



## Inhibition of Acetylcholinesterase activities by detergents in the Nervous system of *Mystus Montanus*

Navnath Eknath Chandanshive

Fergusson College, Pune, Maharashtra, INDIA

Available online at: [www.isca.in](http://www.isca.in), [www.isca.me](http://www.isca.me)

Received 2<sup>nd</sup> May 2015, revised 10<sup>th</sup> June 2015, accepted 15<sup>th</sup> July 2015

### Abstract

*The Acetylcholinesterase is a membrane bound enzyme present in nervous system, muscle and blood. It plays important role as a neurotransmitter and neuromodulator in central and peripheral nervous system respectively. A toxic substance inhibits Acetylcholinesterase activities in animals. When freshwater fish *Mystus montanus* is exposed to different concentrations of detergents, it shows change in normal behaviour pattern as compare with control. It indicates toxicity of detergents to the fish. Acetylcholinesterase activity is correlated with amount of protein content in nervous tissues; it shows significantly decreased with an increase in concentration and period of exposure. It shows that toxicity increases with an increase in concentrations of detergents and period of exposure. Whereas Acetylcholinesterase activity is decreases; this may be due to either decrease in the synthesis of proteolytic enzyme or increased enzyme activity due to induced or intermolecular changes of the enzyme degradation by detergent stress.*

**Keyword:** Detergent, acetylcholinesterase.

### Introduction

The enzyme Acetyl cholinesterase (AChE) is found in different parts of body such as brain, nerve cells, muscles, lungs and erythrocytes. AChE plays a substantial part during the nerve impulses transmission for example a *neurotransmitter* in central and autonomic nervous system and neuromodulator in peripheral nervous system. It is a membrane bound enzyme present on the postsynaptic membrane cholinergic neurons. It has very high binding receptors. When it is present in the peripheral nervous system, it activates the muscles, whereas in the autonomic nervous system act as a major neurotransmitter.

This acetylcholine is inhibited by the stimulation of many deactivating agents; it causes gathering of acetylcholine. The neurotransmission is interrupted by excess stimulation of nicotinic and muscarinic receptors. Therefore, Acetyl cholinesterase deactivators are interacted with the enzyme as a primary target; these are applied as significant drugs and contaminants. The breaking down of acetylcholine is prohibited by a substance called as Acetylcholinesterase inhibitor (AChEI). That stops the Acetylcholinesterase enzyme from contravention of acetylcholine; it increases the level and duration of action of the acetylcholine as a neurotransmitter. AChEI are classified into reversible, irreversible, or quasi-irreversible. The Alzheimer's disease is associated with memory deficits in the brain is due to damage to the cholinergic (acetylcholine-producing) system. The primary cause of depression is an acetylcholine disorder. However, the effect of detergents AChE activity was assessed in brain of the exposed fish.

The acute toxicity of two detergents to fish was determined by the Litchfield and Wilcoxon graphical method. The LC<sub>50</sub> for Det-I 20.0 mg/litre and Det-II 23.5mg/litre. In test solution freshwater fish *Mystus montanus* shows behavioural reactions such as surfacing with gulping of atmospheric air, increase in opercula movement, change in body colour, increased mucus secretion, lethargic movements. A number of behavioural changes have been showed by fish before death like agitation, quick swimming and loss of equilibrium, respiratory pain and bleeding from gill epithelium. These symptoms indicates the disorders of brain and spinal cord, demonstrates the sign of toxicity caused by detergent pollution<sup>1</sup>.

The detergents are widely used in various products and causes physiological disturbance in fish. These disturb the nervous system by deactivating acetyl cholinesterase (AChE), the enzyme that moderated the amount of the neurotransmitter, acetylcholine. The detergents are widely used insecticide is known to cause serious metabolic disturbance in non-target species, like fish. These detergents disturb the nervous system by obstructing Acetyl cholinesterase (AChE)<sup>2</sup>.

The actions of Acetyl cholinesterase and Ca<sup>2+</sup> +, Mg<sup>2+</sup> ATPase and treatment of human erythrocyte membranes with non-solubilizing and solubilizing concentrations of Triton X-100 were studied. Even very low concentration (0.1%) of Triton X-100 shows a significant deactivation of enzymes<sup>3</sup>. A combine action of contaminants on Acetyl cholinesterase activity in several marine species was studied. It has been observed that the

strongest synergy in mixture of various pollutants and inhibit the AChE activity. Some marine species were found more sensitive<sup>4</sup>. It has been reported that Acetyl cholinesterase activity is reduced in the brain of fish *Martes pennant* with environmental contaminations<sup>5</sup>.

The effect of Dodecyl Benzyl Sulphonate (DBS) and Sodium Dodecyl Sulfate (SDS), as well as domestic detergent was studied on the AChE activity of *Daphnia magna* in conditions like vitro and vivo<sup>6</sup>. The interaction of AChE in brain with organophosphate Paraoxon and industrial detergent triton X-100 was explained<sup>7</sup>. When fish exposed to two concentrations i.e. 0.5 and /or 1g/L of SDS, the activity of AChE, GST and CAT was inhibited<sup>8</sup>.

## Material and Method

The freshwater fish *Mystus montanus* were collected from unpolluted area of river Mula upstream of Mahlung. For the experiment moderate sized fishes were selected length varies from 12.3cm to 14.5cm and weight from 18.72gm to 23.86gm. Before conducting toxicity test, these fishes were acclimatised to laboratory condition in a glass tank for seven days as per APHA<sup>9</sup>. Static renewal bioassay tests were conducted in order to evaluate the acute toxicity of Surf excel and Nirma powder.

The 96 hours LC<sub>50</sub> (concentration required for 50 % mortality) values were estimated by the Litchfield and Wilcoxon graphical method<sup>10</sup> and percentage for mortality was calculated using the Abbott's formula<sup>11</sup>. Control group of animals was maintained simultaneously.

Corrected mortality (%) =

$$\frac{\text{Percentage living in control} - \text{Percentage living in treatment}}{\text{Percentage living in control}} \times 100$$

As per graphical method and plots Abbott's formula, the LC<sub>50</sub> values of Surf excel and Nirma for 96 hours were 20.0 mg/litre and 23.5 mg/litre respectively. These fishes are exposed to two different sub-lethal concentrations (33% and 66% of LC<sub>50</sub> values) of detergents i.e. Surf excel and Nirma as per recommendations for periods of 24, 48, 72 and 96 hours<sup>12</sup>. Simultaneously, 10 fishes were kept in a glass container for the same duration as a controlled group<sup>13</sup>. The exposed fishes were scarified for 24, 48, 72 and 96 hours.

After each exposure period Acetylcholinesterase activity was estimated by (Acetyl choline hydrolase EC 3.1.1.7) method given by Wolfgang Pilz<sup>14</sup> and protein is estimated by using Biuret reagent method. Simultaneously, acetyl cholinesterase activity and protein also estimated from the controlled fishes. Three replications have been taken for calculating the mean of protein and acetyl-cholinesterase activity.

Acetyl cholinesterase activity is calculated by formula:

$$\text{AChE Activity} = \frac{\Delta E \times 50 \times 100}{0.961 \times W} = \text{-----} (\mu\text{mole/gram of tissue})$$

ΔE= Difference between Reference and Test reading, 50= volume of test sample solution, 1000= for converting mg into gram, 0.961= for measurement at 490 nm the extinction of the dye is 0.961 μmole. W= wt. of brain tissue taken for enzyme assay.

## Results and Discussion

The Acetylcholinesterase activity in fishes exposed to different sublethal (experimental) concentrations of detergents shows following results.

**Surf excel:** Shows minor decrease in acetyl-cholinesterase activity by 1/3<sup>rd</sup> sublethal concentration i.e. 6.67mg/lit at 24 hrs and 72hrs, whereas at 48hrs and 72 activity was significantly decreased. However with 2/3<sup>rd</sup> lethal concentration i.e. 13.3mg/lit insignificant decrease was observed at 24hrs, then AchE activity decreased significantly.

**Nirma powder:** Exposure of fishes to 1/3<sup>rd</sup> sublethal concentration shows slight reduction upto 72hrs and significant decrease with 96hrs. Though with 2/3<sup>rd</sup> sublethal concentration i.e. 15.67mg/lit demonstrates insignificant decrease upto 48hrs and thereafter significant decrease was observed.

The Acetyl cholinesterase activity is decreased with an increase in concentration and time of exposure with both detergents. It indicates that toxicity increased with rise in concentrations of detergents and period of exposure and is correlated with amount of protein. Acetyl cholinesterase activity decreased may be due to either decrease in the synthesis of proteolytic enzyme or increased enzyme activity due to induced or intermolecular changes of the enzyme degradation by detergent stress. The circulation of residual quantity inhibitor (toxins) in fish treated with detergents may show decrease in Acetyl cholinesterase activity. Our results and findings are in total agreement with the results and findings of many workers.

The detergents are widely used in insecticides and it causes severe physiological disorders in aquatic animals like fish. These detergents disturb the nervous control by preventing the activity of Acetyl cholinesterase (AChE); it restricted the amount of the substance that controls the neuron transmission acetylcholine. It is necessary to understand the influence of pesticides on physiological condition of the organism during impact of toxicity<sup>2</sup>. The studies were conducted on action of mixture of various pollutants on Acetyl cholinesterase activity in several marine species. During this study it is found that the AChE activity was inhibited by strongest interaction in mixture

of various pollutants. Several oceanic species were found more sensitive<sup>4</sup>. The action of Acetyl cholinesterase is decreased with various environmental contaminants in the brain of fish (*Marte spennanti*)<sup>5</sup>.

In crustacean cladoceran *Daphnia magna* effects of two surfactants, namely Dodecyl Benzyl Sulfonate, Sodium Dodecyl Sulphate and a domestic detergent-Y on the Acetylcholinesterase activity is studied. It is found that enzyme activity is significantly suppressed by all chemicals in vitro and vivo conditions. It has been observed that in vitro condition the lowest observed effect concentration (LOEC) values were ranged from 12.5 to 100 mgrl. The correspondent IC<sub>50</sub> -50% inhibition concentration, values ranged from 6.6 to 58.5 mgrl. Although, in vivo LOEC values ranged from 2 to 11.9 mgrl, while EC<sub>50</sub> -50% effect concentration, the values ranged from 11.4 to 56.7 mgrl. Acetyl cholinesterase activity is inhibited by environmental pollutants such as metals, detergents and surfactants<sup>6</sup>. In the brain of Rat Acetylcholinesterase activity was negligible in the presence of 1% triton X-100. This is found that triton X-100 unusually affects the interaction of paraxons and AChE in brain of Rat<sup>7</sup>. The effects antioxidant defense system and Acetyl cholinesterase activity of *Tilapia nilotica* by Trichloroforn and Sodium Dodecyl Sulphate is studied. It is found that in vitro exposure of SDS can hinder activities of GST, CAT and AChE at concentrations of 0.5g/L and 1g/L, it may be due to the denaturation of enzyme by SDS<sup>8</sup>.

Acetyl cholinesterase activity and content was decreased with acidic pollutants. This is suggested that the behavioural symptoms are indicative of disorders of central nervous system that clearly shows the signs of poisoning caused by acid water pollution. Due to the polluted water the pH of the media is altered that causes inhibition of Acetyl cholinesterase activity. Decrease in Acetyl cholinesterase could be due to either decrease in synthesis of enzyme protein or increased enzyme induced or intermolecular changes of enzyme degradation by the acidic stress of the medium. The acetylcholine content in the brain showed elevated levels during exposure to lethal p<sup>H</sup>'s with maximum elevation at the p<sup>H</sup> of 3.4 where the fish survived for 15 hours indicating the block of nervous transmission. They concluded that the intensity of acids in the media seems to have direct relationship with the survival of fishes through brain Acetyl cholinesterase activity. They suggested that the active site of enzyme are known to possess an anionic site, which draws the positive charge of acetylcholine to form anionic bond with the acidic group, and thereby forming the enzyme-substrate complex leading to the expression of enzyme activity<sup>15</sup>.

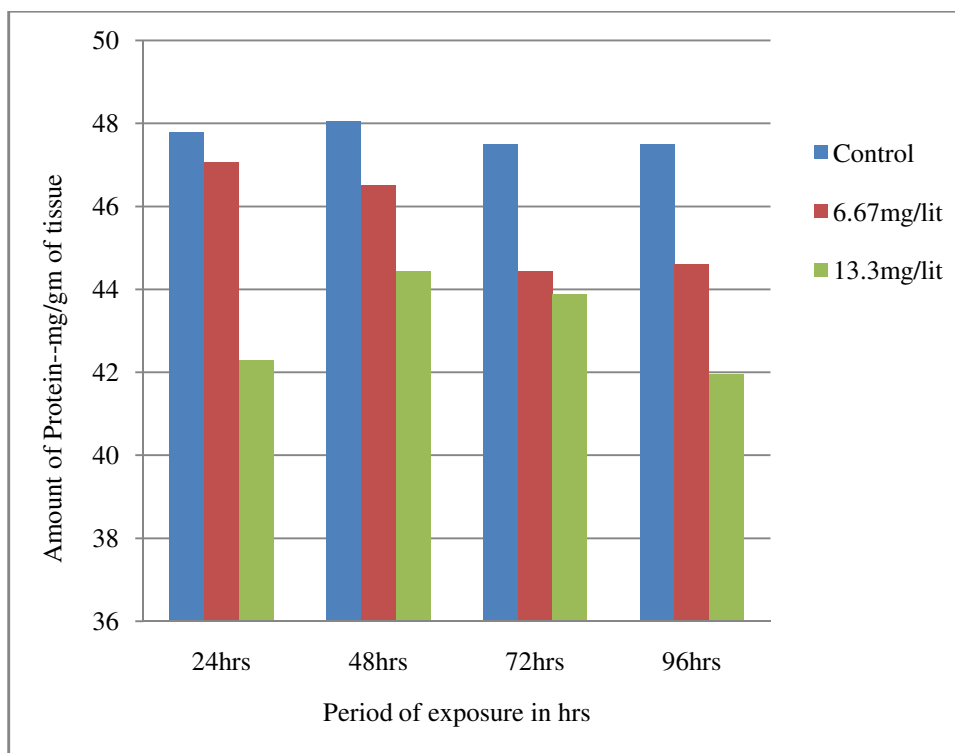
The Acetyl cholinesterase activity was increased on 4<sup>th</sup> day exposure and significantly inhibited on 15<sup>th</sup> day of an exposure. This is suggested that Acetyl cholinesterase activity reflects the

neuronal activity that in turn generally reflects in the metabolic rate of an animal<sup>16</sup>. The non-ionic detergents like Triton X-100, Lubrol-Px, Brij-35, and Tween-80 on have effects on the esterase activity and inhibitor sensitivity of human serum butyl cholinesterase (BuChE). It is observed that a marginal activation of esterase activity is caused by all detergents. The presence of Lubrol-Px, Brij-35, and Tween-80 did not affect the 50% molar inhibition concentration (IC<sub>50</sub>) of the inhibitors tested. However, the IC<sub>50</sub> values were increased for neostigmine, esterine and tetra-isopropyl-pyrophosphormide (acylation site 100 interacting inhibitor) in the presence of Triton X. The interaction of Triton X-100 with the acyl pocket hydrophobic region is able to activate the esterase activity of BuChE<sup>17</sup>.

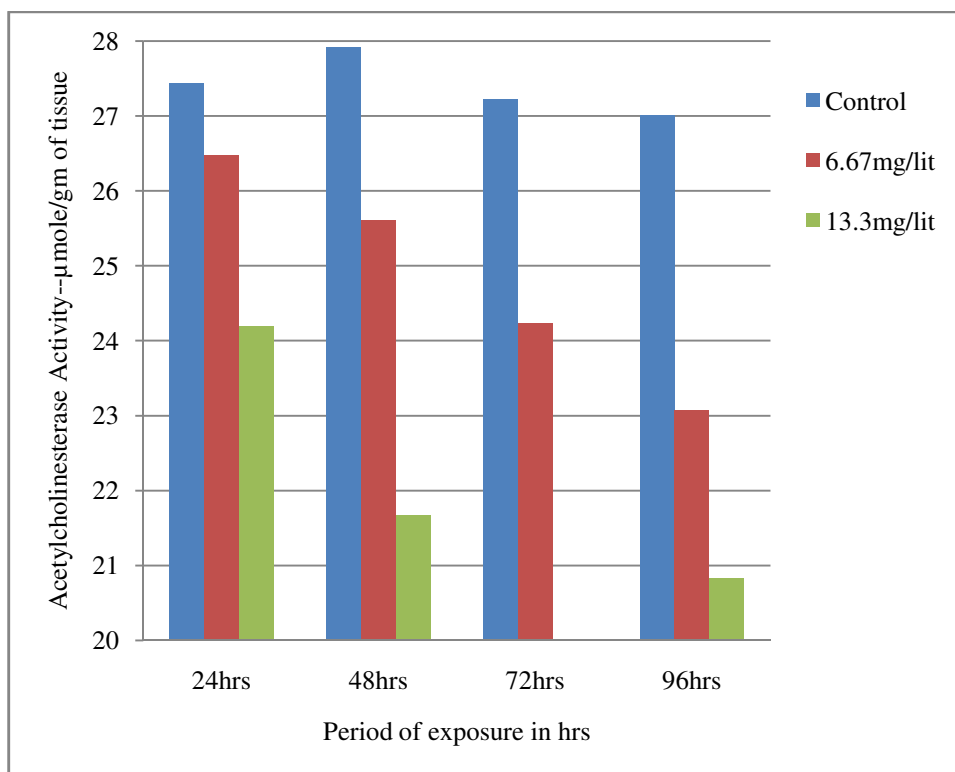
The Acetyl cholinesterase inhibition was not projecting of long-lasting or delayed effects. The severity of illness following repeated exposure may not be proportionally related to the degree of Acetyl cholinesterase inhibition as relationship between neurobehavioral effects, Acetyl cholinesterase inhibition may not be as straight forward as assumed<sup>18</sup>.

The Acetyl cholinesterase activity in erythrocyte, plasma, blood, liver and brain of rats is reduced with sub-acute toxicity test of Anilofos. The arrivals of toxicity symptoms are depend on the rate of decrease in Acetyl cholinesterase activity than on the absolute level of activity reached after exposure. Low variability in the absolute levels of activity reached at the end of the experiment. Toxicity is depending on the rate at which activity of Acetyl cholinesterase was initially reduced; initial inhibition with the highest dose is expected to be much faster than lower doses<sup>19</sup>. The studies on influence of detergents on the Acetyl cholinesterase activity and on the efficiency of its inhibitors, during this studies it is observed that detergents are enough capable to modify the enzymatic action of AChE through an collegial mechanism<sup>20</sup>.

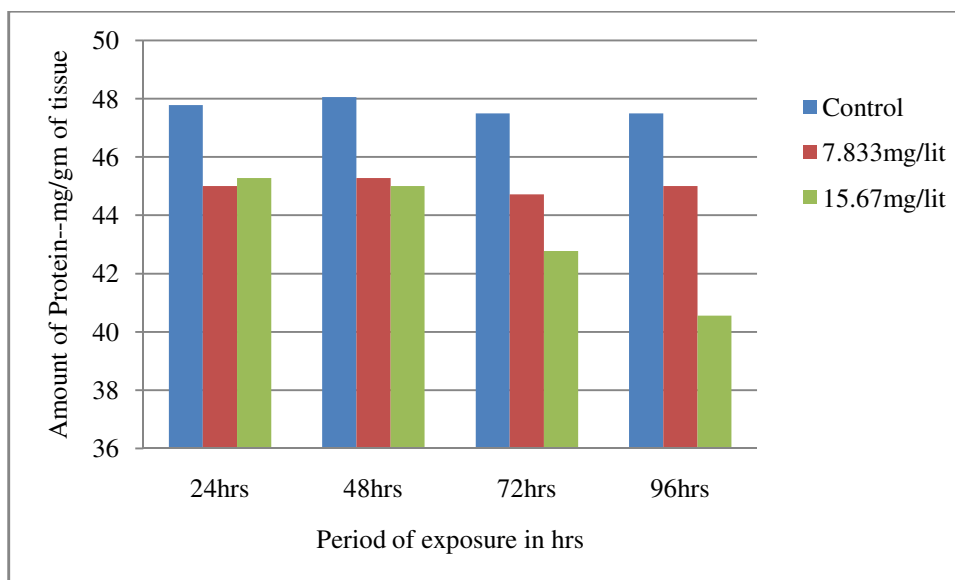
The amount of protein content in brain and gills of freshwater fish *Mystus montanus* was decrease with an increase in concentration of two detergents namely Surf excel and Nirma powder and time of exposure<sup>21</sup>. The activity of Acetyl cholinesterase is significantly modified by various environmental aspects such as pH, Conductivity, DO, turbidity and temperature in nervous system of snail *L. acuminata*. It clearly indicates that by comparing the activity of these enzymes in snail nervous tissue, residing in water body with respect to control one can predict the presence of pollutant<sup>22</sup>. The *in vitro* acetylcholine esterase inhibition activity was evaluated for all synthesized compounds showed a good inhibitory potency with an IC<sub>50</sub> value between 1.0 to 6.4µg/ml, when compared to the control neostigmine with IC<sub>50</sub> value of 8.3µg/ml<sup>23</sup>.



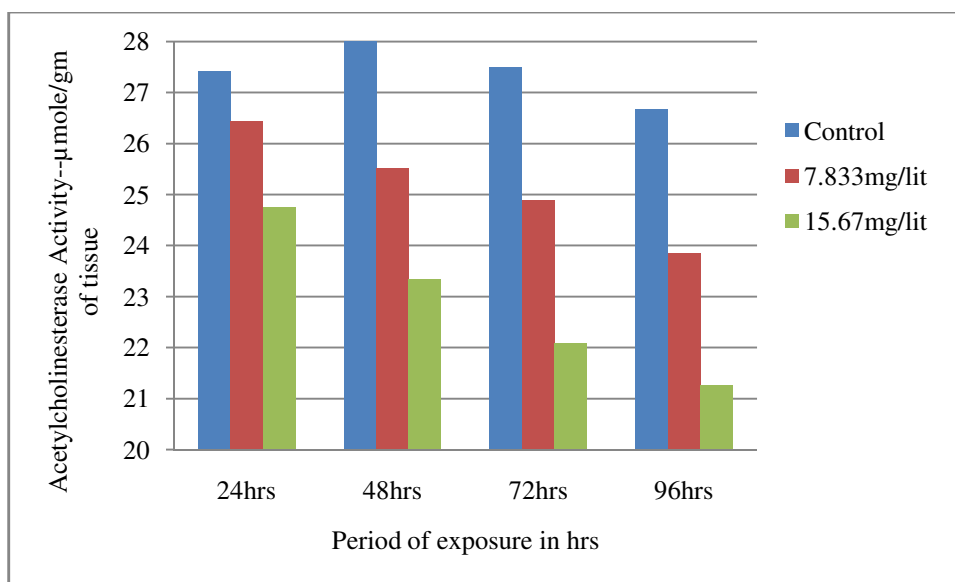
**Figure-1**  
Effects of Surf excel on Protein content (mg/gm) in Brain of *Mystus montanus*



**Figure-2**  
Effects of Surf excel on Acetylcholinesterase Activity in Brain of *Mystus montanus*



**Figure-3**  
Effects of Nirma on Protein content (mg/gm) in Brain of *Mystus montanus*



**Figure-4**  
Effects of Nirma on Acetylcholinesterase Activity in Brain of *Mystus montanus*

**Table-1**  
Effects of Surf excel on Protein content (mg/gm) in Brain of *Mystus montanus*

Concentrations ---in mg/litre	Exposure Time			
	24hrs	48hrs	72hrs	96hrs
Control	47.77767 ± 2.09733	48.05533 ± 1.75533	47.49933 ± 1.443376	47.5 ± 2.5
6.67mg/lit.	47.05533* ±1.071586	46.49967* ± 0.763763	44.4443* ±1.734588	44.61067* ±1.071586
13.3mg/lit.	45.27733* ± 1.734588	44.444* ± 2.096867	43.88867* ± 1.734588	41.944** ± 2.92691

Values are expressed in ---mg/gram of wet weight of tissue, ± = Standard deviation of three observation, \* = Insignificant, \*\* = Significant at 5.5%, \*\*\*= Significant at 1 %, ANOVA table was used for calculation.

**Table-2**  
**Effects of Surf excel on Acetylcholinesterase Activity in *Mystus montanus* at various sublethal concentrations**

Concentrations ---in mg/litre	Exposure Time			
	24hrs	48hrs	72hrs	96hrs
Control	27.43493 ± 1.995495	27.9163 ± 1.506007	27.23077 ± 2.099246	27.00987 ± 1.442298
6.67mg/lit.	26.48367 * ± 2.306323	25.60963 ** ± 1.970888	24.2403* ± 1.834685	23.0789** ± 1.385128
13.3mg/lit.	24.1981* ± 2.335423	21.6673** ± 2.295125	19.96767** ± 1.564502	20.82595** ± 1.595543

Values are expressed as ---  $\mu$ mole/gram of tissue,  $\pm$  = Standard deviation of three observation. \* = Insignificant, \*\* = Significant at 5.5%, \*\*\*= Significant at 1 %, ANOVA table was used for calculation.

**Table-3**  
**Effects of Nirma on Protein content (--mg/gm) in Brain of *Mystusmontanus***

Concentrations ---in mg/litre	Exposure Time			
	24hrs	48hrs	72hrs	96hrs
Control	47.77767 ± 2.097331	48.05533 ± 1.75533	47.49933 ± 1.443376	47.5 ± 2.5
7.833mg/lit.	44.99967* ± 1.6665	45.27767* ± 2.09733	44.722167* ± 1.734588	45.0* ± 2.5
15.67mg/lit.	45.27767* ± 2.545839	44.99967* ± 3.00495	42.77767** ± 2.097331	40.55533** ± 2.097331

Values are expressed in ---mg/gram of wet weight of tissue  $\pm$  = Standard deviation of three observation.\* = Insignificant, \*\* = Significant at 5.5%, \*\*\*= Significant at 1 %, ANOVA table was used for calculation.

**Table-4**  
**Effects of Nirma on Acetylcholinesterase Activity in *Mystus montanus* at various sublethal concentrations**

Concentrations ---in mg/litre	Exposure Time			
	24hrs	48hrs	72hrs	96hrs
Control	27.42737 ± 1.743491	28.37813 ± 0.99375	27.4989 ± 2.320689	26.68037 ± 1.323191
7.833mg/lit.	26.44053* ± 1.388581	25.52287* ± 1.318989	24.8892* ± 1.512647	23.84987** ± 1.415323
15.67mg/lit.	24.75653* ± 1.12027	23.34727* ± 1.490756	22.08693** ± 2.19743	21.26459** ± 1.996707

Values are expressed in ---  $\mu$ mole/gram of tissue,  $\pm$  = Standard deviation of three observation. \* = Insignificant, \*\* = Significant at 5.5%, \*\*\*= Significant at 1 %, ANOVA table was used for calculation.

## Conclusion

The synthetic detergents are used in large quantities for cleaning the household's items, as well as industries for manufacturing and cleaning. As these detergents reach the water body, they modify various environmental factors such as temperature; pH, DO, CO<sub>2</sub>, alkalinity, acidity, BOD, COD, Conductivity, and turbidity of receiving water. The fishes show behavioural changes with these altered environmental factors. In laboratory, when these fishes are exposed to the various concentrations of detergents, they show behavioural changes like swimming movement is lethargic, more secretion of mucus from body and gill epithelium, bleeding through gills, at the base of body appendages (fins) and along the belly. There is loss of nervous control, fish swims along lateral side of body. These behavioural changes are indication of disorder of central

nervous system, shows the sign of poisoning caused by detergent and inhibition of Acetylcholinesterase activity. Results of present investigation clearly indicate that as concentration of detergents and time of exposure is increased, Acetylcholinesterase activity is reduced. In the midstream and downstream of Mula-Mutha river of Pune level of pollution is more than upper limit of water pollution. It has been observed that many fish species have been disappeared from polluted stretch of river. To sustain natural population of fishes and aquatic animals, there is need of proper control and management on use of synthetic detergents. The use of detergent in homes and industries cannot be stopped. There is necessity to develop better method of disposing detergents. There is a need of development of eco-friendly detergents and soaps, so that aquatic fauna of various water bodies will be preserved.

## References

- Chandanshive Navnath Eknath, Studies on Toxicity of Detergents to *Mystus montanus* and Change in behaviour of Fish, *Research Journal of Animal, Veterinary and Fishery Sciences.*, **1(9)**, 14-19 (2013)
- Fukuto T.R., Bull. W.H.O. 44.31, (1971)
- Moore R.B, Manery J.F, Still J and Mankad V.N., The inhibitory effects of polyoxyethylene detergents on human erythrocyte Acetylcholinesterase and  $\text{Ca}^{2+}$  +  $\text{Mg}^{2+}$  ATPase, *Biochem Cell Biol.*, **67(2-3)**, 137-46 (1989)
- Bouquene Gilles and Galgani F., Cholinesterase inhibitions by organophorus and carbamate compounds, *ICES Tech. Mar. Environ. Sci.*, **24**, 54-63 (1996)
- Gerstenberger S.L., Gilbert J.H. and Dellinger J.A., Environmental contaminants and Cholinesterase activity in the brain of fish (*Martes pennanti*) harvested in Northern Wisconsin, *Bull. Environ. Contam. Toxicol.*, **56**, 866-872 (1996)
- Guilhermino L.U, Lacerda M.N., Nogueiraand A.J.A., Soares A.M.V.M., In vitro and in vivo inhibition of *Daphnia magna* Acetylcholinesterase by surfactant agents: possible implications for contamination biomonitoring, *The Science of the Total Environment*, **247**, 137-141 (2000)
- Clint Rosenfeld, Ahmed Kousba and Lester G. Sultatos, Interactions of Rat Brain Acetylcholinesterase with the Detergent Triton X-100 and the Organophosphate Paraoxon, *Toxicological Sciences*, **63**, 208-213 (2001)
- Feng T., Li Z.B., Guo X.Q. and Guo J.P., Effect of trichlorfon and sodium dodecyl sulphate on antioxidant defense system and Acetylcholinesterase of *Tilapia nilotica* in vitro, *J. of Pesticide, Biochemistry and physiology*, **92(3)**, 107-113 (2008)
- APHA, Standard methods for the examination of water and wastewater, 20<sup>th</sup> edition, (1998)
- Litchfield J.T. and Wilcoxon F., A simplified method of evaluating dose effect experiments, *J. Pharmacol. Exp. Ther.*, **96**, 99-113 (1949)
- Abbott's W.S., A method of computing the effectiveness of an insecticide, *J. Econ. Entomol.*, **18**, 265-267 (1952)
- Konar S. K., Laboratory studies on the organophosphorous insecticides, DDVP, and phosphamidon, as selective toxicants, *Trans Amer. Fish Soc*, **98**, 430-437 (1969)
- Burruss R.M., Development and evaluation of on-site toxicity test procedure for fishery investigations, U.S. Department, *Fish. Wild. Serv. Wash.*, **68**, 1-8 (1975)
- Wolfgong Pilz, In: Methods of enzymatic analysis (Ed Bergmeyer) *Academic Press Inc New York* II Edition **2p 840** (1974)
- Krishna Murthy V., Dhananjaya Reddy Y., Manohar Reddy R., Bhaskar M. and Govindappa S., Changes in brain activity of Acetylcholinesterase and behaviour of freshwater fish in acid polluted environment, *Environ. and Ecology.*, **2**, 79-82 (1984)
- Reddy S.L.N. and Venugopal N.B.R.K., Effects of fluoride on Acetylcholinesterase activity and Oxygen consumption in fresh field crab, *Barytel phuasguerini*, **45**, 760-766 (1990)
- Jaganathan L. and Boopathy R, Interaction of Triton X-100 with acyl pocket of butyrylcholinesterase: Effect on esterase activity and inhibitor sensitivity of the enzyme, *Ind. J. Biochem. Biophys.*, **35**, 142-147 (1998)
- Moser V.C., Comparison of aldicarb and methamido phosneuro toxicity at different ages in rat: Behavioural and biochemical parameters, *Toxicol Appl. Pharmacol.*, **157**, 94 (1999)
- Hazarika Archana and Sarkar S.N., Subacute toxicity of anilofos, anew organophosphorus herbicide, in male rats, Effect on some physical attributes and Acetylcholinesterase activity, *Indian J. of Experimental Biology*, **39**, 1107-1112 (2001)
- Zimmermann M, Westwell MS and Greenfield SA, Impact of detergents on the activity of Acetyl cholinesterase and on the effectiveness of its inhibitors, *Biological Chemistry*, Jan; **390(1)**, 19-26, (2009)
- Chandanshive N.E. and Kamble S.M., Detergent induced changes in the protein content of brain and gill of *Mystus monatanus.*, *J. of Association of Zoologists, India*, **2(1)**, 79-85 (2009)
- Singh Nitu, Acetylcholinesterase activities in the nervous system of snail *Lymnaea acuminata* as biomarkers of water pollutants in Ramgarhlake, Gorakhpur, up, India, *International Research Journal of Environment Sciences*, **3(2)**, 20-26 (2014)
- Akilandapuram Velusamy Saranya and Subban Ravi, In-vitro Acetylcholine Esterase Inhibition activity of Chalcones with Phenothiazine Moiety, *Research Journal of Recent Sciences.*, **1(12)**, 40-43 (2012)