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Stress of inorganic pollutant (Nitrate) Induced histopathological alterations in haemeopoetic tissues of *Aspidoparia morar*

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Abstract
The present study examined the histological response induced by inorganic pollutant (nitrate) in a fish, *A. morar* inhabiting Jammu waters. LC_{50} value of nitrate for the studied fish was found to be 2 mg/l. The fish were subjected to three sublethal concentrations of nitrate viz., 25% (0.5 mg/l), 50% (1 mg/l) and 75% (1.5 mg/l) of LC_{50} value of nitrate were employed for the experimental duration of 9 weeks. The hemopoietic tissues were found to be totally damaged by the nitrate intoxication. Liver exhibited fibrosis, necrosis, steatosis followed by degenerative changes. Anterior kidney and spleen, however showed lymphocytic infiltration tubular necrosis followed by vacuolation and degenerative changes. The alterations observed were found to be responsible for hampering the entire metabolic machinery of the fish.

Keywords: Hemopoietic tissues, Nitrate, *Aspidoparia morar*, Histopathological alterations.

1. Introduction
A great variety of pollutants which find entry into water bodies come through domestic, industrial and agricultural effluents [1]. The complexity of the situation created by their presence in aquatic ecosystem becomes apparent only when toxicity is keenly observed in terms of its ramifications and environmental consequence [2]. Among various pollutants, nitrate is of very common usage as fertilizer, which finds its way into the water bodies from the various agricultural fields. Although moderate quantities of nitrate are required as fertilizer to maintain the nutrient budget of water body, but if its level exceeds the limit, it can act as toxicant and may even result in general enfeeblement, retardation of growth and metabolic and pathological changes in physiological process of all the aquatic organisms particularly fishes [3]. Among various physiological processes (besides feeding, osmoregulation, blood), histopathological studies are the ones which, if monitored, can help to establish causal relationship between contaminant exposure and various biological responses. Histopathological investigations have proved to be a sensitive tool to detect direct effects of chemical fertilizers within target organs of fish in laboratory experiments [4]. During the present course of investigations, experiments were conducted to elucidate the effects of sublethal concentrations of nitrate (25%, 50% and 75% of LC_{50} value of nitrate) on the haemopoietic tissues viz., liver, anterior kidney and spleen.

2. Materials and Methods
2.1 Sampling site
The fish, *A. morar* for the present study were netted from Nikowal region of River Tawi, Jammu.

2.2 Histological analysis
The test fishes were dissected open in ringer solution and hematopoietic tissues viz., liver, anterior kidney and spleen were fixed in Bouin's fixative. After post fixation treatment and routine dehydration and clearing, these tissues were embedded in histowax of 54-56 °C. 5-7 µm thick section of these were cut on microtome and stained using haematoxylin eosin stain.

3. Results and Discussion

a) Liver: Liver has the ability to degrade toxic compounds, but its regulating mechanism can be overwhelmed by elevated concentration of toxicants and can definitely result in its structural damages [5]. Liver of the control fishes as shown in the Fig. 1clearly depicts the presence of hepatocytes (involved in such functions as protein synthesis, cholesterol and bile salts synthesis, detoxification, modification and excretion of wastes [6]. Hepatocytes are traversed by sinusoids (Fig. 1) (blood vessel that serves as a location for the oxygen rich, blood [7], Melanomacrophage centres (Fig. 1) acts as depositories for intracellular bacteria, antigen trapping and sequestration of products of cellular degradation and potentially toxic tissue materials such as catabolic breakdown products are some of the other functions of the liver [8]. During present studies, *A. morar* exposed to different sublethal concentrations of nitrate (25%, 50% and 75% of LC_{50} value of nitrate) exhibited various histopathological alterations such as i) fibrosis (Fig. 2) (formation of excess fibrous connective tissue) which became evident from 2nd week, ii) fibrosis was followed in pursuit by necrosis, which implies cell death [9, 10] of liver tissue, which got initiated from 3rd week of the experiment and became pronounced by the 6th week (Fig. 3, 4) iii) steatosis visualized as vacuoles (abnormal retention of lipids within the liver cell) was observed after 8th week which (Fig. 5) was followed by degenerative changes at the end of 9th week of the experiment (Fig. 6). Fibrosis, which was observed from 2nd week of the experiment represents an immediate response of liver's tissue to nitrate toxicity. Fibrosis actually means a scarring process. The clinical condition of fibrosis have earlier also been well documented by Bosetti *et al.* [11] and Burcu *et al.* [12] who considered it to be associated with impaired hepatic tissue function. Present author feels that due to fibrosis, blood vessels may get obstructed and thereby lead to necrosis of the tissue. Necrosis of the hepatic tissue is also similar to those reported for the fish caught in contaminated water and the ones exposed to various chemicals in laboratory conditions [13]. Necrosis implies premature cell death. It results when fish are exposed to various xenobiotics [14, 10]. Necrosis ultimately leads to steatosis, the process describing the abnormal retention of lipids in the liver tissue, which as stated by Burcu *et al.* [12] can cause an imbalance in the homeostatic mechanism of the fish. That steatosis generally accounts for abnormal retention of lipids is clearly authenticated by rising levels of cholesterol and triglycerides content of the fish, *a morar* in blood serum at different time intervals during the experimental period of nine weeks. Present observations on the steatosis of the liver tissue is completely in agreement with the earlier findings of Burcu *et al.* [12] who too observed steatosis in liver of the fish when exposed to different chemical pollutants. They also considered it to be the causative of metabolic disruption of the liver tissue as well. Steatosis of the liver tissue has been observed to result in its total degeneration, which was observed after nine weeks of the experimental period. The degenerative changes in the liver tissue authenticate that its cellular structure has been totally obscured and hence disruption of the normal functioning of the tissue.

b) Anterior kidney: Anterior kidney showed the presence of renal tubules (excretory in function) in addition to hemopoietic tissue (blood forming tissue) (Fig. 7). When exposed to subethal concentrations (25%, 50% and 75% of LC_{50}) of nitrate, anterior kidneys have been found to exhibit various histopathological alterations which include i) lymphocytic infilteration after 2nd week of the experiment (Fig. 8) ii) tubular necrosis observed after 3rd week of the experiment (Fig. 9) , iii) degeneration of haemopoietic tissue during 5th week (Fig. 10) to be followed by tubular vacuolation after the 6th week of the experiment (Fig. 11) and iv) total degenerative cellular architecture observed during 9th week (Fig. 12). Lymphocytic infilteration observed after 2nd week of the experiment in response to nitrate toxicity simply indicate increased synthesis of lymphocytes in the tissue as a mark of stress response and lymphocytic infilteration as stated by Radhakrishnan and Hemalatha [15] is stress related response only. Necrosis, which becomes prominent during 3rd and 4th week of the experiment implies degeneration of the cells. Necrosis along with degenerative changes observed in haemopoietic tissue (observed after the 5th week of the experiment) can thus hamper the haemopoietic/ erythropoietic machinery of the fish. Present view point gets support from the earlier work of Omitoyin *et al.* [16] and Shaheen and Akhter [17] who too advocated necrosis of haemopoietic tissue in the kidneys to be the causative for impairing the erythropoietic machinery of the fish. Tubular vacuolation and subsequent degenerative changes in the haemopoietic tissues of kidneys from as stated by Pal [9] causes an imbalance between the rate of synthesis of substance in the tissue and their subsequent release in the general circulation. Such degenerative changes observed towards the end of the experimental period are clearly indicative of the almost total disruption of the normal functioning of the kidney tissue. This therefore definitely can affect the haemopoietic machinery of the fish.

c) Spleen: Spleen was observed to exhibit an even distribution of red pulp (comprising of erythrocytes), white pulp (comprising of leucocytes) besides presence of melanomacrophage centres (Fig. 13) which as stated by Agius [18] are necessary for synthesising iron containing compounds necessary for haemopoiesis. During present studies, conspicuous changes observed in spleen of nitrate treated fish i) leukocyte infiltration become evident from 3rd week of the experiment (Fig. 14), ii) next conspicuous change observed was the congestion of the splenic tissue (Fig. 15) during 5th week, iii) necrosis was observed to get initialized during 7th week (Fig. 16) iv) vacuolization (Fig. 17) appeared during 8th week and was followed by degenerative changes of the splenic tissue towards the end of the experimental period of 9 weeks (Fig. 18). Lymphocytic infiltration simply indicates that these are possibly being synthesised in quite enough number in the splenic tissue. It simply is suggestive of adaptive mechanism of fish *A. morar* towards the stress of nitrate toxicity. Synthesis and subsequent release of lymphocytes from the splenic tissue (besides anterior kidney) into general circulation seemingly appears to be countering the stress imposed by nitrate toxicity. This is an immunostimulatory response. Congestion of splenic tissue which may cause obstruction/ constriction of the blood vessels. This by affecting can definitely cause the necrosis of the splenic tissue. Necrosis, which is most likely a result of the hypofunction of the concerned organ (spleen) can result in the alterations of various haematological parameters of the fish which has earlier also been reported by Tomova *et al.* [19] and Georgieva *et al.* [20]. Vacuolization according to present author can have far reaching effect on the RBC synthesising machinery of the splenic tissue. Degenerative changes observed towards the end of the experimental period appear to be indicative of the fact that total disruption of the functioning of splenic tissue has occurred.

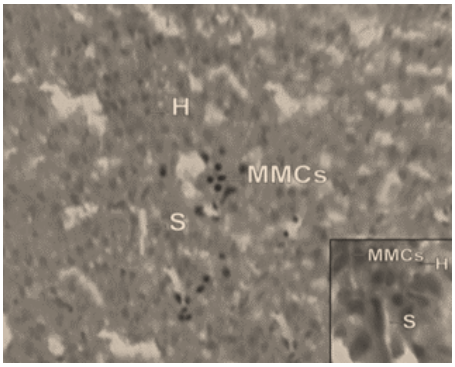

Fig 1: Microphotograph of Liver tissue of *A. morar* from control showing Hepatocytes (H), Sinusoids (S) and Melanomacrophage centres (MMCs) (H&E×1000)

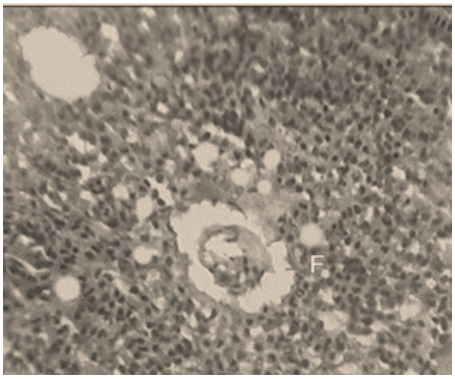

Fig 2: Microphotograph of Liver tissue from nitrate treated fish, *A. morar* showing Fibrosis (F) after 2nd week of the experiment (H&E×1000)

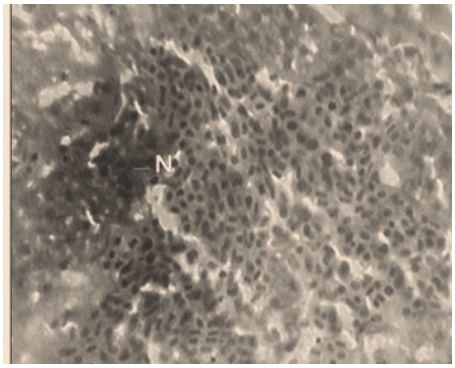

Fig 3: Microphotograph of Liver tissue from nitrate treated fish, *A. morar* showing Necrosis (N) after 3rd week of the experiment (H&E×1000)

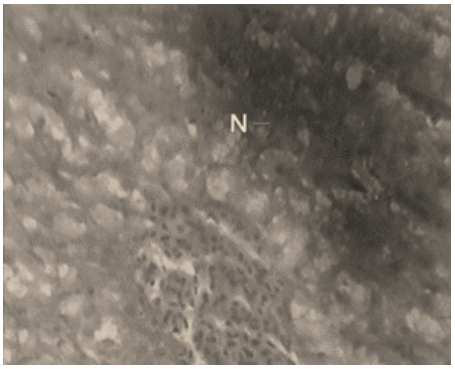

Fig 4: Microphotograph of Liver tissue from nitrate treated fish, *A. morar* showing marked Necrosis (N) after 6th week of the experiment (H&E×1000)

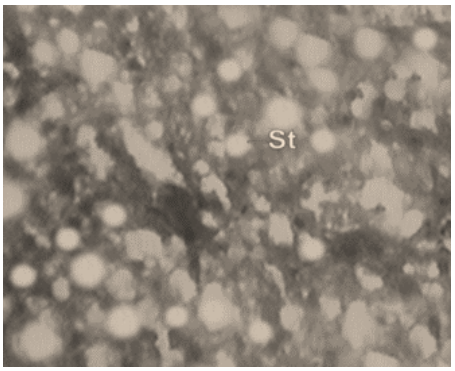

Fig 5: Microphotograph of Liver tissue from nitrate treated fish, *A. morar* showing marked Steatosis (St) after 8th week of the experiment (H&E×1000)

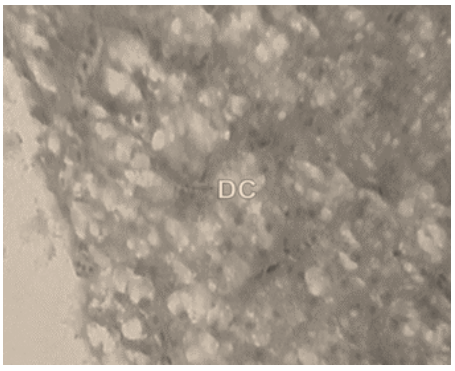

Fig 6: Microphotograph of Liver tissue from nitrate treated fish, *A. morar* showing Degenerative changes (DC) after the 9th week of the experiment (H&E×1000)

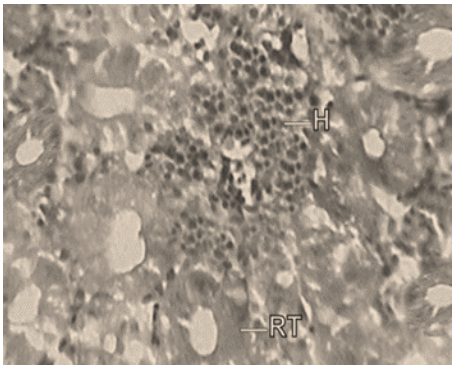

Fig 7: Microphotograph of Kidney tissue from control fish, *A. morar* showing Haemopoietic tissue (H) and Renal Tubules (RT) (H&E×1000)

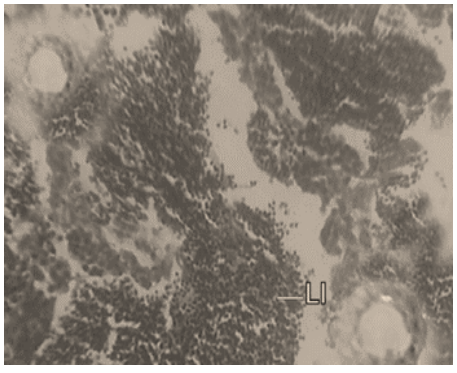

Fig 8: Microphotograph of Kidney tissue from nitrate treated fish, *A. morar* showing Lymphocytic infiltration (LI) after 2nd week of the experiment (H&E×1000)

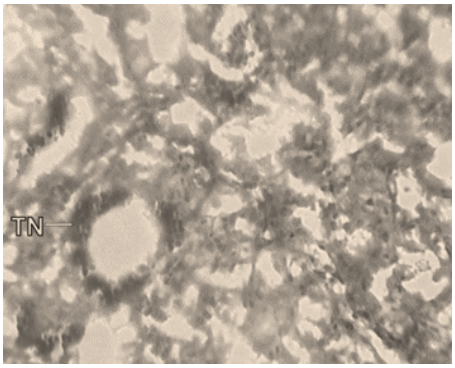

Fig 9: Microphotograph of Kidney tissue from nitrate treated fish, *A. morar* showing Tubular Necrosis (TN) after 3rd week of the experiment (H&E×1000)

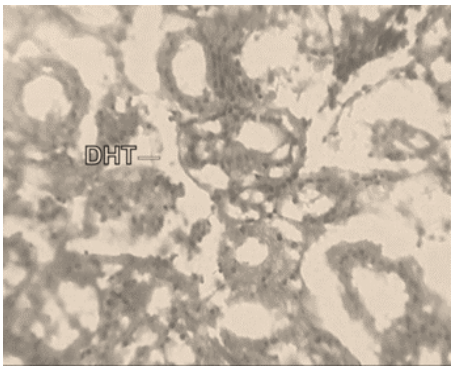

Fig 10: Microphotograph of Kidney tissue from nitrate treated fish, *A. morar* showing Degenerated Haemopoietic Tissue (DHT) after the 5th week of the experiment (H&E×1000)

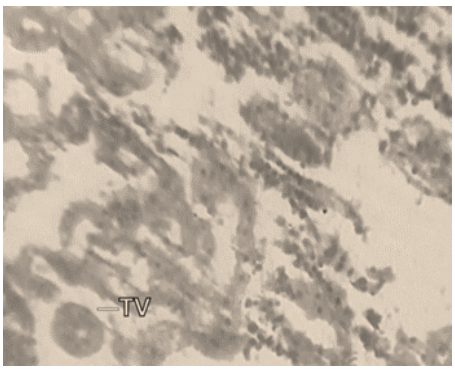

Fig 11: Microphotograph of Kidney tissue from nitrate treated fish, *A. morar* showing Tubular Vacuolation (TV) after 6th week of the experiment (H&E×1000)

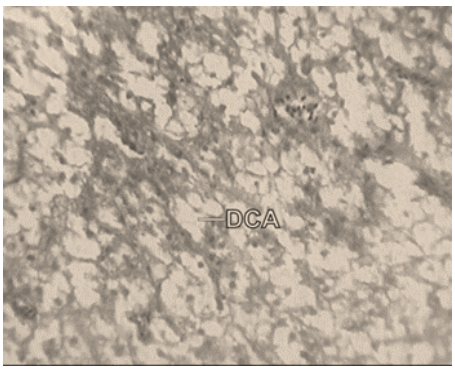

Fig 12: Microphotograph of Kidney tissue from nitrate treated fish, *A. morar* showing Degenerated cellular architecture (DCA) after 9th week of the experiment (H&E×1000)

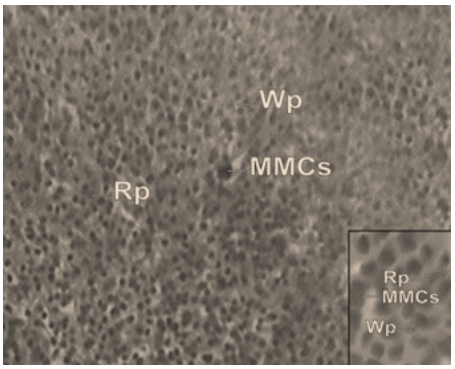

Fig 13: Microphotograph of Splenic tissue of *A. morar* from control showing Red pulp (Rp), White pulp (Wp) and Melanomacrophage centres (MMCs) (H&E×1000)

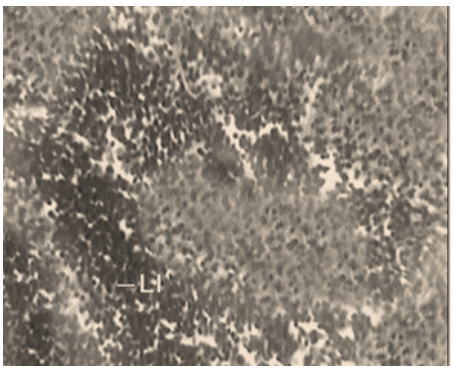

Fig 14: Microphotograph of Splenic tissue from nitrate treated fish, *A. morar* showing Lymphocytic infiltration (LI) after 3rd week of the experiment (H&E×1000)

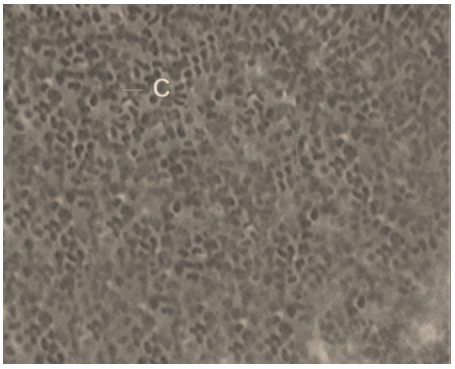

Fig 15: Microphotograph of Splenic tissue from nitrate treated fish, *A. morar* showing Congestion (C) after 5th week of the experiment (H&E×1000)

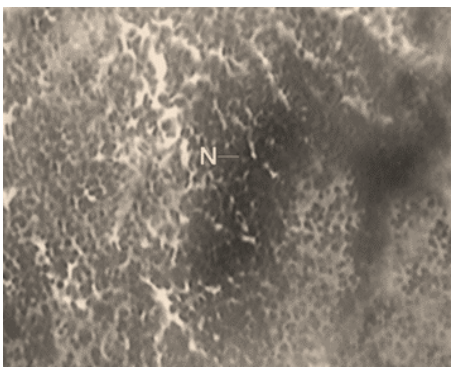

Fig 16: Microphotograph of Splenic tissue from nitrate treated fish, *A. morar* showing marked Necrosis (N) after 7th week of the experiment (H&E×1000)

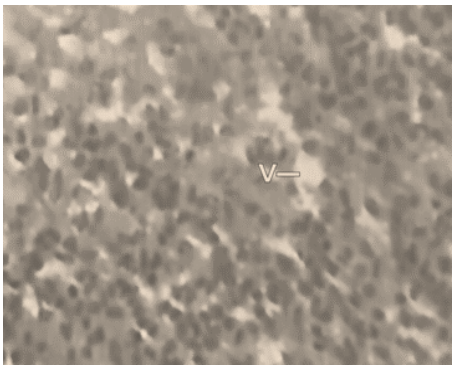

Fig 17: Microphotograph of Splenic tissue from nitrate treated fish, *A. morar* showing Vacuolation (V) after 8th week of the experiment (H&E×1000)

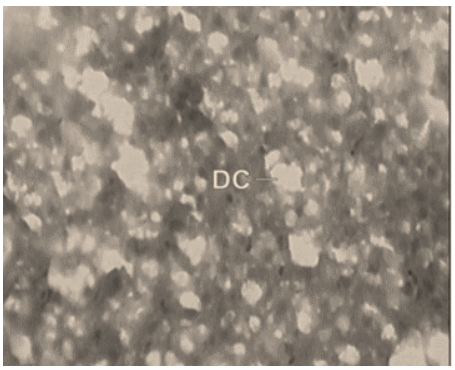

Fig 18: Microphotograph of Splenic tissue from nitrate treated fish, *A. morar* showing Degenerative changes after 9th week of the experiment (H&E×1000)

4. Conclusion

The present investigation thus indicates a direct correlation between pollutant exposure and histopathological disorders observed in several tissues. The histopathological analysis of the tissue when exposed to various sublethal concentrations of environmental pollutants can therefore serve as early warning signal for the survival of the species as well as for environmental protection because changes in haemopoietic tissues can occur much before they (xenobiotics/ pollutants) produce irreversible effects on biota.

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6. References

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