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Investigation on the toxic effects of toilet cleaner Domex on the Fingerlings of *Puntius goninotus* (Bleeker, 1850)

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Abstract

Toxicity of toilet cleaner Domex was assessed on fingerlings of fish *Puntius goninotus* using static toxicity method. Biochemical studies were carried out in fishes exposed to sub lethal concentration (0.03ml/l of 24 h LC50 value).The glycogen, glucose and protein contents of the tissues Viz., muscle and liver were estimated in toilet cleaner Domex exposure and control fishes. The glycogen [(0.10mg/g) in Muscle and (0.25mg/g) in gills] and protein [(0.31mg/g) in Muscle and (0.15mg/g) in gills] levels were found to be depleted in the tissues exposure to sub lethal concentration over the control [0.41 mg/g and 0.16 mg/g respectively]. Whereas the glucose level [(1.31mg/g) in Muscle and (2.32 mg/g) in gills] in the tissue showed an increase on Domex exposure fishes in comparison with the control fishes [(0.13mg/g) in Muscle and (0.08 mg/g) in gills]. The depletion of glycogen level on Domex exposure may be due to stress condition and increased metabolism. The increased in the glucose level on Domexexposure suggests that animal mobilizes the reserve carbohydrate to meets its energy needs. Hence the animal enters in to hyperglycemic condition under stress. Further depletion in total protein content may be the augmented proteolysis and possible utilization of their product for metabolic process. The behavioral changes recorded during the exposure showed decrease in opercular movement, loss of reflex, increase in erotic swimming, increase in mucous secretion all over the body and irregular swimming activity were noticed.

Environmental pollution is becoming a problem of greater concern because it has damaged the quality of water (Langiano and Martinez 2008). The aquatic biota's continue to suffer great chemical stress because of anthropogenic influence. Water pollution is commonly associated with the discharge of domestic, industrial or agricultural effluents. These practices generating a large quantity of residues and un-loading it on water bodies

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are a cheap and efficient way to get rid of most of these contaminants[13,27]. There are hundreds of pollutants that affect the aquatic environment and their effects cause great concern.

Nowadays, the major rivers and aquatic bodies of world were heavily polluted due to domestic, industrial discharge and detergents. A huge amount of various brands of detergent powders, flakes and various shampoos and toilet soaps makes the compositions of the sewage vary in space and time. Sewage contains human and animal excreta, food residues, cleaning agents, detergents and other wastes[5]. Available reports indicate that entry of detergents into aquatic system build up in the food-chain and are responsible for many hazardous effects and even death of the aquatic organisms, including fishes[26]. The synthetic detergents are the most used substances in modern civilization, being used to prepare shampoos, domestic cleaning products and toothpaste and are now one of the largest sources of water pollution[20].

The surfactants are one of the major contents of detergents, commercial surfactants like Linear Benzene Sulphonate (LAS), Alkyl Sulphates (AS), Alkyl EthoxySulphates (AES), Ethoxylates (AE), Alkyl Phenol Ethoxylates (APE), Cetyltrimehtyl Ammonium Bromide (CTAB) etc are synthetic chemicals, which are used in large amounts in detergents, soap, shaving creams, fabric softeners, additives for food, paint, leather and textile items, pesticides, defoliants, antiseptics, disinfectants[23].

These detergents were found to interfere with the food utilization, growth of fishes[22]. Abbas[1]; Adewoye *et al.*,[3] reported that the detergent effluents and discharges have also been noticed to induce severe damage to such vital organs like gills, liver, kidney, skin, heart and brain.

Fishes are very good biosensors of aquatic contaminants and as bio-indicator species respond with great sensitivity to changes in the aquatic environment. Scanning of pertinent literature reveals that detergent related works on fish are still very megre and limited to acute toxicity determination[3].

Hence an attempt has been made to determine the short term (24hours) toxic effect of toilet cleaners to the fingerlings of economically important fresh water fish *Punctius goninotus*. An important consideration for studying the toxicity of toilet cleaners in the fingerlings of *Punctius goninotus* was the paucity of information on the younger developmental stages which are considered to be more susceptible and vulnerable to toxicants and then those of adult stages.

Puntius gonionotus were chosen for this investigation because of its availability throughout the year and as the most cultivable and consumed fish in this area. As the fish occur in fresh water bodies, there may be change of entering of chemical through various channels viz., surface runoff, drift and leeching, that may could cause toxic effects on the fishes and the other aquatic organisms. So it is essential to determine whether Domex affects on non target aquatic organisms especially on the fishes. This type of study helps to understand the toxic effects of toilet cleaners on fishes.

Selection of test organism :

The test organism, fingerlings of *Puntius gonionotus* (weight:5.8±1.3g), (length:6±1.5cm) were collected form Bhadra Reservoir Project, and were transported to the laboratory in well ventilated polythene bags to avoid any injury. The test organism were kept in large plastic containers that has already been washed and rinsed with 5% potassium trioxonitrate to remove any adhered metals and thereafter acclimatization, static bioassay was employed.

Bioassay protocol :

Static bioassay tests were conducted in order to evaluate the acute toxicity of Domex. Dechlorinated tap water was used for acclimation of fish as well as experiment and control. In all treatments, ten fully acclimatized test organisms were held and the same in control stock, as described by Solbe and Peter Collins[24] and Rahman *et al.*,[18]. Fishes were fed twice daily with groundnut oil cake and rice bran with respect to the 10% body weight of fish. The moderate size fishes were selected for experiment and placed in different concentrations of Domex to determine LC_{50} values after 24 hours.

After appropriate toxicity range of the test, solutions were determined by preliminary testing, 5 concentrations of Domex ranging from 0.01ml/l to 0.05ml/l to *Puntius gonionotus*. Observations on survival were made after 24 hours. LC_{50} (concentration required for 50% mortality) values are calculated by graphical method. Experiments were carried out in duplicates and a separate control was maintained.

LC_{50}-24 Determination :

Commercially available Domex (Composition: Sodium Hypochloride, ionic and non-ionic surfactants, Sodium hydroxide, perfume, stabilizer, pigment green 7(C174260) water). brought from the market taken in term of concentration ml/l. In each four trough, each containing 6 liters of water stocked with 10 fish, containing different concentration (0.01, 0.02 and 0.03) of Domex for *Puntius gonionotus*. A control set was run with the same number of fish and the same volume of water but without toilet cleaners. The experiment was run in duplicates. The water was aerated and the feeding was completely stopped during experiment period. Dead fishes were removed immediately and their number was recorded at 24 hrs. LC_{50} was calculated by graphical method.

Toxicity studies:

LC_{50} is a concentration in which 50% of the experimental animals survive. Estimation of LC_{50} by interpretation involving plotting of data in a graph with concentration on X-axis, while percentage on Y-axis. A straight line is drawn between maximum points representing survival at maximum successive concentrations that were lethal to more and less than of the total number of test animals exposed to the toxicant. The concentration at which this crosses the 50% survival line is the LC_{50} value[11]. The LC_{50} value was determined for the Domex to the late fingerlings of *Puntius gonionotus*.

Study of Behavioral response:

The behavioral changes in the fishes

were noted right after the application of testing dose till the end of the experiment. The negative control group was also monitored in the same while for mortalities and changes in behavior including loss of balance, moving in spiral fashion with jerks, lying laterally and opened mouth with rapid opercular movement. Behavioral responses and mortality of the fishes were recorded at the interval of 2 hours and the alternation of behavioral characteristics was recorded.

Biochemical Analysis :

At the end of each exposure period fishes were sacrificed and tissues such as gills and muscle were dissected as removed for biochemical profile analysis. Glucose was estimated by Anthrone method[19], Glycogen was estimated by Kemp's method[9] and Protein content was estimated by Lowery's method[12].

Histology analysis :

For histological examination fish were sacrificed and tissue (gills and muscle) were removed immediately to overcome autolysis; then they were fixed in Bouin's solution (Robert, 2001), dehydrated through graded series of ethanol, cleared in xylene and embedded in paraffin. The paraffin blocks were sent to Prakash diagnosing centre for the sections. These sections were examined under a microscope.

There was no mortality in the control at the end of experiment (24hrs). The estimated 24hrs LC_{50} value of Domex using a static bioassay system for *Puntius gonionotus* was 0.03ml/l (Table-1).

Table-1. LC_{50} values of Domex exposure to fingerlings of *Puntius gonionotus.*

Cleaners	Fish species	Exposure period	Method	LC_{50}
Domex	*Puntius gonionotus*	24	Graphical	0.03ml/l

Behavioral studies :

Different behavioral responses of *Puntius gonionotus* shows at different concentration of Domex was observed throughout the experimental period. The control group showed the normal behavior during the whole experimental period. Application of lowest concentration (0.01ml/ l) of floor cleaner to the fish was observed normal response. The behavioral responses of the test organism during the acute toxicity test were noticed by the sudden change in the organism response to the environment such as erratic swimming, occasional grasping for breath, loss of balance, swimming in a spiral path with jerks and revolving in water. At the highest concentration (0.03ml/l) the more severity of all these responses was observed including the loss of balance, lying laterally at the bottom, swimming down in spiral fashion with jerks rapid, opercular movements with opened mouth (Table 2).

Table-2. Behavioral response of *Puntius gonionotus* fingerling during the exposure to lethal concentration of Domex

Behavior	Concentration in ml/l		
	0.01	0.02	0.03
Erratic swimming	-	+	+++
Gasping for breathing	+	++	+++
Loss of balance	-	++	+++
Swimming in spiral fashion with jerks	+	++	+++
Lying laterally at the bottom	-	+++	+++
Motionlessness	-	++	+++

-=Absent, +=Low, ++=Moderate, +++=High characteristics

Biochemical Analysis :

Glucose :

The variation of glucose content in fish, control and expose trails represented in (Fig. 1) Glucose content (mg/g) in the gills and muscle tissue was estimated after exposing the fingerlings for 24 hours (LC_{50} of Domex for 0.03 ml/l). In that the exposed muscle found to have (1.31mg/g) and gills (2.32mg/g), tissues shows in creased glucose consumption level as contrast to the controlled fingerlings muscle (0.94mg/g) and gills (1.74mg/g).

Glycogen :

Glycogen content mg/g in the gills and muscle tissue was estimated after exposing the late fingerlings for 24 hours LC_{50} of Domex for 0.03ml/l (Table-1 Fig:1) in that exposed muscle (0.1mg/g) and gills(0.25mg/g), gill tissue and muscle tissue found to contain 0.206mg/g and 0.432mg/g respectively.

Protein :

The alteration of protein content in fish in control and exposed trials presented in (Fig. 1) Protein content (mg/g) in the muscle and gill tissue was estimated after exposing the late fingerlings for (24hours LC_{50} of Domex for 0.03ml/l) showed 0.313mg/g and 0.150mg/g respectively. Where in case of control fingerling the protein content in muscle (0.412mg/g) and gills (0.161mg/g).

Histological Analysis :

Analysis of changes in gills :

Histological examination gave significant indication of toxicity of Domex. The effects include gill alteration such as desquamation of the epithelial lining, hemorrhagic and hyperplasia and fusion of the secondary lamellae. While there was no lesion in the control experiment (plate a, b, c, d).

Analysis of changes in muscle :

In this study, the muscle tissue exposed to 24hrs causes damages to the muscle. In treated muscle the nuclear proliferation was observed and also seen different size of the

muscle fibers and disintegration of muscle bundles, atrophy of muscle bundles, marked thickening and separation of muscle bundles. Were as in case of control there was no alteration were observed in the histology of muscle.

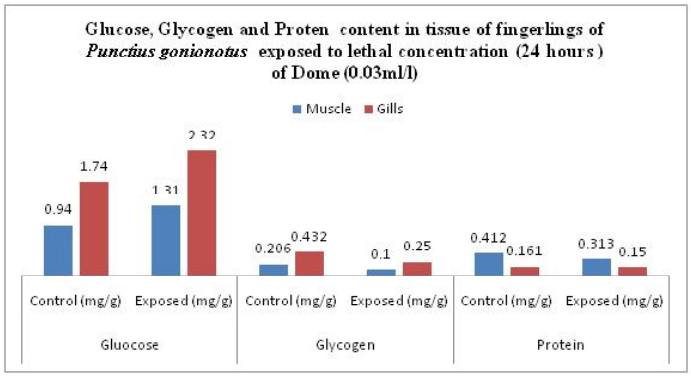

Fig. 1. Biochemical changes in tissues fingerlings of *Punctius gonionotus.*

Analysis of histological changed in muscle and gills of *Puntius gonionotus*

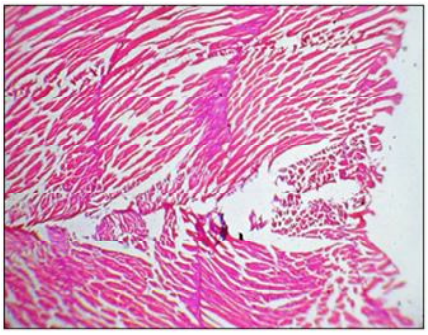

PLATE :a. Muscle of *Puntius gonionotus* Shows no changes (Control)

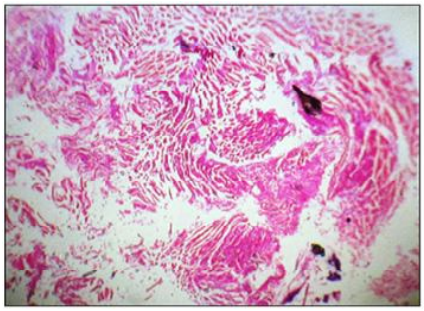

PLATE :b. Muscle of *Puntius gonionotus* exposed shows Muscle bundle degeneration

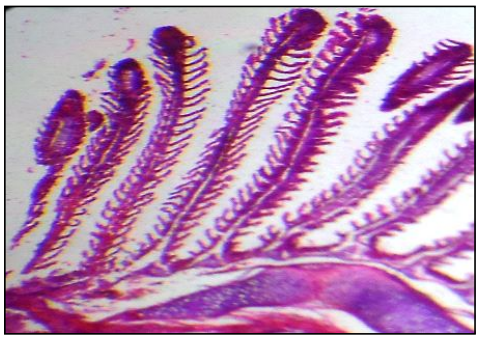

PLATE :c. Gills of *Puntius gonionotus* Shows no changes (Control)

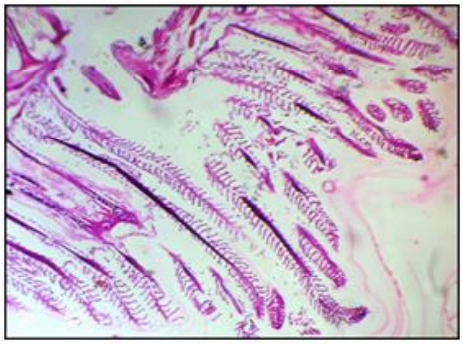

PLATE:d. Gills of *Puntius gonionotus* exposed shows desquamation of epithelial lining

Toxicity studies :

The present studies are in agreement with the following earliest studies reveals that LC_{50} values of the toilet and bathroom cleaner was found that 0.03ml/l concentration of Domex is toxic to the fresh water fishes *Puntius gonionotus*. It is found that the mortality rate increased as the concentration of Domex increased, indicating that the effect of Domex was dose dependent.

Behavioral studies :

The result of the present study clearly reveal that the concentration (0.03ml/l) and shorter exposure period (24hours) of Domex is highly toxic to the fish. The effect of other concentration cannot be ignored. Prolonged exposure to even lower concentration includes behavioral and morphological changes in fishes. These changes and responses indicated stress in fishes which can further lead to death and reduction in fish fauna.

In the present investigation, *Puntius gonionotus* exhibited a variety of behavioral responses like opercular movement was 20-25 times more faster than controlled, loss of nervous control, try to jump out of media. In dead fishes opercula region becomes blackish, hemorrhaging occurs of gill filaments amongst, along the belly, at the base of pectoral, anal and pelvic fins. Body was slimy due to mucus secretion from epithelium of gills. The fishes were surfacing frequently. Affected fishes were swimming on lateral side of the body; nervous control and equilibrium were lost. The body color of dead fishes turn to yellow. In higher concentrations of cleaner swimming movements of fish immediately slow down with the addition of toxicants. During exposure period, the test fish exhibited several behavioral changes before death such as restlessness, rapid swimming and respiratory distress. Opercula ventilation rate as well as visual examination of dead fish indicates lethal effects of the toilet cleaners on the fish. The studies of Omoriege *et.al.,*[15]; Okwuosa and Omoregie[14]; Avoaja and Oti[6]; Oti[16] and Adewoye *et.al.,*[3], corroborated this present study.

Biochemical Analysis :

Glucose :

In the present investigation, the exposer of fresh water fishes *Puntius gonionotus* fingerlings to lethal concentration (0.03mg/g) of Domex have marked metabolic alteration. The total glucose content was increased significantly in gills (2.32mg/g) and muscle (1.31mg/g) tissue sample of exposed fingerlings compare to controlled tissue gills (1.74mg/g) and muscle (0.94mg/g) *Puntius gonionotus* respectively. Increase in the glucose levels during the initial period of exposure indicate the Domex induced stress and the animal needs more sugar to meet the increased energy demand to mitigate the toxic effect of the Domex.

Glycogen :

In the present observation compared to control fish of *Puntius gonionotus* gills (0.432mg/g) and muscle (0.206mg/g), there was decreased level of glycogen in the tissue of gills (0.017mg/g) and muscle (0.036mg/g)

in the fingerlings of *Puntius gonionotus* exposed to lethal concentration of Domex.

The decreased in the glycogen content in the exposed might have been due to the utilization of reserved glycogen to meet the extra energy demand due to the pollutant stress and also suggest the possible onset of glycogenolysis forming free glucose in the exposed tissue (Sreenivasa 2012). Decreased glycogen synthesis is also attributed to the inhibition of the enzymes glycogen synthatase which mediates glycogen synthesis[28].

Protein :

In the present experimental observation, fishes exposed to Domex has showed decrease tendency in the protein content level in gills (0.150mg/g) and in muscle (0.313mg/g) when compared to controlled gills (0.161mg/g) and muscle (0.412mg/g) of fish *Puntius gonionotus* respectively. This indicates that the toxicant has inhibition the protein synthesis during the period, the availability of amino acid have become less, that result in the depletion of protein content. The protein and amino acids were decreased gradually compared to control, when the period of exposure increased. The depletion of protein may also attributed to spontaneous utilization of amino acids in various catabolic reactions inside the organisms in order to combat the stress condition. According to Das and Mukherjee (2003) exposure of fishes for long time to most toxicants interferes with protein metabolism. Decrease in total protein in fish exposed to toxic levels of toxicant could be attributed to either a state of hydration and change in water equilibrium in the fish or a disturbance in liver. Similar findings have been reported in the fish. *Heteropneustes fossils* exposed to rogor, Borah and Yadav[8]; Pechiammal *et al*.,[17].

Histological Analysis :

Gills :

The treated gills of showed damage at the exposure of 24 hr. and exhibit more destruction of the gill filaments. The gills showed desquamation of the epithelial lining, necrosis (Telengioectassiae) of the secondary lamellae, shrinkage of secondary lamellae and also showed hypertrophy and hyperplasia at the base of the secondary lamellae. The histological alterations observed in the present study was in agreement with observation made by Vensa Poleksic and Vesela Karan[29]; Sridhar and Esther Joice[25]; Vijayalakshmi *et.al*.,[30].

Under light microscopy, the fishes (normal) not treated with toilet cleaners showed normal anatomical and surface features of gills arches, filaments and lamellae. The gills arch had two rows of gills filaments and arrow of gill rakers; the former directed towards the opercular opening and the latter towards bucco-pharyngeal chamber. Two rows of gill filaments were directed posterior-ventrally from each arch arranged in a manner that resemble like a comb. The tips of gill filament formed a gill certain forming sieves for the passage of water passing from buccal chamber to opercular chamber. After exposure

of toilet cleaners, sever histo-pathological changes were observed in the epithelial lining of gill arch, gill rakers and gill filaments of fishes. Fusion of gills lamellae was observed at the tip of gill filament with accumulation of blood cells in capillary and infiltration of blood cell in sub-epithelial space. The secondary lamellae of the basal region of filament were also found fused with each other leaving part free, indicating proliferation (epithelial lifting) of inter-lamellar epithelia within the lamellae. The gill rakers, large empty lymphatic spaces were formed around the central row of pilaster cells. The histo-pathological finding of the present study may be correlated with the observation of many researchers. Similar histopathological changes, including lifting of lamellar epithelium and formation of empty space in gill have been seen earlier by some investigators (Konar[10] and Sateesh[21]) in fish exposed to some insecticide and chemical substances.

Muscle :

The muscle in control has no nuclear proliferation and also size of the muscle is same. There is no any alteration in the control but in treated muscle showed damage at the 24hr. and exhibited more destruction of the muscle fibers. The muscle showed different size of the muscle bundles, marked thickening and separation of muscle bundles, edema between muscle bundles and splitting of muscle fibers. Muscle fiber, disintegration of muscle bundles, atrophy so that alteration of histopathology of muscle at the concentration of 0.03ml\l. The histological alterations observed in the present study was in agreement with observation made by VeusaPoleksic and Vesela Karan[29]; Sridhar and Esther Joice[25]; Vijayalakshmi *et.al.*,[30].

Overall, the study has shown that the Domex is toxic to *Puntius goninotus.* Fishes exposed to acute lethal concentrations of the cleaners resulted in significant behavioral, biochemical and histo-pathological alterations. These changes suggest that the treated fish are faced with serious metabolic crisis. The random disruption and structural alterations in gills and muscle in the exposed fishes are indicative of stress mediated production. The results clearly indicate that the addition of cleaners to the water body may be threat to aquatic flora and fauna. Further to sustain its natural population, pollution from washing activities needs proper control and management.

The use of detergent in homes cannot be discontinued. However, better method of disposing the 'after wash' needs to be worked out. There is a need of development of eco-friendly detergents and soaps, so that aquatic fauna of various water bodies will be preserved. If the present rate at which they are introduced into aquatic environment is not checked, then continuous existence of aquatic fauna is in serious threat.

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