprinus carpio made exposed to effect of cadmium at different concentrations for 30 days and they decreased upon extension of exposure time. It was reported in some studies that increases were observed in plasma cortisol, glucose and lactate levels in different fish species exposed to stressors like heavy metals [39–43]. In our study also, increases were observed in glucose, AST, ALT, LDH and cortisol levels, compared with the standard, in the blood of the fish exposed to mercury (Table 1). The differences were found statistically significant for all parameters (except the values corresponding to mercury concentrations of 31.25 μg/L and 62.50 μg/L for glucose) at all concentrations ($P < 0.05$). It is reported in the literature that the equilibrium between oxidants and antioxidants is shifted by aspirin in favor of oxidants to cause damage on antioxidant system and thus, eventually, may cause injuries in liver and kidneys. It is also reported in the literature that an increase in ALT value may indicate damage in liver [1]. It was found in our study that aspirin caused an increase in all blood parameters compared to the standard except for glucose. Erdem and coworkers studied selenium's and zinc's protective effect against damage, which might be caused by aspirin in the body, in 2006. It was found as a result of the study that selenium and zinc play a protective role against the damage caused by aspirin in the antioxidant system. What makes our study distinguished from other studies in the literature is the fact that our study aimed to find what type of changes aspirin causes in blood parameters of the body of an organism containing a very toxic matter like mercury at certain doses. For this purpose, the two groups were compared with each other and the variation between blood parameters was found statistically significant (Table 3). Glucose cortisol values of blood parameters of the fish taking place in Group II have decreased significantly compared with the group I while ALT, and AST, values increased significantly. LDH values increased significantly compared with the fish in Group I except the fish exposed to mercury at concentrations of 500 μg/L and 250 μg/L (Tables 1, 2, and 3). It was reported in the literature that an increase was observed in plasma AST and ALT levels in almost all liver diseases [21, 22]. According to our findings, it may be concluded that aspirin may increase ALT and AST and LDH values to promote liver diseases in the body of an organism if the organism contains a toxic matter like mercury. Dange (1986) [44], and Karataş et al. (2005) [45] determined that stress conditions raise serum glucose and cortisol levels and if the conditions are maintained, they significantly reduce glycogen in liver to cause certain changes in carbohydrate metabolism in their study conducted on various fish species under effect of organic and inorganic pollutants. Chun and Oh (1989) [46] evidenced in a study conducted with Cyprinus carpio that serum glucose concentration is completely related to water quality and the stress caused by pollution in fish has an effect on glucose concentration. Heath (1995) [47] reported that pollution causes stress in fish and other mechanisms, which are responsible for mobilization of energy resources, are activated in case of long-term stress conditions. He also reported that one of these mechanisms is cortisol released by the adrenal cortex, cortisol acts in glycogen storage in liver by reducing the use of glucose by tissues and it keeps blood glucose at high concentrations. According to the existing studies, glucose and cortisol change depending on each other and an increase or a decrease in cortisol is determinative for glucose level in blood. In our study, it was found that cortisol and glucose changed constantly depending on each other and cortisol and glucose values were lower in the fish pretreated with aspirin compared with those made exposed to mercury directly. The degree of the decrease between these two groups was found statistically significant ($P < 0.05$). According to these results, it may be concluded that aspirin may reduce cortisol, which is caused by a toxic material like mercury and causes stress on the fish.

In our study, unlike other studies in the literature, it was found what type of changes Aspirin causes in blood parameters of the body of an organism containing a very toxic matter like mercury at certain doses. According to our findings, as the mercury concentration increases in fish, glucose, AST, ALT, LDH, and cortisol, which are some of blood parameters, increase and the increases are statistically significant. Furthermore, it was concluded that aspirin may increase ALT, and AST, and LDH values to promote liver diseases in the body of an organism if the organism contains a toxic matter like mercury; however, it may reduce the effect of mercury with respect to cortisol.

As a result of the study, it was concluded that aspirin should be used in a more controlled way in the presence of toxic heavy metals like mercury, which might exist in an organism in trace amounts.

Glossary

NSAID: Nonsteroidal anti-inflammatory drugs
AST: Aspartate aminotranspherase
ALT: Alanine aminotranspherase.

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