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# Effect of Sulfamerazine on Oxidative Stress of Rainbow Trout (*Oncorhynchus mykiss*, Walbaum, 1792)

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**Abstract:** The aim of this study was to investigate effects of sulfamerazine on oxidative stress of rainbow trout (*Oncorhynchus mykiss*). The fish were divided into four groups. The first group was fed with diets containing no sulfamerazine; the second group was supplemented with sulfamerazine at 100 mg $kg^{-1}$ for 21 days; the third group was treated with sulfamerazine at 200 mg $kg^{-1}$ for 21 days; fourth group were supplemented with sulfamerazine at 400 $kg^{-1}$ for 21days. Blood samples were taken to determine of the malondialdehyde (MDA) levels and catalase (CAT) activities from fish on the 3rd, 7th, 14th and 21st days of feeding. The levels of MDA were lower in the groups exposed to three different doses of sulfamerazine treatment than in the control group on day 3rd. MDA levels began to increase depending on time at group supplemented with sulfamerazine. MDA levels were found to be higher at fish exposed to 200 mg $kg^{-1}$ dose than those observed in fish exposed to 100 and 400 mg $kg^{-1}$ dose of sulfamerazine ($P<0.05$). CAT activities increased in rainbow trout after application of three different doses of sulfamerazine on day 3rd of exposure, while they decreased after application of sulfamerazine on day 14th, 21st of exposure. CAT activities were found to be higher at fish exposed to 200 mg $kg^{-1}$ dose of sulfamerazine than those observed in fish exposed to 100 and 400 mg $kg^{-1}$ dose ($P<0.05$). It was observed that oxidant-antioxidant status changed in rainbow trout after application of different doses of sulfamerazine.

**Keywords:** catalase, malondialdehyde, rainbow trout, sulfamerazine

# Gökkuşağı Alabalığı (*Oncorhynchus mykiss*)'nda Oksidatif Stres Üzerine Sulfamerazinin Etkisi

**Özet:** Bu çalışmanın amacı gökkuşağı alabalığı (*Oncorhynchus mykiss*)' nda oksidatif stress üzerine sulsamerazinin etkilerini araştırmaktı. Balıklar dört gruba ayrıldı. İlk grup sulfamerazin içermeyen yemle (kontrol), ikinci grup 21 gün süreyle 100 mg $kg^{-1}$, üçüncü grup 21 gün süreyle 200 mg $kg^{-1}$, dördüncü grup 21 gün süreyle 400 mg $kg^{-1}$ sulfamerazin içeren yemlerle beslendi. Balıklardan kan örnekleri beslemenin 3., 7., 14. ve 21. günlerinde malondialdehit (MDA) ve katalaz (CAT) aktivitelerini belirlemek için alındı. MDA düzeyleri, 3. günde sulfamerazinin üç farklı dozunun uygulandığı balıklarda kontrol grubundan daha düşüktü. MDA düzeyleri sulfamerazinle beslenen gruplarda zamana bağlı olarak artmaya başladı. 200 mg $kg^{-1}$ dozunda sulfamerazin uygulanan balıkların MDA düzeyleri 100 ve 400 mg $kg^{-1}$ oranında sulfamerazin uygulanan balıklara göre daha yüksek bulundu ($P<0.05$). CAT aktivitesi uygulamanın 3. gününde sulfamerazinin her üç dozunun uygulandığı balıklarda artarken, uygulamanın 14. ve 21. günlerinde azaldı. 100 ve 400 mg $kg^{-1}$ oranında sulfamerazin

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uygulanan balıklara göre 200 mg $kg^{-1}$ dozunda sulfamerazin uygulanan balıklarda CAT aktivitesi daha yüksek tespit edildi ($P<0.05$). Sonuç olarak sulfamerazinin farklı dozlarının uygulandığı alabalıklarda antioksidan durumun değiştiği gözlemlendi.

**Anahtar kelimeler:** katalaz, malondialdehit, gökkuşağı alabalığı, sulfamerazin

## INTRODUCTION

Several hazards and side-effects are associated with excessive usage of anti bacterial drugs for fish such as immunosuppression, nephrotoxicity, growth retardation, development of resistant bacterial strains, environmental problems such as drug residues in fish farm sediments and residues of drugs in fish products (Saglam & Yonar, 2009; Yonar et al., 2011). The interaction of drugs with lymphoid tissues may alter the functions and balance of the immune system and induce undesirable effects such as immunosuppression, uncontrolled cell proliferation, alternations in other host defence mechanisms against pathogens and it can induce neoplasia. Some drugs have been shown to stimulate immunological processes (Anderson & Zeeman, 1995; Anderson & Jeney, 1992). On the contrary, drugs such as oxytetracycline, oxolinic acid and sulphadiazine have been ascribed to immunosuppressive effects in carp and rainbow trout (Siwicki et al., 1989; Lunden et al., 1998; Lunden & Bylund, 2002; Yonar et al., 2011; Yonar, 2012).

Sulphonamides are among the drugs used to treat bacterial fish diseases. Sulphonamides are structural analogues and competitive antagonists of paraaminobenzoic acid (PABA). They inhibit normal bacterial utilization of PABA for the synthesis of folic acid, an important metabolite in DNA synthesis. The effects are usually bacteriostatic in nature. Folic acid is not synthesized in fish, but is instead a dietary requirement. This allows for the selective toxicity to bacterial cells (or any cell dependent on synthesizing folic acid) over fish cells. Bacterial resistance to sulfamerazine is caused by mutations in the folic acid enzyme that prevents the drug from binding and blocking folic acid synthesis (Treves-Brown, 2000). Sulfamerazine belongs to the group sulphonamides and is found in the 4-amino-N-[4- methyl–2-pyrimidinyl] benzene sulphonamide formulation. It is most often used as part of a synergistic combination with trimethoprim. Its primary activity is against susceptible forms of *Streptococcus*, *Staphylococcus aureus*, *Escherichia coli*, *Haemophilus influenzae* and oral anaerobes. It is commonly used to treat furunculosis, columnaris and bacterial kidney disease infections of fish (Saglam & Yonar, 2009).

Recent evidence indicates that the health of aquatic organisms might also be linked to oxidative stress, and several environmental contaminants may enhance oxidative stres in aquatic organisms (Kolayli & Keha, 1999; Lushchak, 2011). Oxidative stress is a term denoting an imbalance between the production of oxidants and the respective defense systems of an organism. Oxidants encompass oxygen free radicals, reactive nitrogen species, sulphur-centred radicals, and various others (Abuja & Albertini, 2001; Mişe Yonar et al., 2017). Oxidative stress occurs when reactive oxygen species (ROS) overwhelm the cellular defences. ROS are by-products of electron transport chains, enzymes, and redox cycling (Kelly et al., 1998). ROS are biologically important, damaging molecules suc as lipids, DNA, or proteins, and are involved in the pathobiochemistry of degenerative diseases (Sies, 1991). The most widely used assay for lipid peroxidation is the malondialdehyde (MDA) formation, which represents the secondary lipid peroxidation product with the thiobarbituric acid reactive substances test (Draper et al., 1993; Janero, 1990). MDA is the final product of lipid peroxidation. The concentration of MDA is the direct evidence of toxic processes caused by free radicals (Sieja & Talerczyk, 2004; Mişe Yonar, 2019; Eksen & Mişe Yonar, 2021).

Antioxidant defenses, which are generally ubiquitous in animal species and different tissue types, are widely detected in aquatic organisms. Exposure to these contaminants depends on the particular dietary and ecological lifestyles of the aquatic organisms (Livingstone, 1998). Chemical toxic pollutants are important sources of ROS in biological systems (Kappus & Sies, 1981). Oxidative stress and damage to fundamental biomolecules and to antioxidant defenses of organisms are established field in environmental toxicology and ecotoxicology (Kelly et al. 1998; Mişe Yonar et al., 2019). Assaying antioxidant enzymes can offer an indication of the antioxidant status of the organisms and serve as biomarkers of oxidative stress (Kohen & Nyska, 2002). The primary antioxidant protection against these species is provided by the superoxide dismutase and catalase enzymes, respectively (Halliwell & Gutteridge, 2000). Consequently, these antioxidant enzymes contribute to the maintenance of a relatively low level of the reactive and harmful species hydroxyl radical ($OH^{\cdot}$), a chemical product of the reaction between $O_2^{\cdot-}$ and $H_2O_2$. The $OH^{\cdot}$ triggers the lipoperoxidation of membranes, a process that may be potentially dangerous to fish, since they possess a high content of polyunsaturated fatty acids (Kolayli & Keha, 1999; Hidalgo et al., 2002).

Specially adapted enzymes normally counteract damaging effects of oxidative stress, defined as a disruption of the prooxidant-antioxidant balance in favor of the former, leading to potential damage (Sies, 1991). Although the antioxidant enzymes have been characterized in fish and bivalves exposed in vivo or in situ at polluted sites (Di Giulio et al., 1993; Thomas & Wofford, 1993), the effects of sulfamerazine on the activities of the antioxidant system have not been investigated in the any species, including teleost fish. Thus, the aim of the present study was to establish the effect of sulfamerazine on oxidative stress in rainbow trout (*Oncorhynchus mykiss*) after application of different doses of sulfamerazine.

## MATERIALS and METHODS

Healthy rainbow trout having mean length 25.51±1.95 (21.40–28.20 cm) and mean weight 193.90±40.09 (124.20–280.30 g) were obtained from local fish farming. They were brought to the fish diseases laboratory in Fisheries Faculty and acclimatized to the laboratory conditions for 15 days under a natural photoperiod and ambient temperature. During this period, fish were fed ad libitum with pellet feedstuff twice a day. Before starting the test period, all experimental tanks of 650 L capacity, with water recirculation, were cleaned and filled with 500 L of spring water. Dissolved oxygen, pH and conductivity were determined using a digital oxygenmeter and a pH meter. The mean values for test water qualities were as follows: dissolved oxygen 8.01±0.83 mg $L^{-1}$, pH 7.19±0.32, temperature 16.0±1 $^{o}C$, electrical conductivity 168.3±6.2 $\mu Scm^{-1}$, alkalinity 181.6±8.7mg $L^{-1}$ and hardness 180.0±7.0 mg $L^{-1}$ as $CaCO_3$.

The fish were divided into four groups, with 80 fish in each group. After a 2-week acclimation period, the experimental groups were fed with feedstuff containing 100 mg $kg^{-1}$, 200 mg $kg^{-1}$ and 400 mg $kg^{-1}$ sulfamerazine and the control group was fed with a commercial pellet feed not supplemented with sulfamerazine. Three different doses (100, 200 and 400 mg $kg^{-1}$) of sulfamerazine with three replicates were used in the test series. Control units with three replicates were also prepared. The amounts of 100, 200 and 400 mg sulfamerazine (Sigma, S-0800) for per kg fish weight were mixed with feed and given to the fish manually at a rate of approximately 2% fish body weight per day. The use of fish and the experimental protocol were approved by Institutional Ethics Committee for the Local Use of Animals in Experiments.

Blood samples were collected from the caudal vein of anaesthetized (Benzocaine, 50 mg $mL^{-1}$) fish to determine the plasma MDA levels and erythrocyte catalase activities. Blood samples were collected into tubes containing anticoagulant (2 % sodium oxalate). The samples were centrifuged at 200 g for 5 min at +4 $^{o}C$ to separate their plasma and were kept at –20 $^{o}C$ until analyse.

Plasma MDA levels were measured by the modified method of Satoh (1978) based on the reaction with thiobarbituric acid and were expressed as nmol $ml^{-1}$. CAT activity was determined by measuring the decomposition of $H_2O_2$ at 240 nm, according to the method of Aebi (1984). The principle of the assay is based on determination of the rate constant k ($s^{-1}$) or the $H_2O_2$ decomposition rate at 240 nm. Results were expressed as k $gHb^{-1}$, where k is the first-order rate constant.

Data were analysed by analysis of variance (ANOVA) using the General Liner Model procedure of the statistical analysis system with Duncan's multiple-range test. P value < 0.05 was considered to the statistically significant.

## RESULTS

Changes in MDA levels and catalase activities of rainbow trout (*Onchorhynchus mykiss*) in the control group and those exposed to 100 mg $kg^{-1}$, 200 mg $kg^{-1}$ and 400 mg $kg^{-1}$ are presented in Tables 1-2.

The MDA levels on day 3$^{rd}$ of exposed to sulfamerazine were lower than those observed in control group fish. The MDA levels were found to be significant statistically between control group fish with fish exposed to a 100 mg $kg^{-1}$ dose of sulfamerazine on days 3$^{rd}$, 7$^{th}$ and 200 and 400 mg $kg^{-1}$ dose of sulfamerazine on days 3$^{rd}$, 7$^{th}$ and 21$^{st}$ ($P<0.05$).

The increases in MDA levels were observed at fish exposed to sulfamerazine as dependent on time. The MDA levels were found to be lower at fish exposed to 200 mg $kg^{-1}$ dose than those observed in fish exposed to 100 and 400 mg $kg^{-1}$ dose of sulfamerazine ($P<0.05$).

The CAT activities were found to be statistically significant increase between control group fish and fish treated with diferent doses of sulfamerazine on day 3$^{rd}$ of exposure ($P<0.05$). A statistically significant decrease in CAT activities was found between control group fish and fish exposed to 100 mg kg-1 dose of sulfamerazine on day 14$^{th}$ and 21$^{st}$ day of exposure ($P<0.05$). The CAT activities were lower than the levels of control with fish exposed to sulfamerazine on day 21$^{th}$. The CAT activities were found to be higher at fish exposed to 200 mg $kg^{-1}$ dose of sulfamerazine than those observed in fish exposed to 100 and 400 mg $kg^{-1}$ dose ($P<0.05$).

The increases in MDA levels were observed at fish exposed to sulfamerazine as dependent on time. The MDA levels were found to be lower at fish exposed to 200 mg $kg^{-1}$ dose than those observed in fish exposed to 100 and 400 mg $kg^{-1}$ dose of sulfamerazine ($P<0.05$).

**Table 1.** Time-dependent change of the plasma MDA levels (nmol $ml^{-1}$) in sulfamerazine administered groups.

| | Days | | | | |
|---|---|---|---|---|---|
| **Doses** | **0** | **3** | **7** | **14** | **21** |
| **100** | 21.81±1.45$^{a}$ | 6.39±0.44$^{b}$ | 17.34±0.83$^{b}$ | 21.21±1.32$^{a}$ | 15.39±1.595$^{b}$ |
| **200** | 22.18±1.32$^{a}$ | 5.53±0.82$^{b}$ | 13.71±1.21$^{c}$ | 14.79±0.81$^{c}$ | 15.68±0.26$^{c}$ |
| **400** | 22.21±1.36$^{a}$ | 6.96±0.85$^{b}$ | 11.86±1.47$^{c}$ | 21.76±1.27$^{a}$ | 19.47±1.17$^{a}$ |

$^{a, b, c}$: Different letters in the same column indicate statistical difference

**Table 2.** Time-dependent change of the blood CAT activities ($kgHb^{-1}$) in sulfamerazine administered groups.

| | Days | | | | |
|---|---|---|---|---|---|
| **Doses** | **0** | **3** | **7** | **14** | **21** |
| **100** | 45.91±2.37$^{ab}$ | 62.31±3.48$^{b}$ | 57.75±2.64$^{ab}$ | 44.60±3.17$^{a}$ | 35.71±2.03$^{c}$ |
| **200** | 44.43±2.06$^{a}$ | 62.77±3.29$^{b}$ | 47.58±3.85$^{b}$ | 37.31±1.66$^{a}$ | 35.21±3.24$^{c}$ |
| **400** | 42.38±1.74$^{a}$ | 47.21±3.28$^{a}$ | 42.45±1.78$^{a}$ | 31.28±1.92$^{b}$ | 33.22±2.10$^{b}$ |

$^{a, b, c}$: Different letters in the same column indicate statistical difference

A statistically significant increase in CAT activities was found between control group fish and fish treated with different doses of sulfamerazine on the 3rd day of exposure ($P<0.05$). Statistically significant decrease was found in CAT activities between control group fish and fish exposed to 100 mg kg-1 dose of sulfamerazine on the 14th and 21st days ($P<0.05$). The CAT activities were lower than the levels of control with fish exposed to sulfamerazine on day 21$^{th}$. The CAT activities were found to be higher at fish exposed to 200 mg $kg^{-1}$ dose of sulfamerazine than those observed in fish exposed to 100 and 400 mg $kg^{-1}$ dose ($P<0.05$).

## DISCUSSION and CONCLUSION

Oxidative stress is the result of one of three factors: (1) an increase in ROS, (2) a disruption in antioxidant defense systems, or (3) a putative state to repair oxidative damage. Major damage induced by ROS results in changes in cellular macromolecules (membrane lipids, proteins and DNA) and cell function such as changes in intracellular calcium and intracellular pH or cell death (Swann et al. 1991). Lipid peroxidation is a well recognized mechanism of cellular injury used as an indicator of oxidative stress in cells and tissues. The use of lipid peroxidation as a marker of oxidative damage against different drugs is known and reported in the literature (Kelly et al., 1998). For example, Yonar et al., (2011) and Yonar (2012) showed an increase in MDA level in OTC-treated rainbow trout (*Oncorhynchus mykiss*). However, the role of oxidative stress in sulfamerazine toxicity to fish has not been investigated so far. In our study, the increased MDA level can be attributed to the free radicals formed by the application of sulfamerazine, and the changes in the oxidant-antioxidant status can be attributed to the possible accumulation of sulfamerazine in the blood.

The rapid increase in rainbow trout farming has drastically augmented the use of antimicrobials, in particular, antibiotics (Saglam & Yonar, 2009). Radi et al. (1985) state that more than 50 % of the fish tissues are composed of polyunsaturated fatty acids. Therefore, fish tissues are more sensitive to free-radical damage. In most fish, the red muscles are relatively scarce, and other tissues such as liver, blood, and kidney are more important in ROS production. In this study, we determined susceptibility to oxidative damage of the blood. Also, the thiobarbituric acid reactive substances (TBARS) values for erythrocytes after exposure to $H_2O_2$ were found significantly different with respect to normal values.

Greenfield et al. (1991) have confirmed that in red cells both sulphasalazine and 5-aminosalicylic acid (5-ASA) act as antioxidants and inhibit the peroxidation of polyunsaturated fatty acids. This suggests a mode of action by which the documented antioxidant properties of these drugs may be exerting an effect. The ability of drugs to interact with the erythrocyte membrane and terminate free radical reactions may be different, or 5-ASA may have to enter the red blood cell, while sulfasalazine may act extracellularly. It is also possible that sulfasalazine has a greater affinity for free radicals but is therefore consumed more quickly than 5-ASA. This may explain why although sulfasalazine reduces malondialdehyde production at 10 minutes, production at 180 minutes is inhibited by 5-ASA more than equivalent concentrations of sulfasalazine. Inhibition of lipid peroxidation by sulfasalazine

supports the hypothesis that the prodrug sulfasalazine itself is active and scavenges (OH-) radicals and hypochlorous acid. Sulfasalazine and 5-ASA have these properties because they have a phenolic hydroxyl side group that provides scavenging ability but is absent on sulfapyridine. Sulphapyridine inhibited peroxidation at 10-3 M and this drug has some antioxidant activity. The results in this study are in agreement with the results of previous studies. Fish exposed to sulfamerazine as dependent on time display a tendency toward decreased antioxidant enzyme activity. This study has demonstrated that treatment with sulfamerazine leads to oxidative damage in the form of an increase in MDA and a decrease in CAT activity. CAT activity was decreased by affected sulfamerazine toxication in the fish. Sulfamerazine toxicity led to free radicals and oxidative damage.

CAT, an enzyme mostly localized in peroxisomes is, with the glutathione redox cycle, the primary cellular enzymatic defence system against $H_2O_2$, converting it into $H_2O$ and $O_2$. The lack of antioxidant system and changes in the activity of enzymes involved in oxidative stress observed in our system are likely to affect the capacity of cells to defend themselves and respond to pollutant-induced oxidative stress. Indeed, if detoxification of $H_2O_2$ to $H_2O$ by CAT is impaired, a part of excess $H_2O_2$ may be converted to toxic $OH^-$ through the Fenton reaction, depending on free transition metal concentrations (Kehrer, 2000). Thus, CAT is critical for the process of scavenging free radicals. Sulphonamides disrupt CAT activity and lead to accumulation of $H_2O_2$. $H_2O_2$ increases the phagocytic activity of neutrophils. Sulphonamides lead to accumulation of toxic levels of $H_2O_2$ for bacteria by inhibiting their CAT activity. In addition, hydroxyl radical ($OH^-$) and $H_2O_2$ kill cells as well as bacteria (Babior, 1978). CAT activities increased in a dose-related manner following exposure to sulfamerazin on day 3rd. A significant decrease of CAT activity was observed at high doses of sulfamerazine as dependent on time. In this study, significant decreases in CAT activity, which has an oxidant effect, were determined in sulfamerazine treated fish. This effect may be due to the possible increase in H2O2 levels of sulfamerazine. If the oxidative species are not rapidly eliminated, the loss of the physiological integrity of the cell can result in a deterioration in the cell's survivability capacity; this is a phenomenon observed at high doses of sulfamerazine at concentrations where CAT activities are greatly reduced. The decrease in CAT activity with sulfamerazine in our study further is supported by many studies that demonstrate a decrease in CAT activity in an oxidative stress situation (Kelly et al., 1998; Radi et al., 1985; Zigman & Rafferty, 1984). While sulfamerazine acts as an antioxidant in low-dose and short-term applications in rainbow trout, it can cause oxidative damage in high-dose and long-term applications. Fish research has demonstrated that mammalian and piscine systems exhibit similar toxicological responses to oxidative stress (Kelly et al., 1998). Hai et al. (1995) demonstrated a decrease in both reduced glutathione (GSH) content and CAT activity in carp (*Cyprinus carpio* L.) and catfish (*Ictalurus nebulosus*) after treatment with dichlorvos, an organophosphate insecticide known to induce oxidative damage. Similarly, the treatment of catfish (*Ameriurus nebulosus*) with menadione led to a decrease in GSH levels and CAT activity (Hasspieler et al., 1994).

The present study demonstrated that sulfamerazine induces alterations in the activity of CAT enzyme mediating the oxidative stress defence. Sulfamerazine stress can change biochemical data including enzyme activities and the amount of lipid peroxidation products. But these changes are also dependent on the type of sulfamerazine used, the fish species, dose and exposure time.

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