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Pathophysiologic Mechanism of Erythrocyte Morphological Alteration in Freshwater Fish *Channa punctatus* Under Exposure of Nigrosine Black (Acid Black-2 a Tanning Industry Dye)

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Abstract**—** *The present study deals with the pathophysiological effects of Nigrosine black on the morphology of fish's erythrocyte. An exposure of 1/5th LC_{50} of Nigrosine black (378 mg/liter) was produced abnormal morphology in fish blood erythrocytes. After 14 days toxicant produced a spherical shape of erythrocyte with cytoplasmic vacuolation around the periphery of the cytoplasmic membrane of erythrocytes. After the third-week cytoplasmic vacuolation was appear around the lateral side of the nucleus. After the 35th day of the experiment, cytoplasmic vacuolation increased around the nucleus, while in a few erythrocytes, nuclei also showed their acentric condition. Chronic toxicity test (1/20th of LC_{50} i.e. 94.5 mg/ litter), produced cytoplasmic vacuolation and acentric nucleus condition in fish erythrocyte that enhanced after two weeks. Degeneration and fragmentation of cytoplasmic membrane of erythrocytes appeared after the 4^{th} to 6^{th}week of the experiment. Schistocytosis has appeared after the 60th day of the experiment along with a few ghost nucleuses. Pathophysiological condition of erythrocytes showed that it may produce alteration in cytoskeleton protein formation, disturbance in ion transport, gas transport, immune responses, deficiency of G6PD, increased lipid peroxide formation, altered ion permeability of cell membrane, and failure of tubulin polymerization in fishes.*

Keywords**— fish blood erythrocytes, Third-week cytoplasmic vacuolation, acentric nucleus condition, cytoplasmic vacuolation, altered ion permeability.**

I. INTRODUCTION

Dyes are the main constituent of tannery industries effluents (Kavitha and Ganapapthy 2015 and Angelika *et al*.2020**)** along with other toxic chemicals. Annually production of dyes is approximately 384 metric tonnes in India in 2020. In leather industries, approximately 10-12 per cent dye is used and 2-5 per cent disposed of after the tanning process.

Nigrosine Black (Acid Black-2, C.I. No50420, and $C_{22}H_{14}N_6Na_2O_9S_2$) is one of the developed dyes used in the tanning industry for the colouring of hides. Kanpur is an industrial city and about above the 259 tanneries is located near the side of river Ganga at Jajmau area. The large amount of tannery effluent containing the Nigrosine black produced every day in tannery poured into river Ganga and groundwater of Jajmau area. The water is an important constituent of fish and the presence of such chemicals may cause physiological problems in them.

The blood of fish is a fluid tissue and more important and sensitive tissue for various toxicant reflections. Erythrocytes of fish are nucleated hence its play an important role in physiology, immune system, protein signalling and haemostatic condition along with respiration. Few Authors (Jagruti and Anita,2015, Randhir

and Banerjee 2016, Avni and Alkesh 2021) were observe numerical and morphological anomalies in fish erythrocytes under different chemical exposure. According to Vosyliene (1996), the basic quantitative red blood parameter in fish tends to remain stable due to considerable compensatory potential but the morphological alteration in erythrocytes is a biomarker of environmental impact in fish. When these chemicals alter the morphology of erythrocytes, they affect the fish's entire physiology, which is bad for their population.

There is copious literature on the effect of chemicals on fish erythrocytes but no case reported on the effect of Nigrosine on fish erythrocytes morphology. Keeping this point of view in mind we decided to observe the effect of Nigrosine black dye on the erythrocyte morphology of freshwater fish *Channa punctatus*.

II. MATERIAL AND METHODS

2.1 Test Animal

Fish *Channa punctatus* of both sexes with varying weight were collected from local fish farmers from Kanpur city and disinfected by dipping them in 0.01% KMNO4 solution. After collection, the fish were maintained in laboratory aquaria for about 10 days for acclimatization following the method of Dehadrai (1971).

Fish were kept in a large size aquaria (2.5`x1`x1`) contain100 liter of water in each. Commercial fish food was supplied daily with water was 1/10th of their body weight. The water was changed daily with aeration.

2.2 Biochemical parameters of the water samples used in experiment and their methods of analysis.

PARAMETER	METHOD OF ANALYSIS	VALUE	REFERENCES
pH	Digital pH meter	7.36 ± 1.97	
Total hardness	EDTA titrimetric method	283.22 ± 4.57 mg/l	**APHA (2005)**
Dissolved oxygen	Wrinkle method	8.2 ± 0.44 mg/l	**APHA(2005)**
Turbidity	Nephlometric	281 ± 3.85 mg/l	**APHA (2005)**
Temperature	Digital thermometer	28.87 ± 1.47^{O}C	**APHA (2005)**

2.3 Test chemical used in present study.

The test chemical is used in the present studies is Nigrosine Black (Acid Black-2, C.I. No50420, $C_{22}H_{14}N_6Na_2O_9S_2$) obtained from a local vendor.

2.4 Experiment design and schedule of treatment.

Ludmila (1996) was reported LC_{50} (96 hrs.) of Nigrosine black for freshwater fish *Poeciliareticulata* is 1890 mg/l. On the basis of this information, the following exposure duration and concentrations were used with the control group.

1. 1/5th concentration of LC_{50} i.e. 378 mg/liter for 35 days exposure (sub-acute).
2. 1/20th concentration of LC_{50} i.e. 94.5 mg/ litre for 56 day exposure (chronic).
3. Exposed fish in normal water used as controlled with the whole duration of the treated experiment.

All parameters for study in fish will measure at 7, 21, and 35 days for sub-acute while 15, 35, 45, and 56 days for chronic toxicity test along with control, each group have 10 fishes.

2.5 Blood Sampling and the Making of Blood Smear

As per the above-fixed schedule, 6 fishes of each group were anesthetized and sacrificed. Blood of fishes was collected from the caudal peduncle in a heparinized vial for haematological analysis. Blood smears of fish blood were prepared by taking a drop of blood on a glass slide and smeared smoothly and air-dried. Now smear blood slide was fixed with methanol after that blood smear was stained by May Grünwald stain for 5 minutes after that stained slide was washed with 6.8 Ph buffer solutions for 1 minute. After wash with buffer solution blood smear was re-stain with 5% Giemsa stain for 10 minutes, followed by a 10-second wash with Ph. neutral water. After washing the slide, let it air dry, then use one drop of mounting medium on the slide and place a coverslip on it, and then it was photo grouped.

2.6 Analysis of nuclear and cellular morphology of erythrocytes

Morphological analysis of fish erythrocytes were done according to method of Jahan*et al.*,(2019).

III. RESULT

The Control group of fishes was showing normal erythrocytes of *Channa punctatus*, which is oval in shape with a clear cytoplasmic membrane. Pink color cytoplasm filled inside the erythrocytes and oval to round shape nuclei were present at the center of erythrocytes. Cytoplasmic membrane structure and the shape of erythrocytes were also normal in the control group. Few elongated shape erythrocytes were appearing to represent more mature cells. Few leucocytes i.e. heterophils were also seen in blood smear. (**Photo-1**)

A sub-acute toxicity test (1/5th of LC_{50} of Nigrosine black) was produced an abnormal shape of erythrocytes in fish after the 14th day of the experiment. Toxicant Nigrocine black has altered the shape of oval erythrocytes into spherical shape erythrocytes and a few cytoplasmic vacuolation were also seen. Cytoplasmic vacuolation appeared around the periphery of the cytoplasmic membrane. Some immature erythrocytes were devoid of nuclei. (**Photo -2**)

After the 21stday of the experiment, the cytoplasmic vacuolation were appearing around the nucleus of erythrocytes in experimental fish. Few erythrocytes showed more cytoplasmic vacuolation at both terminal ends of the nucleus. Later on, these cytoplasmic vacuoles increased in their size and after fusing with each other, surround the nucleus of the erythrocyte. (**Photo.-3**)

After the 35thday, Nigrosine black toxicity produced progressive cytoplasmic vacuolation in the cytoplasm and around the nucleus. Few erythrocytes were showing the eccentric conditions of nuclei i.e. cytoplasm and nucleus were reach to the opposite pole of erythrocytes. (**Photo.-4**)

The chronic toxicity of Nigrosine black (1/20th of LC50) produced cytoplasmic vacuolation along with little degeneration in erythrocytes on the 15th day of the experiment. (**Photo.-5**)

On the 30th day of chronic exposure produced degeneration of cytoplasmic membrane which changes the shape of erythrocytes. Some hyaline circles also appeared around the nucleus (**Photo.-6**).More cytoplasmic vacuolation and degeneration of the nucleus appeared on the 45thday of the experiment. (**Photo.-7**)

Schistocytosis conditions of the erythrocytes of fish were appearing on the 60th day of the chronic experiment. Degeneration of erythrocytes nucleus was clearly seen along with ghost nucleus. (**Photo.-8**)

3

IV. DISCUSSION

According to Christopher (2004), evolution and interpretation of erythrocyte morphology is an important factor for a complete blood count. Erythrocyte morphology may provide important diagnostic information. In the present study, it is tried to obtain sub-acute and chronic toxicity effect of Nigrosine black dye on erythrocytes morphology of fish Channa punctatus.

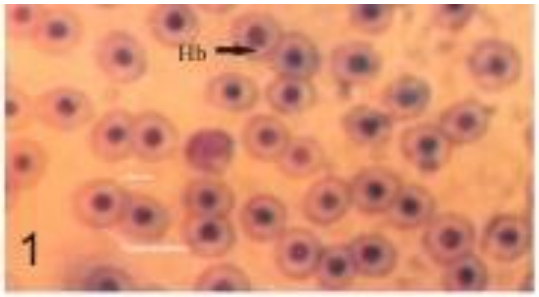
Results of chronic and sub-acute toxicity of Nigrosine black for fish bring out changes into erythrocyte cytoplasmic membrane. It was reported by Christopher (2004) that erythrocyte's shape is maintained by protein Actin, Spectin, and Ankyrin , which is present in erythrocytes in a hexagonal lattice web. In the present study, the changes in the fish erythrocyte plasma membrane may change the management of above mention protein due to exposure to Nigrosine black dye.

It was also observed in the present study that the shape of fish erythrocytes also change after exposure to Nigrosine black which indicates some alterations in ion transporters.Thomas (1998) reported that in fish red blood cells, several ion transporters play a major role in cell volume regulation and intracellular acid-base regulation. Changes in shape and volume of fish erythrocyte in experimental fish indicate that toxicant Nigrosine black changes the function of ion transporter in fish.

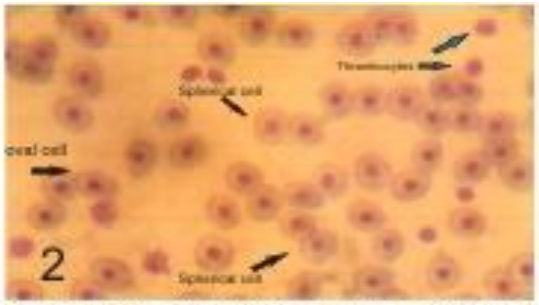
Sanjib*et al*., (2005) reported that absorption of toxic substance in fishes change fish erythrocytes plasma membrane's ion permeability and damage the erythrocyte plasma membrane. In the present study, the same finding was observed which supports our conclusion.

Ford (2013) observe in a study that oxidative hemolysis produced irregular constricted erythrocytes and these cells are known as bite and blister cells and this oxidative hemolysis may also lead to schistocytosis and spherocytosis because of deficiency of G6PD. In the present investigation, Nigrocine black dye also produces oxidative hemolysis in the blood of experimental fish which later on leads to spherocytosis and schistocytosis condition of erythrocytes, hence above findings of Ford (2013) support our findings.

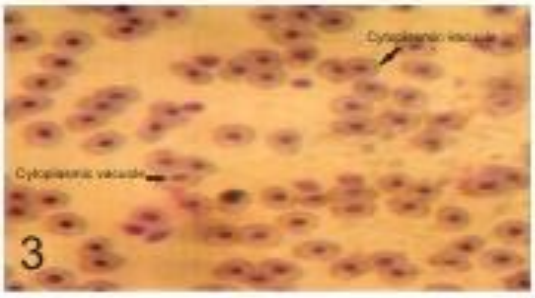
The present study showed that Nigrosine black dye brings out abnormalities in the erythrocyte's nucleus of fish. Few research workers mention in their study that the nucleus of fish erythrocyte plays a vital role in all metabolic and genetic activity (Shahjahan *et al.*, 2020) hence alteration in nucleic material indicate that Nigrosine black toxicant, malfunction the nucleus of erythrocytes also and altered their function.



Photograph-1 RBC of *Channa punctatus* showing normal oval shape with nonnal nucleus, normal plasma membrane and cytoplasm May Grunwald and Giemsa stain X1000

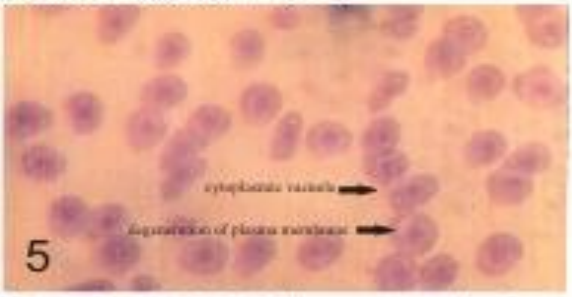


Photograph- 2. RBC of *Channa punctatus* is showing spherical Red blood corpuscles with nucleus. Few large and small thrombocyte also seen after 1/5th LC_{50} exposure of Nigrosine black May at 14 day of experiment Grunwald and Giemsa stain.X1000

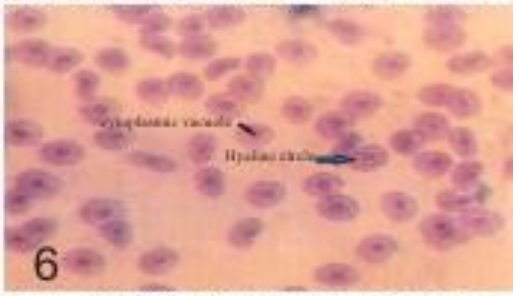


Photograph- 3. RBC of *Channa punctatus* is showing cytoplasmic vacuole in RBCs after 1/5th LC_{50} exposure of Nigrosine black at 21 days of experiment May Grunwald and Giemsa stain.X1000

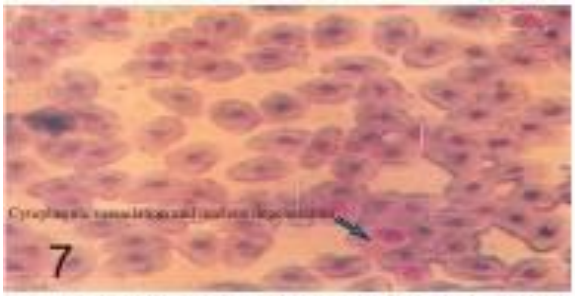
Photograph- 4. RBC of *Channa punctatus* is showing more cytoplasmic vacuole and eccentric position of nucleus in RBCs after 1/5th LC_{50} exposure of Nigrosine black at 35 days of experiment May Grunwald and Giemsa stain.X1000



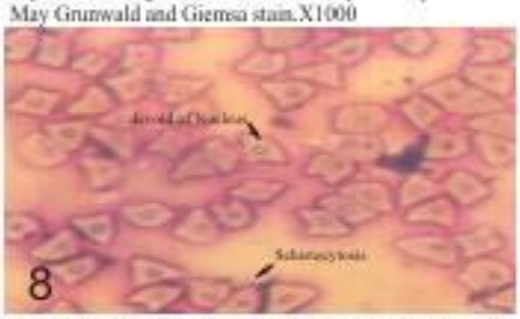
Photograph- 5. RBC of *Channa punctatus* is showing cytoplasmic vacuole around nucleus of RBCs after 1/20th LC_{50} exposure of Nigrosine black at 15 days of experiment May Grunwald and Giemsa stain.X1000



Photograph- 6.RBC of *Channa punctatus* is showing more cytoplasmic vacuole around nucleus of RBCs and some hyaline circle around the nucleus after 1/20th Lc_{50} exposure of Nigrosine black at 30 days of experiment May Grunwald and Giemsa stain.X1000



Photograph-7.RBC of *Channa punctatus* is showing more cytoplasmic vacuole and degeneration of nucleus along with leakage of cytoplasmic membrane which spill hemoglobin of cellafter 1/20th Lc_{50}exposure of Nigrosine black at 45 days of experiment May Grunwald and Giemsa stain.X1000



Photograph- 8.RBC of *Channa punctatus* is showing devoid of nucleus of RBCs , total degeneration and Schistocytosis conditions of the erythrocytes after 1/20th Lc_{50}exposure of Nigrosine black at 60 days of experiment May Grunwald and Giemsa stain.X1000

Sarder*et al.*, (2002) reported that all nucleated cells are capable of presenting an antigen, through major histocompatibility complex (MHC) molecules and nucleated RBC can express MHC and these molecules

have been found on the surface of RBSc, act as immune function. It has been observed that fish erythrocytes act as immune cells (Passantino et al., 2002). Alteration in the RBC of fish in the present study indicates that Nigrosine black also impairs immune function in fishes.

Verónica*et al*., (2018) reported red blood cells of fish are nucleated and these are multifunctional in nature because, RBC is involved in the exchange and transport of gas, along respond to pathogens through cytokinesis, presentation of antigen, phagocytosis, antimicrobial peptides, complement regulatory system, and communication of molecular paracrine with other immune systems. Puente *et al*., (2019) were reported that nucleated Red blood cells of fishes are responsible for the modulation of different sets of genes of expression in the response of stimuli and play an important role in the maintenance of homeostasis of fish's immune system.

Red blood cells are carriers of oxygen and perform the function of respiration among vertebrates. Hemoglobin present in red blood cells is the main component that carries oxygen from the respiratory organs to the main organs of fish and carbon dioxide from organs to respiratory organs. Changes in structure and function of RBC in Nigrosine black toxicant exposed fish also affected the respiration and transportation of gas in experimental fish. This study found that Nigrosine black dye exposure significantly impairs the functions of erythrocyte plasma membrane transport, ion transport, hemoglobin density, nucleus function, and immune function in fishes, which is highly harmful to their health.

V. CONCLUSION

Present study showed that Nigrosine black toxicity produced following in fresh water fish *Channa punctatus*.

1) It is produced abnormality in erythrocyte shape.
2) Nigrosine black is also damage cell membrane of fish erythrocytes.
3) Toxicant is also bringing change in cytoskeleton protein of erythrocyte.
4) Test chemical is also produced abnormality in nucleus of cell and shows deformation in them.
5) It is also altered the gases exchange function in exposed fishes and also altered their transportation properties.

REFERENCES

[1] American Public Health Association (APHA, 2005), Standard methods for the examination of water and waste water, 21st edition, American water works association, Water Environment federation, Washington DC. https://www.scirp.org/(S(czeh2tfqyw2orz553k1w0r45))/reference/ReferencesPapers.aspx?ReferenceID=1870039

[2] Angelika Tkaczyk, KamilaMitrowska, AndrzejPosyniak, (2020) Synthetic organic dyes as Contaminants of the aquatic environment and their implications for ecosystems: *Science of The Total Environment*, Volume 717.13722. https://doi.org/10.1016/j.scitotenv.2020.137222.

[3] Avni I. Parmar&Alkesh I. Shah (2021) Acute toxicity, behavioural response and haematological alterations of *Catlacatla*exposed to Reactive Red 120 textile dye. *Indian Journal of Experimental Biology* Vol. 59, pp. 275-279

[4] Christopher.,M.Mary (2004) Interpretation of red blood cell (Advance level).The 29th World small animal veterinary Association world congress proceeding online. https://www.vin.com/apputil/content/defaultadv1.aspx?pId=11181&catId=30079&id=3852161

[5] Dehadrai, P.V. (1971): Physiological adaptation of air breathing fishes in relation to adverse environmental features. First workshop of the All India Coordinated Research Project on culture of Air breathing fishes, Cuttack September. P.11. http://eprints.cmfri.org.in/8327/1/SEA_FOOD_EXPORT_JOURNAL_VOL_7_(35-41.pdf

[6] Ford. J. (2013) Red blood cell morphology. International journal of laboratory hematology.35,351-357.https://doi.org/10.1111/ijlh.12082

[7] JagrutiKhandubhaiBarot and Anita Bahadur (2015) Toxic Impacts of C.I. Acid Orange 7 on Behavioural, Haematological and Some Biochemical Parameters of *Labeo rohita* Fingerlings.*International Journal of Scientific Research in Environmental Sciences*, 3(8), pp. 284-290,

[8] Jahan, A., Nipa, T. T., Islam, S. M. M., Uddin, M. H., Islam, M. S., and Shahjahan, M. (2019). Striped catfish (*Pangasianodonhypophthalmus*) could be suitable for coastal aquaculture. *J. Appl. Ichthyol.* 35, 994– 1003. https://doi.org/10.1111/jai.13918

[9] Kavitha. P.R. and Ganapathy. G.P. (2015) Tannery process and its environmental impacts a case study: Vellore District, Tamil Nadu, *India.Journal of Chemical and Pharmaceutical Sciences* 8(4):759-764

[10] LudmilaDolezalova (1996). Korostan Black BN3 testing on Eco toxicity. Testing laboratory: VUOS. Report no.: 4220/5A. Owner Company: Synthesia AS.

[11] Passantino .L., M. Altamura, A. Cianciotta, R. Patruno, A. Tafaro, E. Jirillo& G. F. Passantino (2002) Fish immunology. I. Binding and engulfment of *candida albicans* by erythrocytes of rainbow trout (*salmogairdneri* richardson), *Immunopharmacology and Immunotoxicology*, 24:4, 665-678,

[12] Puente-Marin S, Thwaite R, Mercado L, Coll J, Roher N and Ortega-Villaizan MDM (2019) Fish Red Blood Cells Modulate Immune Genes in Response to Bacterial Inclusion Bodies Made of TNFa and a G-VHSV Fragment. *Front. Immunol.* 10:1055. doi: https://doi.org/10.3389/fimmu.2019.01055

[13] Randhir Kumar and T.K. Banerjee. (2016) Arsenic induced haematological and biochemical responses innutritionally important catfish *Clarias batrachus* (L.). *Toxicology*

Reports 3 .148–152.https://doi.org/10.1016/j.toxrep.2016.01.001

[14] SanjibSen Gupta, Ashok Kumar, and Jyoti prakash, Srivastava (2006). Effect of chromium sulphate on haematological factors of the fish *Heteropneustis fossilis.J. Ecotoxicol. Environ. Monit*. 16(4):363-370.http://www.journalcra.com/sites/default/files/issue-pdf/Download%204547.pdf

[15] SardarM .,Rashid, M.M., & M.R., I. (2002). In vitro phagocytic study of blood leucocytes and peritoneal macrophages of walking catfish *Clarias batrachus* against *Aeromonashydrophila* and *Eacherichia coli*. *Bangladesh J. Fish. Res*., 6(1), 35-41

[16] Serge ThomasaStéphaneEgéea.(1998)Fish Red Blood Cells: Characteristics and Physiological Role of the Membrane Ion Transporters ,Comparative Biochemistry and Physiology Part A: *Molecular & Integrative Physiology*Volume 119, Issue 1, Pages 79-86

[17] Shahjahan, M.; SabiaKhatun, M.; Mostarin. Mun.M.;Majharul Islam, S.M.; Uddin, M.H.; Badruzzaman.M. andKhan.S. (2020) Nuclear and cellular abnormalities of erythrocytes in response to thermal stress in common carp *Cyprinuscarpio*. *Front. physiol.*11:543.https://doi.org/10.3389/fphys.2020.00543

[18] Thomas. S. and Egee. S. (1998) Fish red blood cells: characteristics and physiology role of membrane ion transporters. Comp. Biochem. Physiol A mol. Intergr. Physiol.119 (1): 79-86. DOI :10.1016/s1095-6433(97)00404-2

[19] Vosylienė, M. Z. (1996.)The Effect of Long-term Exposure to Copper on Physiological Parameters of Rainbow Trout *Oncorhynchusmykiss*. 2. Studies of Haematological Parameters. *Ekologija* 1: 3–6.

[20] Verónica Chico , Sara Puente-Marin , Iván Nombela , Sergio Ciordia , María Carmen Mena , Begoña Carracedo , Alberto Villena , Luis Mercado , Julio Coll, María Del Mar Ortega-Villaizan (2018) Shape-Shifted Red Blood Cells: A Novel Red Blood Cell Stage.*Cells* 19;7(4):31.https://doi.org/10.3390/cells7040031.