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Histopathological Alterations Induced by Lindane (Gamma-Hexachlorocyclohexane) in a Minor Carp, Aspidoparia Morar Inhabiting Jammu Waters.

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***Abstract*-** The objective of the present study was to identify the degree of damage to the histological architecture of haemopoietic tissues viz., liver, anterior kidney and spleen in *A. morar* exposed to various sublethal concentrations (10%, 20% and 30% of LC_{50} value) of lindane for a period of 60 days. The exposed liver had distention of sinusoids, necrosis, vacuolation and degenerative process in the cellular architecture. Anterior kidneys manifested necrosis, tubular vacuolation, followed by total damage of the tissue. Spleen on the other hand showed deposition of haemosiderin pigments, necrosis, vacuolation followed by proliferation of melanomacrphage centres. Total degeneration of the splenic tissue was observed by the end of the experiment. The results of this histological analysis of various fish tissues indicate a direct correlation between insecticide exposure and histopathological disorders observed.

Index Terms- Lindane, Histological disorders, Haemopoietic tissues, Aspidoparia morar.

I. Introduction

Persistent polluting chemicals are substances which have long life mainly due to their chemical stability. DDT, dieldrin, lindane, endosulfan are well known examples of chemicals which can be found in various compartments of the aquatic environment as well as in living organisms (Bro-Ramussen, 1996). Among different such insecticides lindane is an organochloride insecticide which has been used on a wide variety of crops as well as in public health to control insect borne diseases (with fungicides) and as seed treatment (Ortiz *et al*., 2001).

Aquatic organisms quickly accumulate and store persisting organochlorine insecticide lindane. Fish are very susceptible to bioaccumulation in the tissues as they take up lindane residues from the water through the gills and skin (Gopal *et al*., 1982). Histopathology has been used as a sublethal test for evaluating toxic effects of water pollutants in fish (EIFAC, 1983). Histopathological alterations can also be utilized as tools to get a clear idea as to what extent an organism is affected at tissue or cellular level. Exposure of fish, *A. morar* to various sublethal resulted in various histological alterations in the haemopoietic tissues viz., liver, anterior kidney and spleen. Being haemopoietic, these organs are of vital importance in fish.

II. Materials and Methods

1.1 Sampling site

The fish, *A. morar* (Ham.) for present study were netted from Nikowal region of River Tawi. The area is located towards the southern part of Jammu about 8-12 kms. From main R.S. Pura Tehsil in Jammu district of Jammu and Kashmir.

1.2 Histological analysis

The test fishes were dissected open in ringer solution and their liver, anterior kidney and spleen were fixed in Bouin's fixative.

After the termination of each experiment, the test fishes after collection of their blood were dissected open in ringer saline solution and their liver, anterior kidney and spleen were fixed in Bouin's fixative. For thymus, the lower jaw and snout was removed and the rest of the head was fixed. Later the head was cut into smaller sections.

After post fixation treatment and routine dehydration and clearing, these tissues were embedded in histowax of 54-56ºC. 5-7 µm thick section of these were cut on microtome and stained using haematoxylin eosin stain.

III. Results

Liver histopathology: The normal histology of liver of fish, *A. morar* showed the presence of a) **hepatocytes** (Fig. 1) cells involved in the synthesis of proteins, cholesterol, bile salts and phospholipids. b) **sinusoids** (Fig. 1) (the blood vessels similar to capillaries but with discontinuous epithelium) and c) **melanomacrophage centers (MMCs)** (Fig. 1) (pigment containing cells and are normally located in the stroma of haemopoietic tissue of the liver).

During present studies, *A. morar* after exposure to sublethal concentrations of lindane exhibited many alterations in liver tissue, the chief being i) distention or widening of blood sinusoids observed after 15^{th} day of the experiment (Fig. 2), ii) necrosis and vacuolation (during 35^{th} day of the experiment) (Fig. 3), iii) degenerative process was observed in cellular architecture during 50^{th} day of the experiment (Fig. 4) iii) total degeneration of the tissue by the end of 60^{th} day of experimental period (Fig. 5).

Kidney histopathology: Microscopic examination of head kidney of control group showed the presence of a) **haemopoietic tissue** (blood forming tissue) (Fig. 6) and

b) **renal tubules** (excretory in function) (Fig. 6).

Compared to control group, cellular structure of kidney exposed to lindane manifested changes viz., i) necrosis of kidney tissue which was observed after 20th day of the experiment (Fig. 7), ii) tubular vacuolation by 30th day of the experiment (Fig. 8), iii) Signs of tubular degeneration observed after 40 days of the experiment (Fig. 9) iv) total damage of the cellular make up of kidney structure by the end of experimental period of 60 days (Fig. 10).

Spleen histopathology: Microscopic examination of spleen from the control fish showed the presence of i) red pulp (comprising of erythrocytes) (Fig. 11) ii) white pulp (comprising of leucocytes) (Fig. 11) and iii) melanomacrophage centres (Fig. 11) (which are the important component of reticuloendothelial system and act as main repository for iron containing compounds (Agius, 1979).

Upon treatment with lindane presently normal histology of *A. morar* was observed to get disrupted resulting in various histopathological effects which include i) deposition of haemosiderin pigments observed after 15th day of the experiment (Fig. 12), ii) necrosis and vacuolation which became more marked during 35th day of the experimental period (Fig. 13), iii) proliferation of MMCs by 45 days of the experiment (Fig. 14) and iv) inflammation and total degeneration of the splenic tissue by the end of the experiment (Fig. 15).

IV. Discussion

The histopathological alterations in the liver tissue under the influence of lindane toxicity resulted in distention or widening of blood sinusoids which evidently means that haemopoiesis being one of the synthetic metabolic processes get hampered by insecticide intoxication.

Necrosis of the hepatocytes which implies cell death, is an advanced and usually irreversible stage of degeneration characterised by dead hepatocytes (Pal, 2006). Distention of blood sinusoids evidently means that haemopoiesis being one of the synthetic metabolic processes possibly get hampered by the insecticide intoxication. In this context findings of Gupta (2008),Vinodhini and Narayanan (2009), Pathan *et al*., (2010) who also reported severe necrosis of hepatocytes and widening of blood sinusoid to hamper the haematopoietic machinery of the fish lends a strong support for the present viewpoint.

Random distribution of vacuoles in the hepatocytes of affected fishes as observed presently indicate an imbalance between the rate of synthesis of substance in the parenchymal cells and their release into the circulation. In tune to present findings Kabir and Begum (1978), Shastry and Sharma (1979), Gingerich (1982), Vinodhini and Narayanan (2009), Oliva *et al*. (2010) and Velmurugan *et al*. (2007) too, observed vacuolation of the hepatocytes in their fishes exposed to different xenobiotics. This, all of them stressed, certainly affect the release of substances in the general circulation.

Since liver is the site of various metabolic activities its the total degeneration which was observed after 60 days of the experimental duration completely disrupt the metabolic processes of the liver (including haemopoiesis) and hence result in the malfunctioning of the liver tissue.

The various histopathological changes observed in the kidney tissue are suggestive that kidney are malfunctioning. This simply is indicative of the fact that haemopoietic machinery possibly gets affected under the influence of lindane toxicity and hence may result in inhibition of further release of erythrocytes in the general circulation.

In this context, the observations of Bucher and Hofer (1993) and Ranzani-Paiva *et al*. (1997) can be quoted who too behold that histopathological alterations in kidneys are the most plausible causatives for decline in haematological parameters on exposure to various xenobiotics. As an important organ of immunity response elaboration (Zapata and Cooper, 1990), pathological changes induced in kidney tissue by lindane toxicity present author states, by affecting defense system, can definitely disturb homeostasis and health of fishes.

Lindane induced deposition of haemosiderin pigments in the splenic tissue which is one of the breakdown products of haemoglobin from senescent erythrocytes as reported by Zapata and Cooper (1990). According to Hibiya (1982) deposition of haemosiderin causes disease haemosiderosis when because of increase in the rate of destruction of erythrocytes in spleen there is resultant decline in the number of mature erythrocytes in the circulating blood. Deriving support from these findings deposition of haemosiderin pigments observed during present studies, it appears may represent the diseased condition called haemosiderosis.

Vacuolation of the splenic cells is indication of disruption of the synthetic machinery of this organ and definitely the normal mechanism of release of substances form the tissue into the general circulation.

Proliferation of MMCs in the spleen as observed presently finds association with either normal ageing or to prolonged starvation or infectious diseases (Reviewed by Couillard *et al*., 1999). Lindane intoxication, it appear, induce a sort of stress condition which may then cause the proliferation of MMCs. MMCs proliferation are known to affect the erythrocyte synthesis and can block haemopoiesis or erythropoeisis in fish (Gupta, 2008). All the above alterations lead to the total damage of the tissue thereby hampering its normal functioning.

V. Conclusion

All the histopathological observations indicated that exposure to sublethal concentrations of lindane caused structural effects in the liver, anterior kidney and splenic tissues of *A. morar*. Liver, anterior kidney and spleen histopathological alterations, such as those observed in this study may result in severe physiological problems ultimately leading to the death of the fish. In conclusion, the findings of the present histological investigations demonstrate a direct correlation between pesticide exposure and histopathological disorders observed in several tissues.

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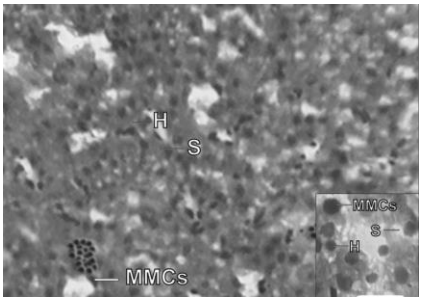

Fig. 1 Microphotograph of liver from control fish showing Hepatocytes (H), Sinusoids (S) and melanomacrophage centres (H&E×1000)

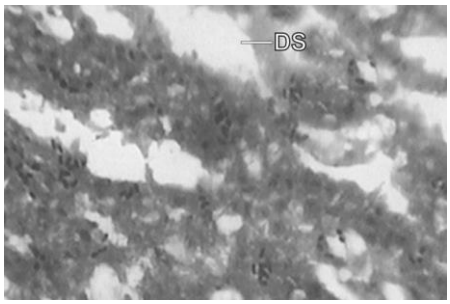

Fig. 2 Microphotograph of liver tissue from lindane reated fish showing distended blood sinusoids (DS) after 15th day of the experiment (H&E×1000)

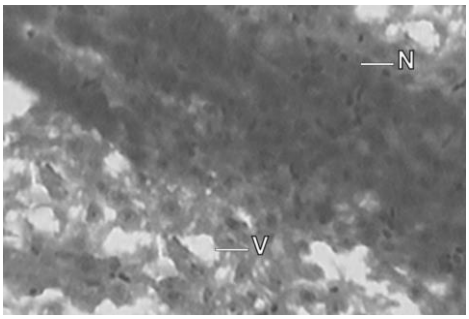

Fig. 3 Microphotograph of liver tissue from lindane treated fish showing Necrosis (N) and Vacuolation (V) after 3th day of the experiment (H&E×1000)

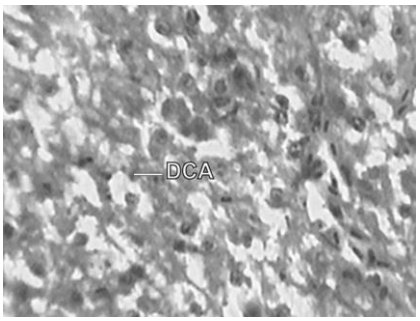

Fig. 4 Microphotograph of liver tissue from lindane treated fish showing Degenerative cellular architecture during 50th day of the experiment (H&E×1000)

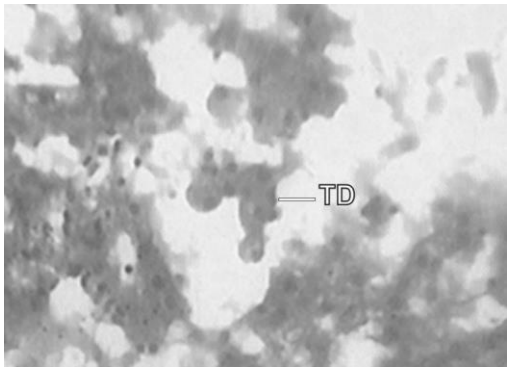

Fig. 5 Microphotograph of liver tissue from lindane treated fish showing Total degeneration of the tissue after 60th day of the experiment (H&E×1000)

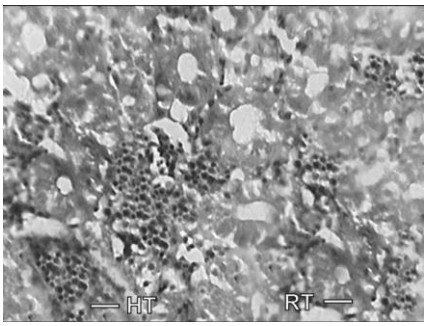

Fig. 6 Microphotograph of kidney tissue from control showing haemopoietic tissue (HT) and renal tubules (RT) (H&E×1000)

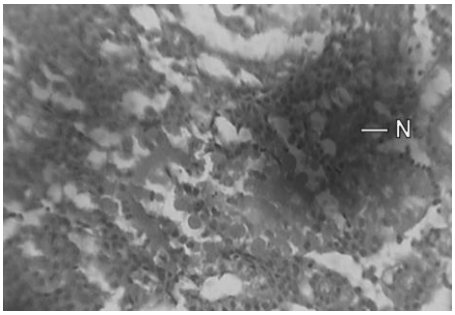

Fig. 7 Microphotograph of kidney tissue from lindane treated fish showing Necrosis (N) after 20th day of the experiment (H&E×1000)

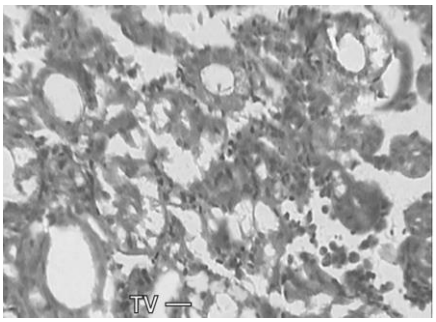

Fig. 8 Microphotograph of kidney tissue from lindane treated fish showing Tubular vacuolation (TV) after 30th day of the experiment (H&E×1000)

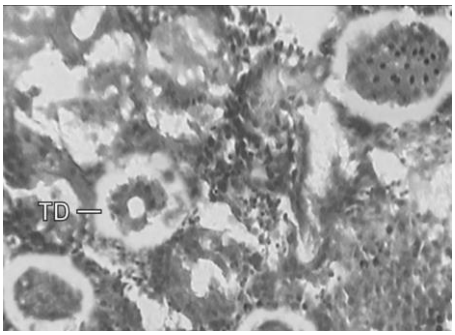

Fig. 9 Microphotograph of kidney tissue from lindane treated fish showing Tubular degeneration (TD) after 40th day of the experiment (H&E×1000)

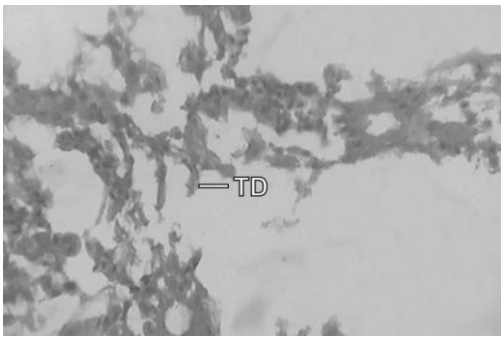

Fig. 10 Microphotograph of kidney tissue from lindane treated fish showing Total degeneration (TD) of the tissue after 60th day of the experiment (H&E×1000)

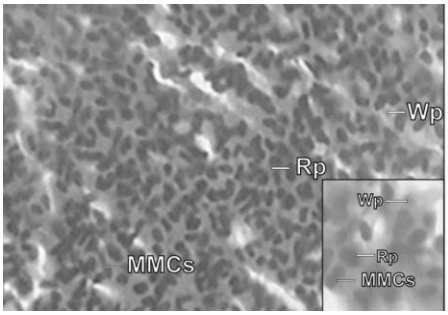

Fig. 11 Microphotograph of splenic tissue from control showing Red pulp (Rp), white pulp (Wp) and melanomacrophage centres (MMCs) (H&E×1000)

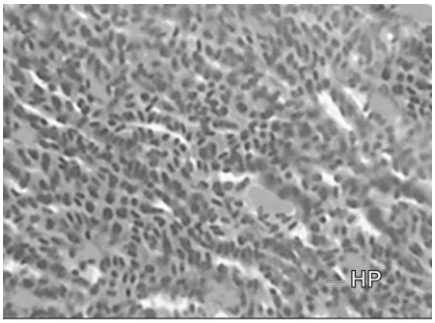

Fig. 12 Microphotograph of splenic tissue from lindane treated fish showing deposition of Haemosiderin pigments (HP) after 15th day of the experiment (H&E×1000)

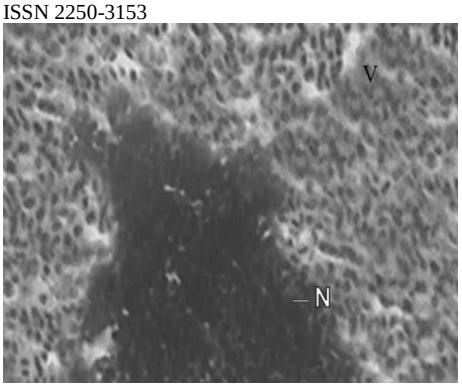

Fig. 13 Microphotograph of splenic tissue from lindane treated fish showing Necrosis (N) and Vacuolation after 35th day of the experiment (H&E×1000)

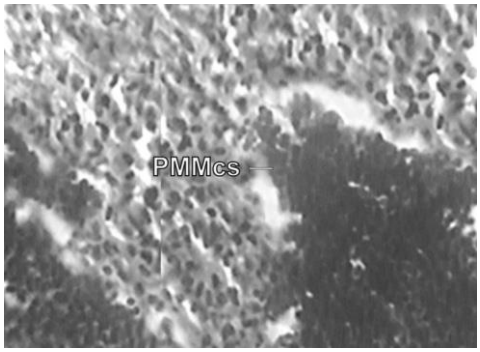

Fig. 14 Microphotograph of splenic tissue from lindane treated fish showing Proliferation of MMCs after 45th day of the experiment (H&E×1000)

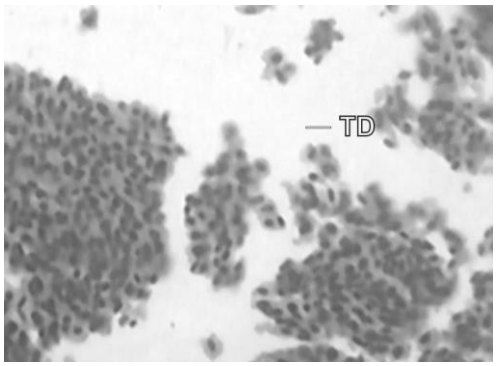

Fig. 15 Microphotograph of splenic tissue from lindane treated fish showing Total degeneration of the tissue after 60th day of the experiment (H&E×1000)