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Surfactant-induced lipid peroxidation in a tropical euryhaline teleost *Oreochromis mossambicus* (Tilapia) adapted to fresh water

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Exposure to anionic (sodium dodecyl sulfate , SDS), cationic (cetyl trimethyl ammonium bromide CTAB) and non ionic (Triton X-100) surfactants at a sub lethal concentration of 1 ppm resulted in severe oxidative stress in the hepatic, renal and cardiac tissues of fresh water adapted Oreochromis mossambicus . Hepatic catalase showed significant increase ($P<0.001$) in all the surfactant exposed fish, but the renal enzyme was significantly increased only in CTAB dosed fish ($P<0.001$) and the cardiac enzyme showed significant increase in Triton ($P<0.05$) and CTAB dosed fish ($P<0.001$). SOD levels were significantly increased ($P<0.001$) in hepatic, renal and cardiac tissues of all the surfactant-treated fish. Glutathione reductase also was significantly increased ($P<0.001$) in the hepatic and renal tissues of surfactant dosed fish except cardiac tissues of CTAB exposed animals. Glutathione levels in the tissues studied were significantly higher in the surfactant treated animals ($P<0.001$) whereas malondialdehyde levels were significantly elevated only in the hepatic tissues of animals exposed to Triton ($P<0.001$) . The surfactants based on their charge, antioxidant profile and in vivo metabolism may be arranged in the order of decreasing toxicity as CTAB > Triton > SDS. Thus it may be inferred from the present study that the antioxidant defenses and the in vivo metabolism of the surfactants are key factors in deciding the surfactant toxicity.

Lipids are a major constituent of membranes comprising 20-80% of the membrane mass. In addition to the fundamental role of providing compartmentation, the cell membrane lipids are involved in the responses of cells to a number of external stimuli like hormones, growth factors and neurotransmitters[1]. Deleterious effects of various chemicals on the membranes are known[2]. An often neglected group is that of the surfactants which constitute an important component of detergents, pesticides, herbicides, shampoos, cleaners and other products of day to day use. Available toxicity data for surfactants largely comprise works relating to mortality, larval development and reproductive capacity[3-8]. But reports are almost lacking on deleterious effects of surfactants on cell membrane specially with reference to peroxidation.

Lipid peroxidation is oxidative destruction of poly unsaturated fatty acids (PUFA) in the cell membrane. The cells have built-in antioxidant systems to check this deleterious process. These include enzymes like catalase, superoxide dismutase, glutathione reductase etc as well as non enzymatic molecules like glutathione, vitamin E, carotene etc[9]. Catalase, a porphyrin containing enzyme , destroys hydrogen peroxide by catalyzing its two electron dismutation to water and oxygen. Superoxide dismutase catalyses the two electron dismutation of superoxide radical to hydrogen peroxide and oxygen. Glutathione reductase catalyses the reduction of oxidized glutathione at the expense of NADPH to its reduced form. The reduced glutathione because of its sulfhydryl group can serve as a proton donor to the free radicals. Also it is the coenzyme of glutathione peroxidase. The attack of free radicals on PUFA produces intermediates like conjugated dienes and finally the cytotoxic aldehyde malondialdehyde. It can directly interact with DNA causing alkylation of bases, can induce inter/inta strand scissions and contribute to mutations.

The present work focuses on the peroxidative effects of three surfactants viz anionic (sodium dodecyl sulfate/SDS), cationic (cetyl trimethyl ammonium bromide/CTAB) and non ionic (Triton X-100) on liver, kidney and heart of the euryhaline teleost *Oreochromis mossambicus* (Tilapia) adapted to freshwater. These surfactants are commonly used in a large number of cleaning agents, pesticides, herbicides etc. Their concentrations in the natural environment like river water and sediments range between 1-10 ppm for sodium alkyl sulfates, 0.01-2.6 ppm for non ionics and 5-50 µg /L for cationics[10-12]. Hence the surfactants selected in the present study represent simple compounds belonging to these 3 groups, which are an often neglected group of aquatic pollutants.

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Materials and Methods

Fish species weighing 15±3 gm and 8.5±0.5 cm long were collected from Rice Research Institute, Vyttila. They were fed on a commercial diet *ad libitum* and were acclimated in aquarium tanks for a month before the experiment. Six fish each were maintained in a sub lethal surfactant concentration of 1 ppm (1/10 of 96 hr LC50) in aerated fibre glass tanks and a control group was also maintained without any surfactant. The surfactant concentrations were prepared by dissolving the respective surfactants in tap water and diluted to obtain the required concentration of 1 ppm (APHA)[13].

The tap water used had dissolved oxygen content of 7-8 ppm ,hardness-below detectable limits, *p*H 7, temperature 25°±3°C and salinity 0 ppt. During experimental period of 30 days the animals were fed on the same diet so as to avoid the effects of starvation on normal physiological processes and antioxidant status. The water in experimental tanks was replaced every 48 hr with water containing fresh surfactant so as to avoid any possible degradation of the surfactant. Fishes were deprived of food 24 hr before assay. They were killed by pithing (by damaging the brain and severing the spinal cord between the head and the trunk region using a sharp needle) and the tissues viz liver, kidney and heart were removed, washed in ice-cold sucrose (0.33M), blotted dry and weighed.

The marker enzymes in lipid peroxidation-catalase (CAT), superoxide dismutase (SOD) and glutathione reductase (GR) were assayed. Also the levels of the antioxidant glutathione (GSH) and malondialdehyde (MDA) were estimated.

Catalase was assayed by the method of Maehly and Chance [14]. The enzyme extract was prepared by homogenizing the tissue in the phosphate buffer and centrifuging at 5000 rpm. Specific activity was expressed as international units/mg protein, 1IU=Change in absorbance/min/extinction coefficient (0.021). Superoxide dismutase (SOD) was assayed by the method of Kakkar *et.al*[15]. Glutathione reductase (GR) was assayed by the method of Bergmeyer[16] . Enzyme activity was expressed as units/mg protein. One unit was defined as the change in absorbance/minute. Reduced Glutathione(GSH) was assayed by the method of Patterson & Lazarow[17]. The tissue extract for glutathione estimation was prepared in phosphate buffer *p*H 7.5. Malondialdehyde was assayed by the method of Niehaus and Samuelsson [18]. Protein was estimated by the method of Lowry *et.al*[19].

Statistical analysis— Testing of statistical differences between test and control groups were done using one way analysis of variance (ANOVA). Multiple comparison test (Tukeys test) was done to check whether the surfactants differed significantly from the control as well as amongst themselves[20].

Results and Discussion

The results are presented in Table 1.

ANOVA showed that there were significant differences between control and surfactant treated groups with respect to all the parameters tested. Subsequent comparisons were made between the individual test groups by a multiple comparison test (Tukeys test). Control group was compared with each of the three surfactant groups, also the surfactant treated groups were compared between themselves.

Hepatic catalase activity showed an overall significant change (F=521.7, $P<0.001$) as obtained by ANOVA. The hepatic catalase activity was significantly increased ($P<0.001$) in all the surfactant treated groups when compared with the control. Comparison between the surfactants revealed that SDS and Triton induced similar changes . However CTAB mediated effects were significantly different when compared to the effects of SDS and Triton. The renal enzyme also showed an overall significant change (F= 40.01, $P<0.001$, by ANOVA) . However multiple comparison revealed that significant increase ($P<0.001$) was noted only in animals exposed to CTAB when compared with the control. Here too animals exposed to triton and SDS showed similar enzyme activities which were not significantly different from one another. Cardiac enzyme also showed an overall significant change (F=21.96, $P<0.001$, by ANOVA). The enzyme was significantly ($P<0.001$) elevated in animals exposed to Triton and CTAB. A comparison between the effects of these surfactants was found to be significantly ($P<0.001$) different from one another.

ANOVA showed that there was an overall significant change in superoxide dismutase activity in hepatic (F=3309.88, $P<0.001$), renal (F=16228.77, $P<0.001$) and cardiac tissues (F=2571.14, $P<0.001$) of all the surfactant treated groups when compared to control ($P<0.001$). Comparison between the surfactants revealed that hepatic and cardiac enzyme activities were significantly different ($P<0.001$) in all the surfactant treated groups. The renal enzyme in fish exposed to either CTAB or SDS did not show significant differences in enzyme activity when

Table 1 — Effects of sodium dodecyl sulfate (SDS), Triton X-100 and cetyl trimethyl ammonium bromide (CTAB) on hepatic, renal and cardiac enzymes

[Values are mean ± SD from fish in each group]

Tissue	Enzyme	Control	SDS	Triton	CTAB
Hepatic	Catalase#	12.1 ± 1.25	$32.01 \pm 1.52^{*c}$	$35.28 \pm 3.5^{*b}$	$78.31 \pm 5.8^{*bc}$
	SOD**	12.51 ± 0.31	$16.6 \pm 0.26^{*ac}$	$14.1 \pm 0.28^{*ab}$	$26.6 \pm 0.4^{*bc}$
	GR+	2.4 ± 0.13	$6.8 \pm 0.17^{*ac}$	$4.4 \pm 0.19^{*a}$	$4.1 \pm 0.2^{*c}$
	GSH++	1256 ± 48.6	$2534 \pm 50.2^{*ac}$	$1920 \pm 65.7^{*ab}$	$2110 \pm 68.6^{*bc}$
	MDA$	0.063 ± 0.02	0.097 ± 0.03^{a}	$0.295 \pm 0.02^{*a}$	0.08 ± 0.03^{b}
Renal	Catalase#	5.29 ± 1.85	4.8 ± 1.7^{c}	8.3 ± 2.3^{b}	$17.35 \pm 5.5^{*bc}$
	SOD**	12.11 ± 0.15	$24.2 \pm 0.12^{*a}$	$13.9 \pm 0.18^{*ab}$	$24.3 \pm 0.3^{*b}$
	GR+	4.1 ± 0.11	$1.9 \pm 0.14^{*}$	$6.5 \pm 0.15^{*}$	$2.2 \pm 0.18^{*}$
	GSH++	1302 ± 52.5	$3706 \pm 63.8^{*}$	$2280 \pm 59.7^{*}$	$3040 \pm 63.6^{*}$
	MDA$	0.008 ± 0.01	0.004 ± 0.02	0.021 ± 0.03	0.006 ± 0.03
Cardiac	Catalase#	10.38 ± 2.5	9.74 ± 3.12^{ac}	$13.8 \pm 2.95^{*ab}$	$17.5 \pm 3.2^{*bc}$
	SOD**	15.38 ± 0.41	$23.7 \pm 0.53^{*ac}$	$20.02 \pm 0.36^{*ab}$	$27.9 \pm 0.29^{*bc}$
	GR+	2.3 ± 0.2	$5.2 \pm 0.21^{*ac}$	$3.5 \pm 0.18^{*a}$	2.4 ± 0.61
	GSH++	750 ± 50.2	$1650 \pm 52.9^{*ac}$	$1000 \pm 48^{*ab}$	$1580 \pm 60.5^{*bc}$
	MDA$	0.009 ± 0.03	0.008 ± 0.04	0.021 ± 0.04	0.0045 ± 0.03

one IU=Change in absorbance at 230 nm/min, expressed /mg protein

** units/mg protein

+ X 10^{-3} units/mg protein

++ nmoles/100 g wet tissue

$ mmol/100 g tissue

control compared with SDS, Triton and CTAB is represented as "*" if significant at $P<0.001$, significant differences at $P<0.001$ between SDS and triton, triton and CTAB and that between SDS and CTAB are represented as" a","b" and "c" respectively. No symbol- not significant

[ANOVA followed by Tukeys test]

compared with each other, but there were significant increases ($P<0.001$) in the enzyme activity in animals exposed to triton when compared with SDS and CTAB dosed groups.

Glutathione reductase also showed an overall significant change in hepatic (F=18.99, $P<0.001$), renal (F=86.24, $P<0.001$) and cardiac (F=36.58, $P<0.001$) tissues. The enzyme activity was significantly increased ($P<0.001$) in the hepatic, renal and cardiac tissues (except cardiac tissues of CTAB) of surfactant treated animals when compared to control. The effects of triton and CTAB on the enzyme levels in hepatic and cardiac tissues were not significantly different from one another whereas SDS mediated effects were significantly ($P<0.001$) different from that of triton and CTAB ($P<0.001$). The renal enzyme was influenced alike by all the surfactants.

There was an overall significant change in reduced glutathione content in hepatic (F=32.78, $P<0.001$), renal (F=4626.89, $P<0.001$) and cardiac (F=1022.52, $P<0.001$) tissue by ANOVA. There were significant increases ($P<0.001$) in hepatic, renal and cardiac tissue levels of reduced glutathione in all the surfactant treated groups when compared with the control group. SDS dosed fish had the highest and the triton dosed fish had the lowest hepatic glutathione content. The renal and cardiac glutathione content in all the three surfactant treated animals were significantly different ($P<0.001$). But there were no significant differences between the hepatic glutathione levels in animals exposed to triton and CTAB.

ANOVA showed an overall significant change in the hepatic malondialdehyde levels (F=310.48, $P<0.001$). However group comparisons by Tukeys test revealed that malondialdehyde levels were significantly different ($P<0.001$) from the control group only in hepatic tissues of animals exposed to triton. The animals exposed to SDS and CTAB had malondialdehyde levels comparable to that of control. Also there were no significant differences between SDS and CTAB with respect to the hepatic levels of malondialdehyde.

Toxicity of oxygen is due to the production of oxygen derived free- radicals, the most common ones being superoxide (O_2^-), hydroxyl free radical (OH^-) and the singlet oxygen. Under normal conditions also free radicals are produced during several physiological processes. During mitochondrial respiration 1-5% free radicals[21] are produced and

immune response by activated phagocytes[22] also produces free radicals. These normal levels of free radicals are scavenged by the normal amounts of antioxidant enzymes . But a substantial increase in the levels of these highly reactive radicals occurs when the animal is subjected to stress conditions like environmental chemicals/pollutants[23]. This is reflected in increased production of the antioxidant enzymes.

The antioxidant profile in surfactant-dosed fishes revealed significant increase in the levels of catalase, superoxide dismutase and glutathione. The antioxidant enzymes viz catalase and superoxide dismutase showed the highest increase on exposure to CTAB . Also the glutathione content and glutathione reductase were significantly increased, but malondialdehyde levels were not significantly high. This could be due to the increase in glutathione which can prevent formation of MDA[24]. Cationic surfactant interacts with the cell membrane in two possible ways –hydrophobic interactions with the hydrophobic residues and hydrophilic interactions with ionic groups of membrane proteins and lipids[25] . The negative charge on the phospholipids might also have resulted in enhanced interactions. Also the surfactant is highly polar and it is thought that the fish species does not metabolise it[26].

Triton X-100 was found to resemble CTAB in its toxic effects but differs in that it is non ionic and also subjected to metabolism. The levels of catalase and superoxide dismutase were lower than in CTAB group, but MDA was significantly higher. It was also observed that the glutathione content was the lowest in the Triton dosed group. The increased MDA in this group may be due to the decreased glutathione content. This depletion of GSH may be due to its increased conversion to oxidised glutathione (GSSG) by the enzyme glutathione peroxidase or/and utilization for conjugation reactions by the hepatic biotransformation enzyme-glutathione-S-transferase (GST) . Also studies have shown that nonionics like alkyl phenol ethoxylates are metabolized by the fish species *in vivo* to 4-nonyl phenols which are excreted as glucuronide conjugates and hydroxylates[27]. Gadagbui *et.al*[28] also support the view that tilapia is more likely to excrete xenobiotics as glutathione conjugates or mercapturic acids because of its high GST activity. Thus increased GSH utilization and comparatively lower levels of catalase and superoxide dismutase could account for increased oxidative stress and increased MDA in this group. Being nonionic Triton X -100 is capable of hydrophobic interactions with the cell membrane through its long alkyl chain and also hydrophilic interactions through its ethylene oxide chain.

SDS, the anionic surfactant, is a short chain alkyl sulfate . The levels of catalase and superoxide dismutase were comparatively lower in this group than in the other two surfactant-treated groups. But the levels of glutathione reductase and GSH were significantly increased in all the tissues studied. The levels of malondialdehyde were comparable to that of control. These factors together imply that SDS-induced stress in these fishes may be overcome to a large extent by an increased production of the chain-breaking antioxidant GSH as well as increases in the levels of catalase, superoxide dismutase and glutathione reductase. Being anionic, SDS interactions with the cell membrane are limited to the cationic sites on the cell membrane lipids and proteins. Also negative charge of the SDS may repel similarly charged phospholipids . The surfactant may also be metabolized to some extent by beta or omega oxidation in the hepatic tissues and excreted as carboxylic acid derivatives[26].

Thus it may be inferred from the statistical data analysis that the extent of peroxidative damage induced is in the order CTAB>TRITON>SDS. Being cationic CTAB has more affinity for the negatively charged membrane lipids, and also is not metabolized. Hence it is regarded as the most toxic. Triton X-100 is metabolized and excreted as glutathione conjugates. This results in increased peroxidation and more malondialdehyde due to GSH depletion and is the second toxic compound. SDS is subjected to beta/omega oxidation and also SDS induces increased GSH which probably helps the animals to overcome the stress to a large extent.

Thus, from the present study it is inferred that the exposure to surfactants is stressful. The increases in the levels of malondialdehyde coupled with the increased production of catalase, superoxide dismutase, glutathione reductase and glutathione reflect the cell membrane-directed toxicity of the surfactants used and essentially serve as biomonitors of surfactant -induced oxidative stress.

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