Histopathological changes in liver, kidney and muscles of pesticides exposed malnourished and diabetic rats

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Histopathological changes were observed in liver, kidney and muscles of normal, protein-malnourished, diabetic as well as both protein-malnourished and diabetic albino rats when exposed to a mixture of monocrotophos, hexachlorocyclohexane and endosulfan at varying intervals. The examination revealed hepatotoxic, nephrotoxic and muscular necrotic effects in pesticides exposed rats. Toxicity was aggravated in protein-malnourished and diabetic animals and more so, if the animals were both diabetic and protein-malnourished.

Keywords: Diabetic, Histopathology, Pesticide, Protein-malnourishment

Monocrotophos, hexachlorocyclohexane (HCH) and endosulfan are routinely employed in agriculture in India and are well documented to induce pathological changes in tissues1-4. However, pre-existent nutritional conditions are an important factor in determining the susceptibility to pesticide toxicity5-7. Moreover, low protein diets are reported to influence insulin mediated glucose uptake by peripheral tissues and the ability of insulin to suppress the hepatic glucose output8. This aspect of pesticide toxicity is of great importance, particularly in the developing countries, like India, where protein malnutrition and diabetic conditions are rampant. It is, therefore, aimed to investigate cellular changes in liver, kidney and muscle of malnourished and diabetic rats following exposure of mixture of pesticides.

Materials and Methods

Animals—Adult albino rats of either sex (150-200 g) were procured from the animal colony of Central Drug Research Institute, Lucknow and later reared and bred in the department for the present study. These were maintained under standard husbandry conditions, fed on a standard diet and given water ad libitum. The investigation was conducted upon getting clearance from ethical committee of the Institute. It was carried out under groups I, II, III and IV, representing normal, diabetic, protein malnourished and diabetic-protein malnourished group of animals, respectively and comprising 6 rats in each group. Accordingly, the rats were randomly divided into two broad dietary groups and maintained for 30 days on formulated isoenergetic diets at 20 (group I) and 5% (group III) protein level (Praveen Feeds, Ranital Chowk, Jabalpur).

Diabetic conditions were induced into some randomly selected animals, from both the dietary groups as per Nimenibo9. After keeping the animals on fast for 12 h, alloxan monohydrate (BDH Chemical Co. Ltd., India), dissolved in 0.15 M N-saline, was injected ip once at a dose level of 120 mg/kg body weight. The blood glucose level of hyperglycemic rats was allowed to stabilize for 7 days. Diabetes mellitus was confirmed in rats by testing their blood glucose level using diagnostic kits. Glucose level was determined by glucose oxidase-peroxidase enzymatic method10 using the Span diagnostic kit procedure, manufactured and marketed by Span Diagnostics Ltd., Surat (Gujarat, India). Only the rats with their blood glucose level exceeding 200 mg% were considered diabetic and formed groups II and IV. The blood glucose level was monitored at weekly interval to ensure the stability of diabetic status.

Pesticide treatment—Pesticide mixture of monocrotophos (0.156 mg/day), endosulfan (0.2 mg/day) and HCH (1.3 mg/day), representing the level of pesticide residues consumed in composite
diet\textsuperscript{11}, was suspended in coconut oil (0.088 mg/ml) and stored in the refrigerator (4°C) till use. Daily dose of pesticide mixture (0.044 mg/day), as adopted earlier\textsuperscript{12} was administered to the rats for 0, 15, 30 and 60 days by oral intubation. Animals in the control group received coconut oil alone in a similar manner.

**Histopathological study**—Rats, 6 in each group, were sacrificed after fasting overnight upon termination of their feeding schedules, as indicated above. Tissues from liver, kidney and muscle from hind limb were excised and fixed in 10% formaline saline. Besides, the spleen of group IV animals was also included for examination. After fixing for 3 to 4 days, the tissues were cleared and embedded to paraffin wax (60°C-62°C). Uniform sections of 5 µm thickness were cut and stained with hematoxylin and eosin by routine procedures. The stained sections were examined for pathological changes at ×200 or ×400 magnifications.

**Results**

The microscopic studies showed varying degree of cellular changes from mild to marked in diabetic, protein-malnourished and protein malnourished-diabetic rats following exposure to pesticides mixture at varying intervals (Figs 1-12).

**Liver**—Lobular structure in rats unexposed to pesticide and in pesticide exposed animals at zero

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Figs 1-4—Section of liver of 1: protein-malnourished albino rat showing fatty change (arrow marked) after repeated oral exposure of pesticide mixture, 2: diabetic albino rat showing fatty change (arrow marked) after repeated oral exposure of pesticide mixture, 3: protein-malnourished-diabetic albino rat showing necrosis (N) and degeneration (D) of parenchymatous cells, dilation of sinusoids at 45 days after repeated oral exposure of pesticide mixture, 4: protein-malnourished-diabetic albino rat showing central lobular necrosis (N) at 45 days after repeated oral exposure of pesticide mixture. [Figs 1-3 × 200, Fig. 4 × 400].
interval remained unaltered. The central vein appeared intact and sinusoids and Kupffer cells were normal. However, liver from rats suffering from diabetes (group II), protein-malnourishment (group III) and protein malnourishment-diabetes (group IV) depicted marked cellular changes on being repeatedly exposed to pesticide mixture once daily. The specimens revealed congestion of central vein and swelling of hepatocytes. Pesticide poisoning caused focal necrosis, dilation of sinusoids, fatty changes and congestion of sinusoids around central vein regions. Changes were severe in group IV animals. Enlarged hepatocytes, vacuolation, pyknosis and degeneration were evident in all the rats under health stress (Figs 1, 2, 3 and 4).

Kidney—Kidneys of unexposed animals showed well-demarcated cortex and medulla, and intact capsule with well formed glomerular tuft. Degenerative changes were observed in proximal or distal convoluted tubules; glomerular tuft and Bowman’s capsule were normal. The interstitial tissue and blood vessels were normal with no inflammatory changes. The kidney of pesticide exposed rats of groups II, III and IV showed demarcated tubular necrosis; being severe in the rats of group IV (Figs 5-7). The parenchyma revealed degeneration, desquamation and necrosis. Changes increased with duration of pesticide exposure. These were most

Figs 5-8—Section of kidney 5: protein-malnourished albino rat showing necrosis (N) at 60 days after repeated oral exposure of pesticide mixture, 6: diabetic albino rat showing marked necrosis (N) and maximum haemorrhage (H) at 45 days after repeated oral exposure of pesticide mixture, 7: protein-malnourished-diabetic albino rat showing severe congestion (C) and necrosis (N) after repeated oral exposure of pesticide mixture, 8: protein malnourished-diabetic albino rat showing increased Bowman’s space after 45 days of repeated oral exposure of pesticide mixture [Figs 5-7 × 400; Fig. 8 × 200].
severe at 60 days post-treatment in group IV, indicating complete necrosis of tubular epithelium and haemorrhage was prominent in the glomeruli. Increased Bowman’s space was evident in group IV rats (Fig. 8).

**Muscle**—Unexposed or exposed rats at zero interval showed normal muscular architecture. However, necrosis was severe in animals under health stress and exposed to mixture of pesticides (Figs 9, 10 and 11).

**Spleen**—Marked and extensive haemorrhage was noticed in the spleen of animals belonging to protein malnourished-diabetic group (Fig. 12).

**Discussion**

Histopathology of tissues revealed mild to marked cellular changes upon administration of pesticides mixture to adult rats. The magnitude of manifestation depended on the health status of experimental animals. Histopathological changes in tissues of animals exposed to various chemical agents have been reported\(^2\sim^4,^{13,14}\). On the contrary, information on such changes under variable health condition is lacking and therefore, the present results cannot be compared with. However, microscopic changes in architectural pattern of tissues in protein-malnourished adult rats upon monocrotophos poisoning, as reported earlier\(^4\), were alleviated in exposed animals when allowed to feed on high protein diet.

**Liver**—Normal architectural pattern of liver without degenerative and necrotic changes in

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Figs 9-11—Section of muscle 9: protein-malnourished albino rat showing necrosis (N) after repeated oral exposure of pesticide mixture, 10: diabetic albino rat showing necrosis (N) at 30 days after repeated oral exposure of pesticide mixture, 11: protein-malnourished-diabetic rat showing severe necrosis (N) at 45 days after repeated oral exposure of pesticide mixture [Figs 9 & 10 × 200; Fig. 11 × 400].

Fig. 12—Section of spleen of protein-malnourished-diabetic albino rat showing severe necrosis (N) at 45 days after repeated oral exposure of pesticide mixture [× 200].
unexposed rats or exposed at zero interval, indicated that chemical agents in exposed animals have not induced any injury that could have induced impairment in metabolic activity of liver. The occurrence of fatty changes in groups II, III or IV suggested inhibition of some lipid metabolic enzymes, causing thereby, a disturbance in metabolic activity required for maintenance of tissue.

Prominent fatty changes with necrosis in portal areas indicated that some toxic metabolites may be transported from intestine to liver, resulting in these changes. The presence of definite necrosis indicated capability of the toxic metabolites causing cell death.

Kidney—Not much histological changes were observed in cellular structure of unexposed rats or exposed at zero interval. However, widened capsular space, degeneration and necrosis of renal tubular epithelia were noticed in exposed animals under varying health stress conditions. These damaging changes reflected action of toxic metabolites of pesticides under study. Microscopic changes were more pronounced in poisoned animals suffering from both diabetes and protein malnourishment, indicating a severe nephro-toxic effect of chemical agents at the present dosage employed.

Muscles—In pesticide poisoned rats, degeneration leading to necrosis of skeletal muscles was observed, pointing to myotoxic nature of pesticides. Degenerative changes were more pronounced when the rats were under strained health conditions, more so in group IV animals and this was expected since both diabetes and protein-malnourishment have adverse effects on muscle architecture and its activities. The present findings on biochemical changes in muscles under the present conditions are well correlated with the observed pathological findings.

Spleen—The spleen of group IV animals was noticeably enlarged and was therefore, included for examination specifically. Marked and extensive haemorrhage was noticed that indicated impairment in lymphatic system of exposed animals.

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References