SUMMARY

- The median lethal concentration (LC50) of sodium nitrite to an Indian major carp, *Cirrhinus mrigala* for 24 and 96h were found to be 28.31 and 19.95 mg/L respectively. 1/10th of the LC50 value for 24h (28.31) was taken as sublethal concentration.

- During acute treatment of nitrite, the fish *Cirrhinus mrigala* showed various behavioural changes such as restlessness, abnormal swimming, behaviour, loss of balance, copious secretion of mucous and spreading of excess mucous all over the body surface, hyper excitability, loss of scales, floating upside down with abdomen directed towards, rapid opercular movement, gulping of air, asphyxiation and finally jerky movement before the death.

- The main toxic action of nitrite on aquatic animals is due to the conversion of oxygen-carrying pigments to forms that are incapable of carrying oxygen, causing hypoxia and ultimate death.

- In the present study the significant increase in plasma T₄ and T₃ level of fish *Cirrhinus mrigala* during acute and sublethal treatment might have resulted from activation of hypothalamus–pituitary–thyroid (HPT) axis by nitrite. The elevated T₄ levels may be due to a change in the equilibrium between T₃ and T₄, maintained by peripheral monodeiodination processes, causing a ‘backing up’ of plasma T₄ levels. The observed increase in T₃ levels might be a response to maintenance of the basal metabolic rate of the fish to ensure its survival.

- The significant decrease in plasma T₄ during the initial period of sublethal treatment indicates a decrease of the thyroid gland activity.

- In the present study the significant decrease in plasma sodium ion of fish *Cirrhinus mrigala* during acute and sublethal treatment might have resulted from disruption of Na⁺/K⁺–ATPase due to nitrite toxicity.

- The observed increase in plasma potassium ion during acute and sublethal treatment indicates K⁺ efflux from erythrocytes and skeletal muscle tissue, leading to extracellular hyperkalemia. Significant decrease in plasma potassium
level during sublethal treatment might have resulted from inhibition of the Na\(^+\)/K\(^+\) -ATPase due to nitrite toxicity or structural alteration in renal tubules in the kidney of fish.

- The significant decrease in plasma chloride ion during sublethal treatment may be due nitrite-induced hypertrophy and hyper functionality of chloride cells and gill Na\(^+\)/K\(^+\) ATPase activity or may be due to inhibition of carbonic anhydrase by nitrite toxicity.

- In general significant decrease in plasma sodium, chloride and potassium level during acute and sublethal treatment of nitrite might have resulted from the structural damages in the gills due to nitrite toxicity and also affecting the transport processes such as the Na\(^+\)/K\(^+\) -ATPase. The gills, due to their intimate contact with the environment are important effectors of osmotic and ionic regulation may be a target for nitrite action.

- In the present study during acute and sublethal treatment the hemoglobin and hematocrit values of fish *Cirrhinus mrigala* were decreased both at acute and sublethal nitrite treatment. It may be attributed to the destruction of blood cells or a defect in the Hb molecules during nitrite toxicity. Further nitrite might have affected the hematopoietic system and biochemical pathway of heme formation. The observed decrease in hematocrit value in the present study indicates the anemic condition of fish due to nitrite toxicity.

- The significant increase in Hb and Hct value during initial period of nitrite toxicity perhaps attributed to the erythropoiesis reactivation mechanism induced by the spleen and liver to compensate the cerebral hypoxia induced by nitrite.

- Significant decrease in RBC count during acute and sublethal nitrite treatment may be due to a decrease in nonspecific immunity of the fish due to stress or it may be due to accumulation of nitrite in the organs like gill, liver and kidney which may cause internal hemorrhage resulting in a decrease in the number of red blood cells. Further impaired osmoregulation and gill damage during nitrite exposure resulted in hemodilution, which lead to a decrease in the number of red blood cells through haemolysis.
In the present investigation, the reduction in WBC count during acute and sublethal treatment (after 21st day) may be attributed to immunological suppression of the cells by nitrite toxicity. In contrast the significant increase in WBC cells during sublethal treatment (upto 21st day) indicated an immediate stimulation of the immune system to protect the fish against infections that might have been caused by nitrite.

The significant increase of MCV and MCH during acute and sublethal treatment indicates swelling of red blood cells due to hypoxia or impaired water balance due to nitrite intoxication. The increases in MCV and MCH suggested that the anemia was the macrocytic nesmochromic type. Whereas the observed decreases in MCV value during sublethal treatment (except 7th day) indicate that the erythrocytes have shrunk, either due to hypoxia, stress or impaired water balance.

The significant increase of MCHC value during acute and sublethal treatment may be due to congenital sphaerocytosis. Decrease in MCHC value during acute and sublethal treatment is probably an indication of red blood cell swelling and/or to a decrease in hemoglobin synthesis.

In the present study the observed increase of plasma glucose level of fish *Cirrhinus mrigala* during acute and sublethal treatment indicates a stress response triggered by the presence of nitrite in water or might be due to hypoxic condition caused by the nitrite in water. Further high plasma level may be an indicative of simultaneous activation of the HPI and HSC axes by nitrite and induction of different compensatory responses or may be attributed to the hepatocellular damage and kidney failure as a result of toxic effect of nitrite.

The significant increase in liver glycogen content of fish *Cirrhinus mrigala* during acute and sublethal treatments indicates impairment in the carbohydrate metabolism or a decrease in the rate of glycogenolysis.

The observed reduction in muscle glycogen content of fish *Cirrhinus mrigala* during acute and sublethal treatment indicates the utilization of stored glycogen to meet the high energy requirement under the toxicant nitrite stress.
In the present study the observed decrease in plasma protein level during acute and sublethal treatment might have resulted from an increase in protein catabolism or impaired protein synthesis due to liver disorder or nephrosis.

The significant increase in gill Na\(^+/K^+\)-ATPase activity during acute toxicity indicate the direct toxicity of nitrite on ATPase function or a compensatory response to maintain serum Na levels at a constant level (in the present study the plasma Na levels was decreased both in acute and sublethal nitrite treatment).

In contrast, the decrease in gill Na\(^+/K^+\)-ATPase activity during sublethal treatment indicates disruption in its cellular and ionic regulation and salt uptake. Gills Na\(^+/K^+\)-ATPase activity depression could also result from gill destruction, mainly of chlorine cells. Since the gills are primary target organ for toxic action of nitrite it affect the major target molecules the ion dependent ATPase, which lead the disturbances in ion homeostasis.

In the present investigation the observed epithelial necrosis and desquamation of the gill epithelium of fish Cirrhinus mrigala exposed to acute and sublethal nitrite toxicity are direct responses induced by the action of nitrite. The defense responses noticed are lifting up of the epithelium and lamellar fusion. The lifting of the epithelium increases the distance through which the toxicant has to travel to reach the blood stream. Lamellar fusion could be protective in that it diminishes the amount of vulnerable gill surface area. Gill hyperplasia might serve as a defensive mechanism leading to a decrease in the respiratory surface and an increase in the toxicant blood diffusion distance. Histological damage to gill surfaces by nitrite is attributed to high accumulations in gills, irritation due to elevated mucous secretion, increased ventilation volume and decreased gill oxygen uptake efficiency.

The severe destruction of hepatic cells and formation of inter cellular spaces in liver of nitrite treated fish could be possibly due to sudden withdrawal and utilization of stored glycogen from the liver cells of fish to meet the energy demand during nitrite stress. The vacuolation of hepatocytes might indicate an imbalance between rate of synthesis and rate of release of substance in the hepatocytes. Increased cellular and nuclear volume of hepatocytes can be considered...
as responses to the stressor agent, since they indicate the activation of the liver functions and do not interfere with the hepatic performance, but rather indicate the intensification of hepatocytes metabolic activity under adverse conditions.

- In the present investigation during acute and sublethal treatment, the high incidence of cytoplasmic vacuolation, associated with granular and hyaline droplet degeneration and necrosis in the proximal and distal tubules characterize degenerative changes in the renal tissue, normally related to the presence of nitrite in the filtrate from the glomerulus. Moreover, these degenerative changes may be due to altered metabolic activity or due to nitrite ion-renal tissue interaction. The toxic action of nitrite may be direct on the cellular components or occur by inducing stress responses.

- In the present study the observed histological lesions of gill, liver and kidney of fish, *Cirrhinus mrigala* may be nonspecific responses of fish to nitrite toxicity. The magnitude of the above alterations showed a great relationship with degree and duration of stress.

- The present study provided evidence that the waterborne nitrite induced changes in hormonal, ionoregulatory, hematological, biochemical parameters and altered morphological structure of gill, liver and kidney both at acute and sublethal concentration. These biomarkers offer a rapid and sensitive means of monitoring towards the impact of nitrite ion on aquatic biota and ultimately whole of the ecosystem.

- Further more these biomarkers can be used to revise rapid, effective screening assays, which can complement other testing techniques by significantly reducing the number of samples that may require a more elaborate, definitive or specific evaluation. Biomarker-based techniques do have a major role to play in the overall effort of environmental monitoring and protection.
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*Originals not seen