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Effect of Paper Mill Effluent on Lipid Profile of Freshwater Snake Headed Fish, *Channa punctatus* (Bloch, 1793)

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Abstract: Industrial effluents reaching the aquatic ecosystem through various routes like run-off, leaching, and direct discharge from factories are major causes of environmental pollution. These were reported to have a negative metabolic impact on different non-target aquatic organisms. Fish is a close inhabitant of the aquatic environment, serves as a useful model for assessing the effect of chemicals mixed in the aquatic environment. *Channa punctatus* Bloch (Actinopterygii: Channidae), one of the most common edible fish, if exposed to industrial effluents containing harmful substances, maybe a serious threat to human health. In the present study, an attempt was made to understand the effect of sublethal concentrations of paper mill effluent on lipid profiles of *Channa punctatus* after exposure to 96 hours. A significant increase was observed in total serum lipid, cholesterol, and phospholipid with decreased triglyceride levels. A significant increase was observed in the cholesterol and triglycerides levels, whereas total lipids and phospholipid levels showed a marked decline in the liver. Thus present study concludes that the estimation of the lipids profile of fish will certainly detect early signs of stress physiology concerning their habitat.

Keywords: *Channa punctatus;* cholesterol; phospholipids; triglycerides; paper mill effluent. **Abbreviations:** KMnO₄-Potassium Permanganate; V_E-Volume of Effluent; V_{DW}-Volume of Dilution Water; FAO-Food and Agriculture Organization; S.E.-Standard Error (S.E.); LC₅₀,-Lethal Concentration with 50% mortality; M..-Molarity; mg/l-Milligram per Liter; L-Liter; Hrs.-Hours.

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1. Introduction

Rapid urbanization and industrialization in India resulted in a substantial increase in the liquid waste traditionally discharged in open land or into nearby natural water bodies, causing many environmental problems, including a threat to aquatic biota [1-4]. Industrial discharges and wastes are the undesired outcomes of economic development and technological advancement [5-7]. In India, only 60 % of 13,500 million liters or more of industrial effluent discharged daily by industries is treated, and rests remain untreated [8-10]. The industrial wastewater carrying various noxious and toxic contaminants and heavy metals discharged into rivers, streams, and other aquatic reservoirs regularly and disturbed the cell's normal functioning [11-14]. This may lead to alternation in aquatic animals' functional biochemical and physiological mechanisms and is likely to affect the growth and production of aquaculture

adversely [15-17]. Many of the toxic substances released from these industries are lipophilic and accumulate in fatty tissues of aquatic animals like fishes or become protein-bound, so it is of importance to know the critical concentration above which human beings are affected, and the commercial fish species become unsuitable food [18-21].

After agriculture evolution, the intensive application of pesticides without proper disposal management has led their excess residues to reach the neighboring aquatic ecosystem through various routes like run-off, leaching, spray-drift, industrial effluent, and its inhabitants, mainly fish [22-24]. The effluent drainage from different industrial units poses a serious threat to the aquatic flora and fauna [25-27]. Industrial effluents carry many toxic substances capable of affecting the genetic material of the organisms, leading to immediate and inherited mutation [28-30]. A variety of somatic diseases like cell death, immunological impairment, ineffective tissue repair, premature aging, and carcinogenesis may also result from the chemicals in the effluents [31-33]. As per some earlier reports, the industrial effluents exposure among fishes exhibited different behaviors such as schooling where fishes form groups; surfacing, i.e., frequent movement to water surface; hanging due to loss of balance, opercular movement rate, and convulsions [34-36]. Due to effluents exposure, these behaviors among fishes were subjected to effluents exposure were subjected sensory organs, neuromuscular coordination, and affect the fish metabolism in diversified manners [37, 38].

India has 20th rank among the paper-producing countries in the world. The most important problem the paper industries are facing today is the disposal of tremendous volumes of wastewater [39-43]. Paper and pulp mills use a huge quantity of freshwater during scouring, bleaching, digesting, rinsing, cortication, lime treatment, and other finishing processes and return a large quantity of wastewater containing undesirable impurities [44-48]. The Indian paper industry consumes about 250 m³ of freshwater per ton of paper and generates the corresponding wastewater [49-51]. In India, around 905.8 million m³ of water is consumed, and around 695.7 million m³ of wastewater is discharged annually by this sector [52, 53]. So paper mills are normally located at the banks of rivers to ensure an adequate supply of water and a means of disposing of effluents, which are generally characterized by high concentrations of chemical oxygen demand, biological oxygen demand, suspended solids, extreme pH, and elevated temperatures [54-57]. The discharged effluents made the water unfit for human consumption and create health hazards in various aspects if consumed directly or indirectly [58-62]. The use of the biochemical approach has been advocated to provide an early warning to potentially damaging changes in stressed organisms [63-66]. Accordingly, the health of aquatic organisms is influenced by the physiological activities taking place in their body [67-69]. Lipids play an important role in the architectural dynamics of the cell and transport mechanism across the cell membrane [70-73]. Lipids also contribute to energy production as they have high caloric values and play a vital role in the biochemical adaptations of animals to stress conditions [74-78]. Hence, the present investigation is aimed to study the effect of sublethal concentrations of paper mill effluent on the lipid metabolism of *Channa punctatus*.

2. Materials and Methods

2.1. Procurement of test fish.

Healthy specimens of snake-headed fish, *Channa punctatus* Bloch (Actinoptrygii: Channidae) with bodyweight 45±5 g and body size 12±5 cm, were collected from a local fish farm Balrampur (Uttar Pradesh), India, and were transported to the laboratory. The fishes were

carefully examined for any injury and then kept in 1 % solution of KMnO₄ for few hours to get rid of dermal infection. These were further kept in a large plastic jar containing 50L of clean tap water and acclimatized for 15 days to the laboratory conditions. During these periods, the fishes were fed on boiled egg yolk and commercial fish food.

2.2. Collection of paper mill effluent.

The treated effluent samples were collected from Yes Paper Mill Ltd. Darshan Nagar, Ayodhya (Uttar Pradesh), India, in a polyethylene container. The percent concentration of test solution has been calculated using the formula after FAO [79]:

Where, V_E = Volume of effluent; V_{DW} = Volume of Dilution water.

2.3. Plan of the experiment.

The LC₅₀ for treated paper mill effluent for 96 hours was 15% after Prakash and Verma [80]. Based on reference LC₅₀, fishes were exposed to sublethal concentrations (10%) of paper mill effluent for the period of 24, 48, 72, and 96 hours. A control group was also maintained in an identical environment for the same periods *viz.* 24, 48, 72, and 96 hours. The fishes of both groups were regularly fed with commercial food, and the medium was changed daily to remove feces and food remnants. The fishes were sacrificed immediately at the end of 24, 48, 72, and 96 hours in both experimental and control groups.

2.4. Blood sample collection.

The blood samples were collected from live fishes through a cardiac puncture in both experimental and control groups at 24, 48, 72, and 96 hours exposures. These were allowed to stand for some time and, after that, centrifuged at 3500 rpm for 10 min to obtain serum.

2.5. Tissue sample collection.

The liver of both experimental and control groups fishes just after 24, 48, 72, and 96 hours exposures were dissected out and washed thoroughly with 0.9 N saline solutions then homogenized and centrifuged at 3500 rpm for 20 minutes to obtained supernatant. The serum and supernatants were used for the analysis of total lipids [81], cholesterol [82], phospholipid [83], and triglycerides by the method after Foster and Dunn [84].

2.6. Statistical analysis.

The biostatistical analysis was performed using advanced numerical tools and the data presented in the manuscript as mean \pm standard error (S.E.) unless otherwise stated. Student's t-test calculated the statistical significance of the difference between the control and experimental group.

3. Results and Discussion

Lipid is an important energy source and an essential component of the cell membrane (phospholipids and cholesterol). Besides this, they also play a significant role as messengers in

signal transduction pathways and molecular recognition processes [74]. Hence, any changes in lipid metabolism would signal to impairment of these crucial pathways. In the present study, total lipids, total cholesterol, and phospholipids levels in serum were significantly high in 96 hours effluent exposed fishes compared to control (Table 1). Similar observations have also been made in freshwater fishes inhabiting polluted water by some researchers [85, 86]. Cholesterol, phospholipids, and triglycerides combine to form total lipids; hence increase in serum phospholipids and cholesterol level is directly proportional to total lipids [87, 88]. Liver dysfunction and disturbance of lipid metabolism also favor elevation in phospholipids and cholesterol [89, 90]. Being an important structural component of plasma membrane, lipids maintain fluidity, so membrane degeneration of hepatic cells could be another possible cause of their elevation [91, 92].

In the present study, an elevated level of serum cholesterol in *Channa punctatus* exposed to paper mill effluent might indicate liver dysfunction, which normally esterifies cholesterol and excrete a part of it with the bile (Table 1) [92, 93]. Katti and Sathyanesan demonstrated that blood cholesterol elevation might be due to the inhibition of enzymes involved in lipid metabolism by toxic effluents leading to the slow removal of lipids from blood [94]. High cholesterol levels in the serum could also be due to lipid transport from the synthesis site for subsequent utilization either through oxidation or a process of the gradual instauration of lipid molecules [86, 95, 96].

In the present study, serum triglycerides level was significantly declined in test fish compared to control after 96 hours exposure to sublethal concentration of paper mill effluent (Table 1) [97, 98]. Low triglycerides level in blood was also reported in copper sulfate exposed fish, *Labeo rohita* [99]. It has been reported that constant energy demand leads to the mobilization of triglycerides since these serve as lipid depots [74, 100]. A decline in triglycerides could also be correlated to their utilization in membrane biogenesis or might be due to improper synthesis of triglycerides in the hepatic cells [74, 101].

Table 1. Alterations in serum lipid profile level in paper mill effluent exposed fish, *Channa punctatus* (N=6).

Experimental	Treatment of fish to sublethal concentration (10%) at different periods			
Group	24 hrs	48 hrs	72 hrs	96 hrs
Total Lipid(mg/dl)				
Control	474.21±0.42	472.57±0.34	473.51±0.29	471.28±0.37
Treated	482.87±0.25	496.24±0.26	512.28±0.21	532.18±0.31*
% increase over control	+1.83%	+5.01%	+8.19%	+12.92%
Total Cholesterol (mg/dl)				
Control	142.32±0.41	140.58±0.25	141.26±0.29	141.59±0.28
Treated	163.28±0.24	179.26±0.27	192.25±0.31*	218.32±0.06*
% increase over control	+14.73%	+27.51%	+36.09%	+54.19%
Phospholipids (mg/dl)				
Control	187.11±0.31	188.12±0.22	187.52±0.32	188.54±0.28
Treated	193.87±0.12	205.12±0.16	219.34±0.27	241.58±0.19*
% increase over control	+3.61%	+9.04%	+16.97%	+28.13%
Triglycerides (mg/dl)				
Control	138.58±0.21	138.15±0.16	137.87±0.19	138.21±0.22
Treated	122.25±0.16	114.98±0.18	99.28±0.17*	79.44±0.12*
% decrease over control	-11.98%	-16.77%	-27.99%	-42.52%

^{*}significant at 5% level of student 't' test

Lipid content is an essential organic constituent of the tissues of all animals. It plays a key role in energy metabolism as they are the best energy producers of the body next to carbohydrates [102, 103]. It acts as a reversed depot of energy from where it is supplied and when required [94]. Chezhian *et al.* reported that lipids are vital to embryogenesis, providing two-third of energy by oxidation [104].

Treatment of fish to sublethal concentration (10%) at different periods **Experimental** Group 24 hrs 48 hrs 72 hrs **96 hrs** Total Lipids (mg/l) 6.11±0.18 Control 6.15±0.11 6.18 ± 0.12 6.13±0.17 6.05±0.24 Treated 5.84 ± 0.23 5.69±0.28 5.46±0.31* % decrease over control -1.63% -5.50%-6.87% -10.93% Total Cholesterol (mg/l) 4.09±0.18 4.07±0.17 4.08 ± 0.15 4.08 ± 0.14 Control 4.35±0.15 4.49±0.21* Treated 4.24 ± 0.17 4.29 ± 0.14 +3.67% +5.15% +10.05% % increase over control +6.44% Phospholipids (mg/l) Control 18.28±0.14 18.54±0.19 18.35±0.18 18.44±0.15 Treated 17.62 ± 0.11 17.22 ± 0.15 16.69±0.17 16.25±0.32* -9.04% -11.88% % decrease over control -3.61% -7.12%Triglycerides (mg/l) 3.14 ± 0.07 3.15±0.08 3.16 ± 0.12 3.14 ± 0.15 Control 3.28±0.09 3.69±0.17* 3.98±0.19* Treated 3.45±0.34 % increase over control +4.46% +8.70%+14.36% +26.75%

Table 2. Alterations in lipid profile of liver in paper mill effluent exposed fish, *Channa punctatus* (N=6).

In the present study, a significant decline in total lipid content in the liver was recorded in freshwater test fish, *Channa punctatus* exposed to sublethal concentration of paper mill effluent (Table 2). Some workers also observed that industrial effluent decreases the total lipid content in different tissue of fishes [105-107]. A decrease in total lipid in tissues might be due to a sudden decrease in glycogen content in the same tissue, an intermediate energy source during toxic stress conditions [108, 109]. After glycogen, lipid content may be used for energy production to overcome toxic stress. Another region of depletion of lipid content may be due to lipolysis or the mitochondrial injury, which impaired the function of TCA cycle and the fatty acid oxidation mechanism [110-112]. The decreased level of tissue lipid content may be due to liver dysfunction or mobilization of glycerol or inhibition of oxidative phosphorylation [113, 114].

In the present study, a significant increase in triglycerides and cholesterol was noticed in the liver of test fish *Channa punctatus*, while there was a significant decrease in the phospholipid in the liver after 96 hours of exposure effluent (Table 2). A similar result was also observed in the reproductive tissue of *Channa striatus* exposed to tannery effluent by Sivachandran and Sultana [115]. In the present investigation, the increased level of liver triglyceride and cholesterol suggests that paper mill effluent interference in the process of steroidogenesis or vitellogenesis in the test fish [116]. The accumulation of triglycerides in the liver is due to impaired glycerol utilization, associated with disturbances in the biochemical process in hepatic cells [117]. The available glycerides are utilized to provide energy during effluent stress condition and their survival [118]. In the present study, decreased phospholipid level in the liver of effluent exposed fish was the sign of structural damage of hepatic cell membrane [119].

4. Conclusions

Like glycogen, Lipids are also a storage form of energy in aquatic fauna. The present study plays an important role in industry effluents risk assessment using a fish model that is a key indicator of environmental toxicity. The significant increase in serum lipid with decrease in liver of test fish, *Channa punctatus* Bloch (Actinoptrygii: Channidae) after exposure to sub lethal concentration of paper mill effluent. A decrease in the total lipid content of the liver with elevated serum lipid level in effluent exposed test fish suggests that lipid might have been

^{*}significant at 5% level of student 't' test

utilized in energy production for other metabolic functions during toxic stress conditions. The changes in biochemical constituents of serum and tissue are important to indicate the susceptibility of organ systems to toxicants by changing their function. The results may help understand the toxicity of the industrial effluents in the natural aquaculture system and may work as pre- alarming indicators of chemotoxicity in the freshwater fish, *Channa punctatus*. It can be concluded that paper mill effluent was toxic to the aquatic animals, and thus it is imperative to understand its ecotoxicological implications. However, the effluent is being regularly added to the water bodies in the natural environment, and the fish remains continuously in contact with a higher concentration of the chemicals. Thus, there is a greater risk in nature than observed under the experimental conditions. Therefore, further studies are required to arrive at a definite conclusion and to find out the active principle of the hazardous potential of the paper mill effluent.

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Conflicts of Interest

The authors declare no conflict of interest.

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