Indian J. Applied & Pure Bio. Vol. 38(3), 1267-1275 (2023). **ISSN: 0970-2091**
A Web of Science Journal

Effect of Dairy effluent and Neem oil on the physiology of *Clarias gariepinus* (Burchell)

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Abstract

Dairy industry is one of the major industries contributing to the economy of various communities and also to economic growth of the nations. However, the effluent from these dairy farms is frequently dumped in to the water bodies, leading to heavy water pollution, and thus making the waters unfit for aquatic life. Aquatic animals, particularly fish seem to be greatly affected by the contaminants, thus disturbing the food chain and also impacting the ecological balance. In this regard, the present study was undertaken, to study the impact of dairy effluent on fish. Hardy fish belonging to the species, *Clarias gariepinus* was selected for the study and was exposed to different concentrations of diary effluent. Various scientific studies have suggested that neem oil can be quite effective as an absorbent of dairy effluent and hence the fish were also exposed to exclusive neem oil treatment and also to a diverse combination of effluent along with neem oil moiety. Four different tissues - liver, gill, brain and muscle were selected for the study. Fishes were divided in to six different groups and were exposed to various concentrations of effluent, neem oil and also a combination of effluent with neem oil. Important biochemical compounds such as total free sugars, total protein and total lipids were estimated in different groups of fish. All the six groups of fish, showed a significant ($P < 0.001$) decline in total free sugars, total protein and total lipids. This decline may be attributed to gluconeogenesis, occurring in various tissues, so as to tide over the unfavorable conditions and survive in the effluent waters. Though neem oil was used to neutralize the effluent, it however did not have much of an impact on the effluent waters. This may be because the concentrations of neem oil may not be sufficient to nullify the effluent. Further studies may be conducted in future to effectively treat the dairy effluent using neem oil.

Key words : *Clarias gariepinus*, dairy effluent, neem oil, Total free sugars, Total Protein, Total Lipids.

Dairy industries generally release their waste into the nearby streams orland without any prior treatment causing serious pollutionproblems[20]. The dairy waste is white in color and usually the pH is alkaline. Rapid conversion of lactose to lacticacid reduces the pH of the effluents causing additional load ontreatment processes[18]. Dairy effluents-decompose readily and rapidly leading to depletion of dissolved oxygen ofthe receiving water bodies and resulting in anaerobic conditions and strongfoul odours[10]. If the wastes of a dairy plants are not treated properly beforetheir disposal to main sewage, the dairy industry will be contributing a large share to the total water pollution. Hence waste disposal only after its treatment is an importantconsideration in every dairy plant[11].

Dairy wastes often contain high levels of milk constituents such as casein, lactose, fat, inorganic salts, as well as detergents and sanitizers, all of which contribute significantly to high BOD and COD levels[12]. The high levels of suspended particles and dissolved solids indicate a considerable risk for contamination. Discharge of such pollutants into inland surface water will result in oxygen loss in the water bodies, harming aquatic life and producing unsanitary anaerobic conditions. Previous studies suggest that, dairy effluent is a serious problem, and must be treated before it is discharged in to the water bodies. In this connection, neem can be considered as a suitable absorbent of dairy waste as suggested by Vikas *et al.,*[23]. Studies suggest that neem oil contains several triterpenoids, Azadirachtin, Salannin,Sterols, Alkaloids, flavonoids, glycosides etc.[7,21] and therefore may be a suitable agent to degrade the dairy effluent. Neem oil was chosen for this study because it is biodegradable, safe, efficient, versatile, environmentally friendly, inexpensive and a natural biopesticide.

As dairy effluent is known to impact the water bodies and the aquatic life, the present study was attempted, to study the effect of dairy effluent upon aquatic animals, specifically fish, and also to study the impact of neem oil as an effective biodegradable agent of the dairy effluent. Fish exposed to environmental toxicants may also develop acute and chronic stress, which are characterised by a complex range of maladaptive behaviours and a condition of re-established homeostasis[4]. Such circumstances may disrupt physiological and biochemical processes, having a negative impact on the health and welfare of fish[3]. This study evaluated the toxicological effects in the African Catfish, *Clarias gariepinus*, a regularly consumed tropical freshwater fish, in order to bring to light the dangers posed by tropical fishes owing to dairy effluent exposure. This fish is widely cultivated both inside and outside of its natural range of tropical and subtropical environments[1]. They are also among the most widely cultivated fish in the world[5]. As a result of their widespread occurrence in both natural and artificial environments, they unintentionally come into contact with farming runoff, hence they serve as indicator species in this study.

This investigation was therefore aimed at elucidating the biochemical and haematological responses of *Clarias gariepinus* to acute toxicity of the dairy effluent and neem oil individually and in combination.

The freshwater fish, *Clarias gariepinus* (Burchell) wascollected from the Dharga fish pond, Red hills, Chennai, in cleanc ontainers of 10 litres capacity ensuring that they were not harmed either physically or physiologically during collection and transportation. 50 individual fisheswith weight (200 - 250g) and length (20 – 30 cm) were acclimatized to laboratory conditions, for a period of one week maintaining them in the 20 liters of dechlorinated and aerated tap water at 25°C. They were fed daily with pelleted commercial feed. Water in the fish tanks was changed. The fishes were maintained free from any contamination.

Treated dairy effluent was collected from a central Dairy industry (Aavin Nam) located in Madhavaram about 25 kms from Chennai, Tamil Nadu. Dairy effluent was collected in 40 litres capacity polythene containers and was broughtto the laboratory with due care and stored in therefrigerator at 20°C until further analysis. The dairy effluent was collected from the outlet of effluent treatment plant (ETP) of dairy.

The neem oil is a biodegradable, safe, efficient, versatile,environmentally friendly and above all low priced and a natural pesticide. Crude neem oil was obtained from International Instituteof Biotechnology and toxicological Research (TIBTR), Paddapai.

The bioassay procedure was carried out to calculate theLC_{50} of dairy effluent. The $LC_{50}$of effluent on the fishfor 96hr was calculated according to Weil,[25].

After calculating LC_{50} values the animals were segregated into6 groups where in each group contained 6 fishes.Group Iwere maintained in tap water,Group II fishes in water with 8% dairy effluent,Group III fishes in water with 16% dairy effluent, Group IV fishes in water with 6% Neem oil, Group V fishes in 8% dairy effluent mixed with 2% neemoil and Group VI fishes in 16% dairy effluent mixed with 4% neemoil.

The fishes were starved prior to the experiment for a period of 24 hrs. After 96 hours, the animals both control and experimental were sacrificed by decapitation without anaesthetization. The tissues - brain, liver, gill and muscle selected for the study. The tissues were excised following the procedure of Vijayan and Brownson[22].

The biochemical constituents like total protein, total free sugar and total lipids were analysed for acute toxicity in the selected tissues following standard procedures. Total Protein was estimated following the procedure of Lowry *et al.,*[9], total free sugars following Roe,[19] and total lipids following Folch *et al.,*[6].

Blood samples from fish of all six groups were used for haematological investigation. The haemoglobin was determined following the method of Sahil, 1962. Total WBC count and Total RBC count was estimated by methods outlined by Davidson and Hendry, 1969 and Dacie and Lewis, 1969. ThePacked Cell Volume (PCV) was determined using Wintrobe tube following the method of Mukherjee, 1998.

Statistical analysis was carried out with two-way analysis of variances (ANOVA).

In the present study, *Clarias gariepinus* was exposed to different concatenations of dairy effluents, neem oil and also a combination of effluent and neem oil. The results for acute toxicity (96 hr) were obtained for all groups. Total protein content in the Liver, gill, brain and muscle of *C. gariepinus* when exposed to the dairy effluent,neem oil and combination of both, neem oil showed a slight decrease in almost all the combinations. Statistical analysis of protein in the Liver, gill, brain and muscle of 6 different groups show highly significant variation at $P < 0.001$ level. The observations are illustrated in Table-1 and Figure 1.

This study sheds light on the biochemical changes in various tissues of the freshwater fish, C*larias gariepinus* through acute toxicity studies on exposure to dairy effluent and neem oil individually and also combination of both. Four different tissues were selected for the study *viz*: liver, gills, brain and muscle and biochemical investigation on three major metabolites (protein, free sugars and lipid) was performed on the tissues. All the tissues showed decline in these three metabolites on exposure to the dairy effluent which may be attributed, to the high contamination due to the dairy effluent which can have a drastic polluting impact upon natural waters[13]. Significant decline in total free sugars was observed in all the groups exposed to dairy effluent and neem oil in all the four tissues studied. This may be due to the hypoxic pollution stress caused by dairy effluent. Under the impact of the stress, to survive the undesirable environment, there may be synthesis of steroid hormones, such as glucocorticoids, or catecholamines,[14,24] by the adrenal glands of fish. These steroid hormones, may induce gluconeogenesis, thereby causing a decline in free sugar levels in tissues and may cause a rise in blood glucose levels as suggested by Amutha *et al.,*[2]. In the present study, fish were exposed to different concentrations of dairy effluent in combination with neem oil as neem oil is considered to be a natural absorbent, of dairy waste[23]. Though neem oil was used in combination with the dairy effluent, significant effect of neem oil as an absorbent of effluent was not observed. This may be because the percent of neem oil may not be sufficient enough to degrade the effluent. There was an overall decline in the proteins, free sugars and also in the lipid content of all the tissues of *Clarias gariepinus*. Under the influence of dairy effluent stress,the freshwater fish, *Clarias gariepinus* might switch to gluconeogenesis, thereby causing a fall in proteins, sugar and lipid levels so as to supply glucose to the effluent stressed fish. As the dairy effluent causes high BOD levels in the environment they are discharged, there may be release of glucocorticoids and catecholamines, which may induce gluconeogenesis, leading to the overall decline of three important energy components from various tissues.

The sharp decline in the haematological parameters in groups IV, V and VI may be due to stress induced hypoxia caused by the damage in the gills[15]. The decline in RBC likely reflects dysfunction of the hematopoietic system which is regarded as the susceptible indicator of environmental contaminants[17]. The reduction in WBCs indicates autolysis produced by hemolytic enzymes leaked out by cells under toxicant stress[8]. While decreased haemoglobin in dairy effluent-exposed fish may be attributed to disruption of haematological process and rapid breakdown of erythrocyte cell membrane, the decrease in PCV in blood indicated the existence of a toxic agent[16].

Table-1. Total Protein in different tissues of *Clarias gariepinus*

S.No	Different Groups	Liver	Gill	Brain	Muscle
1	Group I (control)	12.80	10.37	10.66	12.79
2	Group II (8% (DE)	10.46	9.01	8.38	11.61
3	Group III 16%(DE)	10.87	8.56	8.19	10.90
4	Group IV 6% (Neem Oil)	10.80	8.99	8.11	9.41
5	Group V 8% (DE+Neem oil)	7.92	8.86	8.05	8.67
6	Group VI 16%(DE+Neem oil)	7.94	8.98	8.34	7.92

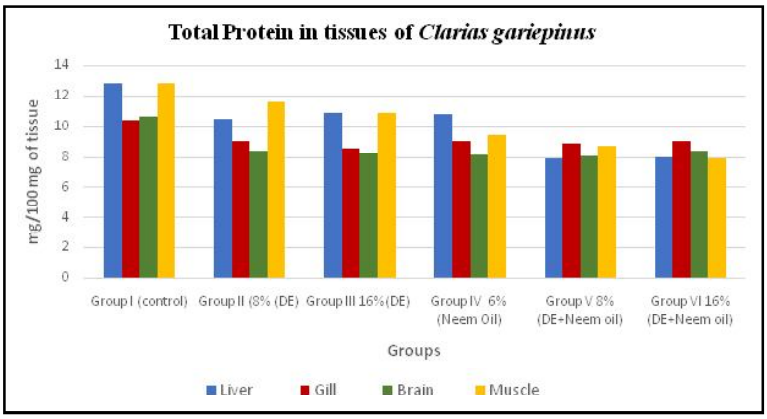

Figure. 1: Total Protein in different tissues of *Clarias gariepinus*

Total free sugars have been estimated in different tissues of *Clarias gariepinus*. Total free sugarsin the Liver, gill, brain and muscle of *C.gariepinus*when exposed to the dairy effluent,neem oil and combination of both observations were similar to the one observed in proteins. There was a slight decrease in almost all the combinations. Statistical analysis of protein in the Liver, gill, brain and muscle of 6 different groups show highly significant variation at $P < 0.001$ level. The observations are illustrated in Table-2 and Figure 2.

Table-2. Total free Sugars in different tissues of *Clarias t gngariepinus*

S.No	Different Groups	Liver	Gill	Brain	Muscle
1	Group I (control)	13.90	13.27	13.23	10.81
2	Group II (8% (DE)	13.28	10.86	12.19	10.04
3	Group III 16%(DE)	10.91	10.98	11.81	9.50
4	Group IV 6% (Neem Oil)	11.06	11.98	11.99	8.96
5	Group V 8% (DE+Neem oil)	10.25	10.07	7.94	8.15
6	Group VI 16%(DE+Neem oil)	10.80	10.44	8.40	7.94

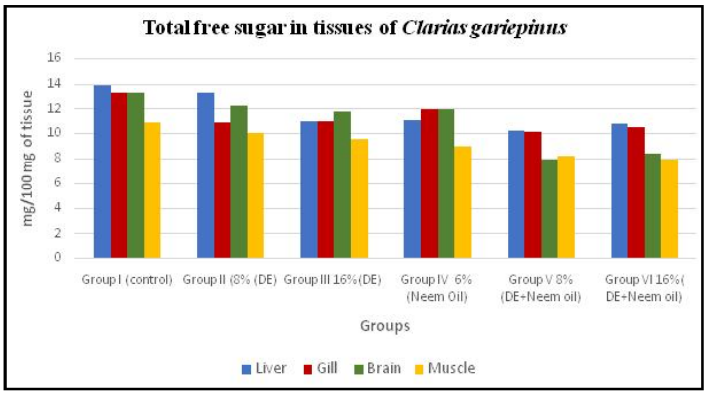

Figure. 2: Total free sugars in different tissues of *Clarias gariepinus*

Total tissue Lipids have been estimated in different tissues of *Clarias gariepinus*. Lipid content in the Liver, gill, brain and muscle of *C. gariepinus* when exposed to the combination and oil of dairy effluent,neem effluent and dairy effluent have been illustrated. Our observations were similar to the one observed in proteins. There was a slight decrease in almost all the combinations. Statistical analysis of protein in the Liver, gill, brain and muscle of 6 different groups show highly significant variation at $P < 0.001$ level. The observations are illustrated in the Table-3 and Figure 3**.**

Table-3. Total Lipids in different tissues of *Clarias gariepinus*

S.No	Different Groups	Liver	Gill	Brain	Muscle
1	Group I (control)	10.91	10.91	9.98	9.46
2	Group II (8% (DE)	9.93	9.32	7.85	8.37
3	Group III 16%(DE)	9.03	8.85	9.9	7.23
4	Group IV 6% (Neem Oil)	8.97	8.07	9.08	7.14
5	Group V 8% (DE+Neem oil)	8.47	7.83	9.68	7.65
6	Group VI 16% (DE+Neem oil)	8.03	6.07	7.78	6.93

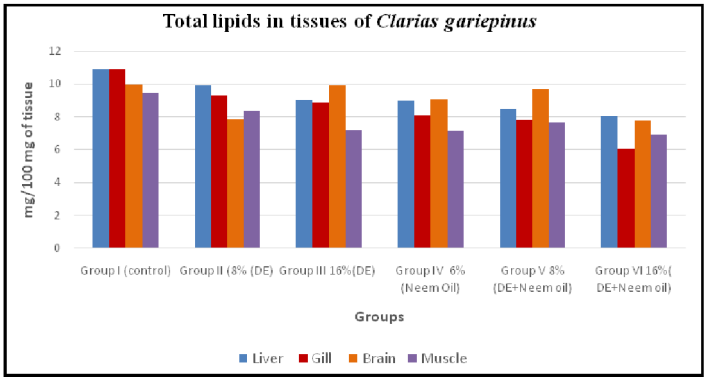

Figure. 3: Total Lipids in different tissues of *Clarias gariepinus*

The hematological analysis highlighted that Group IV, V and VIhave showed significant decline in Hb, RBC, WBC and PCV of *Clarias gariepinus*. Sharp decline of WBC was observed in Group IV. Decline in all four parameters were observed in Group IV and V, while there was a slight improvement in Group VI.

Table-4. Hematological analysis of *Clarias gariepinus*

	Group I (Control)	Group II (8% DE)	Group III (16%DE)	Group IV (6% Neem Oil)	Group V 8% (DE+ Neem oil)	Group VI 16% (DE+ Neem oil)
Hb (g/dl)	12.56	12.60	10.40	6.53	7.43	7.53
RBC (in millions)	2.55	2.75	2.37	1.64	1.88	1.91
WBC	210966	198966	153133	132933	177266	16966
PCV	35.23	29.40	28.67	20.13	20.13	22.77

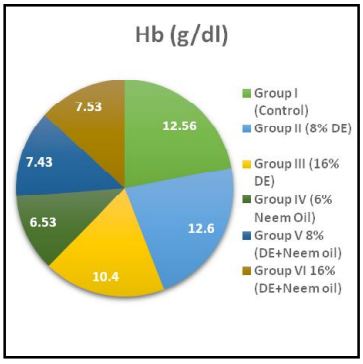

Fig. 4: Hb content in *Clarias gariepinus*

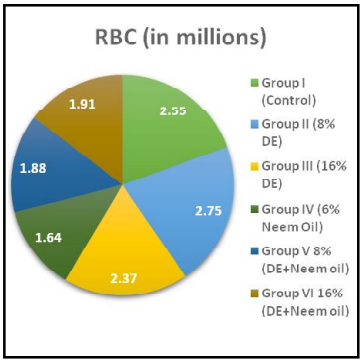

Fig. 5: Total RBC in *Clarias gariepinus*

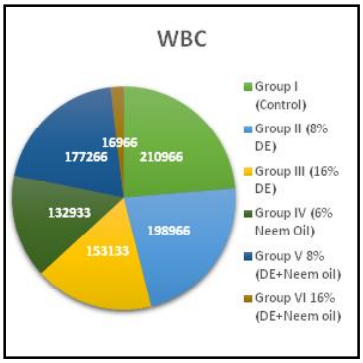

Fig. 6: Total WBC in *Clarias gariepinus*

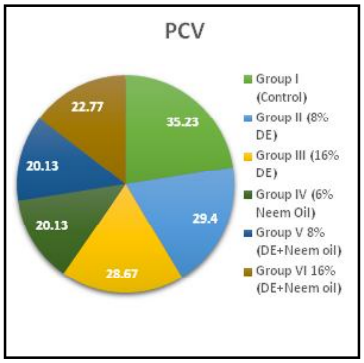

Fig. 7: PCV in *Clarias gariepinus*

Though neem oil has been known for its toxic degradation properties, it was not found to be effective when used as a natural degrading agent and absorbent of dairy effluent. The percentage of neem oil may have contributed to its ineffectiveness in degrading the dairy effluent. Hence, it is suggested that different percentages of neem oil be used along with the dairy effluent to prove its significance as a natural degradation agent.

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