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Biochemical and Haematological Changes in Fish Exposed to Sublethal Concentrations of Linear alkylbenzene sulphonate (LAS)*)

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Summary

This study reports haematological parameters and biochemical changes in liver, kidney, and gill after exposing rainbow trout (Oncorhynchus mykiss) to linear alkylbenzene sulfonate (LAS). Rainbow trout were exposed to LAS for a 54-day period. The three treatments included a control (unexposed), a treatment group I (0.2 mg/L LAS) and treatment group II (0.4 mg/L LAS). Below stated enzyme levels were determined at the end of the study; lactate dehydrogenase (LDH), aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP). Enzyme activities (ALT, AST, ALP) in LAS exposed fish decreased in the liver and kidney. But LDH activities in LAS exposed fish increase in the gill. Although erythrocyte number was increased in the experimental groups, this is not statistically significant. Histopathologically observed, there wasn't any significant change in all organs of trial groups when compared to control group.

Keywords: rainbow trout, linear alkyl benzene sulfonate, enzymes, liver, kidney, gill

Synthetic detergents may cause ecological disturbances. Linear alkylbenzene sulfonate (LAS) is the most widely used synthetic organic chemical in the detergent industry and textile production (8, 10, 26). These pollutants cause various physical and physiological alterations in fish. Invertebrates and fish are sensitive indicators of pollutants present in the water (25). Detergents cause destruction in gill epithelium, impairment of chemoreceptor organs, and damage to epidermis and pharyngeal walls (4).

Tovel et al. (24) noticed the accumulation of higher concentrations of surfactants in gill, liver, kidney and gall bladder of fish. LAS is highly toxic for organisms living in water and even with the concentrations of 0.01-1 mg/L may produce sensitivity in microalges, invertabrates and fish (26).

Subacute studies in fish show that the gills and the locomotive muscles are the sites most vulnerable to LAS toxicity. Low levels of LAS induce behavioural changes, such as a disruption in avoidance responses and attraction. (7, 22). It is known that the primary effect of LAS in fish is directly on the gills (23). Concentrations of LAS (0.6-6.5 mg/L) exposed fish have demonstrated marked structural lesions on gills and there were some findings associated with acute toxicity (1).

However it has been reported that in lesser concentrations it works as a stress factor and causes a decrease in weight gain and behavioural abnormalities characterised by different swimming motions (10). Some enzymes (glucose-6-phosphate and lactico-, malico- and isocitrico- dehydrogenase acid and alkaline phosphatase) were highly inhibited after exposure to sublethal concentrations of the LAS (28). Roy (21) noticed that after exposing fish to detergents over a certain period of time the activities of all enzymes in opercular epidermis cells are inhibited.

Misra et al. (16) observed that fish fingerlings could also be affected by exposure to sublethal concentrations of LAS (0.005 ppm) for 24, 48, and 96 h under stable laboratory conditions. LAS may impair metabolic processes in fingerling carp, because increased exposure to LAS resulted in significant increases in lactic acid and decreases in glycogen and sialic acid along with the inhibition of acid and alkaline phosphatase activity.

The application of these biochemical assays to fish may be beneficial in the detection of organ damage and functional impairment due to environmental pol-

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lutants. It may also provide toxicologists another animal on which to evaluate the toxicity of the growing list of chemical compounds in our environment (19).

The aim of this study is to investigate the metabolic effects of prolonged sublethal LAS concentrations on rainbow trout.

Material and methods

In this study 120 rainbow trout (Oncorhynchus mykiss) with a mean weight of 40-60 g were used. The fish were obtained from Sapanca Fisheries Station of Istanbul University. The fish were weighed before the study and separated into three groups, each containing 40 fish. During the course of the study the fish were held in a 2000 L Fiberglas tank. The photoperiod was 12 h light/12 h dark and water in the tank was saturated with oxygen (11 ± 2°C). They were allowed to acclimatise for 3 weeks before the onset of the experiment and were fed daily with commercial rainbow trout pellets (2% body wt).

The fish were divided into three groups of 40 fish each and held in 500 L fibreglass tanks filled with aerated and oxygenated water. Prior to the experiment the fish were individually weighed. The fish were chosen according to their weight, so that the initial weight of fish in each group was not different. While no chemical compound was applied to the water of the first group (control), 0.2 mg/L LAS and 0.4 mg/L LAS were added to the second and third group's water for 54 days. LAS levels applied to the experimental groups were measured with the Anilin Blue method (5) and LAS levels were kept constant throughout the study. Linear alkylbenzene sulphonates (LAS C_{11-12}) were donated by Henkel Company (Henkel Co. Ltd., Istanbul, Turkey).

At the end of the experimental period, the fish were bled by caudal puncture with a heparinised syringe for haematological analysis. Organ samples of fish that were chosen randomly from each group were obtained after having been anaesthetized (with lidocaine hydrochloride) and their organs weighed. Some tissue was taken immediately and frozen in ice for measurement of biochemical parameters and pathological examinations.

Preparation of tissue homogenates. Regarding the gills, the tissue was treated for 30 s in a mixer (Braun model, Germany) before the homogenisation step, while homogenisation of liver and kidney with 12-15 passes of a teflon pestle in 1 mM EDTA and 1 mM 2-mercaptoethanol, at pH 7.0 was made and centrifuged at 3000 g for 20 min at 4°C. The resultant supernatant fluid was centrifuged at 10,000 g for 20 min at 0°C and the resultant clear supernatant fluid was frozen at –20°C or used immediately for determining the enzyme activity (18). LDH (Bio-Clinica LTLD-0640), ALT (Bio-Clinica LGP-24), AST (Bio-Clinica LGO-24), and ALP (Bio-Clinica LAL-23) were determined at a temperature of 25°C using enzyme-kits of Bio-Clinica. Co. by spectrophotometer.

Haematological analysis. Red blood cell (RBC), thrombocyte counts were taken with the hemocytometric method. Packed cell volume (PCV) is determined with microhematocrit and the haemoglobin (Hb) level with oxihaemoglobin method (11).

Pathological analysis. The samples taken from liver, kidney, spleen, heart, gill, and integumentary systems of the fish were examined. The samples of liver, kidney, spleen, heart, gill, stomach and gut were taken from all groups and fixed with a 10% formalin solution. The samples were put through routine procedures, stained with haematoxylin-eosin and examined with a light microscope.

Statistical analysis. Differences among the groups (control and treatment groups) were statistically tested with variance analysis and the averages of the groups were analysed by Duncan's test.

Results and discussion

The results of this experiment are given in table 1. Sublethal concentrations of LAS (0.2 and 0.4 mg/L) decreased enzyme activities in the liver and kidney. AST and ALP levels were significantly lower, but alterations in ALP levels were insignificant compared

Tab. 1. Liver, kidney and gills enzyme activities (U/g fresh wt) of rainbow trout after it was exposed to sublethal concentrations (0.2 mg/L and 0.4 mg/L) of linear alkylbenzene sulfonate (n = 15)

Organs	Parameters	Control Group (Unexposed) x ± SE	Treatment Group I (0.2 mg/L LAS) x ± SE	Treatment Group II (0.4 mg/L LAS) x ± SE
Liver	AST	212.30 ± 8.71^a	50.76 ± 3.66^b	40.40 ± 5.60^b
Liver	ALT	174.50 ± 20.01^a	96.11 ± 11.82^b	67.73 ± 7.72^b
Liver	ALP	29.88 ± 2.84^a	27.87 ± 3.53^a	21.56 ± 2.60^a
Kidney	AST	39.36 ± 4.76^a	21.13 ± 1.76^b	8.19 ± 3.84^c
Kidney	ALT	47.12 ± 7.81^a	38.80 ± 2.68^a	16.86 ± 1.45^b
Kidney	ALP	32.47 ± 5.24^a	22.69 ± 1.68^b	20.82 ± 2.09^b
Gill	LDH	72.36 ± 6.13^a	117.89 ± 8.84^b	125.89 ± 8.47^b

Explanations: a, b, c – means with different superscripts in the same row are significantly different ($p < 0.05$)

Tab. 2. Effects of sublethal concentrations (0.2 mg/L and 0.4 mg/L) of linear alkylbenzene sulfonate on some haematological parameters of fish (n = 15)

Parameters	Control Group (Unexposed) x ± SE	Treatment Group I (0.2 mg/L LAS) x ± SE	Treatment Group II (0.4 mg/L LAS) x ± SE
RBC(× 10^4/mm^3)	7.40 ± 1.32^a	6.78 ± 1.73^a	7.56 ± 2.16^a
PCV (%)	28.82 ± 4.60^a	27.26 ± 7.17^a	27.37 ± 6.98^a
Hb (g/dl)	3.56 ± 0.77^a	3.51 ± 1.06^a	3.62 ± 0.94^a
Thrombocytes (× 10^3mm^3)	5.84 ± 3.90^a	5.64 ± 3.95^a	6.65 ± 5.34^a

Explanations: a, b, c – means with different superscripts in the same row are significantly different ($p < 0.05$)

to the control group. Activities of LDH in the gill were significantly higher in experimental groups compare to control group. As it can be observed in table 2, there weren't statistically significant differences between the control and treatment groups concerning the number of erythrocytes, thrombocyte, haematocrit and haemoglobin. When histopathological findings were evaluated, no significant changes between the experimental and the control groups have been determined.

Synthetic detergents cause various physical, haematological and biochemical alterations in fish. These detergents may impair metabolic processes in fingerling carp (16). Effects of surfactants are generally attributed to their ability to react directly with proteins leading to enzyme inhibition (8).

Gupta et al. (8) suggested that in vitro treatment of LAS probably indicates that even lower concentrations of surfactants cause cellular damage and inhibit enzyme activities. In the present study rainbow trout were exposed to two different sublethal doses (0.2 mg/L, 0.4 mg/L) in order to determine the effect of LAS on some biochemical parameters. Alterations were noted in the levels of ALT, AST, ALP in the liver and kidney as well as LDH in gills. The liver was chosen as the primary organ to focus upon because of its importance in intermediary metabolism and possible significance in the biotransformation of exogenous compounds (19). Determinations of transaminases AST and ALT have proved useful in the diagnosis of liver and kidney diseases in fish (13). Fish receiving LAS in their water at 0.2 and 0.4 mg/L doses had decreased ALT and AST activity in the liver compared to the control group. The decreases of liver transaminases levels were statistically significant ($p < 0.05$). Liver ALP activity showed a decrease in both the 0.2 and 0.4 mg/L groups compared to the control but this was not significant. The decreases of enzyme activities could have been due to the toxic effect of LAS on the liver, causing enzyme inhibition.

Favilli et al. (6) observed the four dehydrogenase are highly inhibited by synthetic detergents. Misra et al. (16) also stated that the impairment in the activities of ALT, AST, and, ALP could be part of an overall biochemical manifestation of toxicity. The decreases in the activities of ALT and AST indicate disruptions of linkage between carbohydrate and protein metabolisms providing a source of keto acids for the Krebs cycle and gluconeogenesis (8).

ALT, AST, and, ALP activities in kidneys, likewise the enzyme activities in the liver, demonstrated an important decrease in groups receiving 0.2 and 0.4 mg/L compared to the control.

Misra et al. (16) discovered a decreased level of acid and alkaline phosphate activities. They noticed significant alterations in ALP activities after exposure to LAS. This result proves that sublethal concentrations of a detergent may cause metabolic disturbances in fish fingerlings. Inhibition of ALP reflects alterations in protein synthesis and uncoupling of oxidative phosphorylation (27). Gupta et al. (8) reported that all the cationic detergents are active on ALP. Ecotoxicants can affect mucus and goblet cells on the gill surface (3). Sublethal concentrations of detergents damage the gill epithelium of fish by changing the lipid composition of the tissue and affecting mucus production (2, 15, 21, 23). LDH has long been used to demonstrate tissue damage in fish for a long time (17).

In this study LDH activity of the gills of rainbow trout exposed to two different sublethal doses (0.2 mg/L and 0.4 mg/L) were increased ($p < 0.05$) when compared with the control group. This indicates that LAS has a high potential to interfere with aerobic mechanisms (29). Sublethal effects of LAS include pathological changes of gills, decreased growth, hypertrophy, hyperplasia and impaired swimming activity (12). Important factors influencing the toxicity of detergents consist of their molecular configuration, the water hardness, the dissolved oxygen concentration, the age and species of the affected organism (8). It has been stated that increased motility of fishes results in an increase of the metabolism, cause some changes in the enzymatic reactions, and thus leads to a greater energy expedinture. It has been reported that the accelerated metabolism leads to an increase in lactic acid levels by means of increased glycogenolysis and increased LDH activity (8). Mazeaud et al. (14) have reported an increase in plasma corticosteroids and catecholamines in the circulation due to physiological stress in fish. It has likewise been stated that this increased energy request could be related with the enzymatic system in the metabolism. This might indicate that the enzyme production mechanisms in the cells are disturbed (29). However, it is known that detergents alter enzyme proteins and membrane permeability. Sublethal doses of LAS undoubtedly promote increased levels of LDH in the epidermis of gills. This indicates that the integrity of the aerobic processes is diminished and a compensatory increase in anaerobic oxidation takes place.

The presence of detergent in the water increases the respiration rate of fishes and especially expands muscle mass and contraction at the buccal and opecular areas. The increased opecular gill motility of fishes clearly indicates the decrease of dissolved O_2 in the water and physiological stress. Toxicants attack gill epithelia and impede the diffusion of oxygen and can affect the osmoregulatory function of gills. Swimming capacity was significantly reduced when exposed to LAS (10). It was determined that LAS added at 0.4 mg/L doses to fish's water caused statistically non-significant increases on erythrocyte number in the second treatment group compared to the control. We explain this increase of erythrocyte parameters by the decrease of O_2 level of the water and the effects of the hypoxia on fish. As a matter of fact it is known that hypoxia has a direct effect on the control of erythropoiesis and the increase of erythrocyte production (9). The thrombo-

cytes in white perch were the first white blood cells to show a significant relationship to water quality (20). In this study, the thrombocytes tend to increase in rainbow trout, which were exposed to 0,4 mg/L LAS compared to other groups, control and trial. Although LAS causes biochemical changes, in liver and kidney tissues, in this study there weren't significant histopathological changes in the organs observed. In fish exposed to the lowest concentration of detergent, the histopathological changes are not detectable thus denoting that a correlation of the enzyme patterns with the degree of aerobic metabolism in the epithelial cells cannot be established (30).

In conclusion, two different sublethal doses of LAS application did not cause any pathological changes. But the LAS application affected the liver and kidney tissues by decreasing the enzyme activity and it also affected the gills by increasing LDH activity.

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AMASS S. F., PACHECO J. M., MASON P. W., SCHNEIDER J. L., ALVAREZ R. M., CLARK L. K., RAGLAND D.: Procedury hamujące transmisję wirusa pryszczycy do świń i owiec przez personel kontaktujący się z zakażonymi świniami. (Procedures for preventing the transmission of foot-and--mouth disease virus to pigs and sheep by personnel in contact with infected pigs). Vet. Rec. 153, 137--140, 2003 (5)

Wirus pryszczycy rozprzestrzenia się głównie drogą aerozolową i przez kontakt bezpośredni ze zwierzętami zakażonymi. Zakażone świnie wydalają wirus do 10. dnia po zakażeniu. Wirus pryszczycy przeżywa w kale 103 dni, w gnojowicy bydła przez 70 dni w 17°C i 84 dni w 4°C. Najskuteczniejszym sposobem likwidacji pryszczycy jest eliminowanie z hodowli chorych zwierząt. Jednakże człowiek musi zdiagnozować chorobę i podjąć działania uniemożliwiające kontakt zwierząt wrażliwych ze zwierzętami chorymi i zanieczyszczonymi przez wirus pomieszczeniami. Celem zbadania skuteczności procedur bioasekuracyjnych w zapobieganiu transmisji wirusa pryszczycy, szczep 0/0K/35/2001, badacze przez 45 min. badali zakażone świnie, a następnie kontaktowali się z tymi zwierzętami, w stosunku do których albo nie stosowano procedur bioasekuracyjnych, albo je stosowano (mycie rąk, czysta odzież ochronna; prysznic i czysta odzież ochronna). Wirus pryszczycy zidentyfikowano w wydzielinie jamy nosowej jednej osoby bezpośrednio po sekcji zakażonej świni. Nie występował on w próbkach pobranych po 12 i 48 godz. Personel po kąpieli pod prysznicem i zmianie odzieży ochronnej nie przenosił zakażenia.

G.