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Morphofunctional Changes in the Liver of Male Mice After Chronic Treatment with Phosphamidon

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Phosphamidon, (dimethyl phosphate, ester with 2-Chloro N. N-diethyl 3-hydroxycrotonamide) is a plant systemic insecticide which is largely used against sucking insects. Many attempts have been made to study the effects of different organophosphorous insecticides on mammals and other animals, but the studies carried out so far, are limited and mainly devoted to the study of toxicity of pesticides and of its main metabolites, by-products and formulations. Organophosphates and chlorinated insecticides cause pathological and biochemical changes in the liver of mammals as reported by Dikshith and Datta (1972); Kimbrough and Gaines (1968); Munro et al.(1974) and Sarin and Saxena (1978). No such information is available on the phosphamidon, which is a very popular insecticide among the Indian farmers. The present investigation includes the effects of chronic exposure of phosphamidon on histopathological and biochemical changes in the liver of white albino mice.

MATERIALS AND METHODS

A group of 30 male mice (Av. Wt. 35 g) was given phosphamidon (technical grade) at the dose level of 35 ppm in drinking water ad libitum for 60 days.Another group of 30 male mice was simultaneously observed as control. All the animals were kept on the commercial standard diet supplied by Hindustan Lever, Delhi. The weight of animals was recorded weekly.

Ten animals from each group were sacrificed by cervical dislocation on the 30th and 60th day of the treatment. Liver was removed quickly, weighed and one set of tissue was fixed in Bouin's fixative for histopathological examination. Sections cut at 6 µm, stained with hematoxyline and eosin were used for pathological studies. Blood was taken directly from the heart for bilirubin, SGOT and SGPT estimation.

Biochemical estimations of enzymes and proteins were done colorimetrically on Systronics 102 balance cell colorimeter on the wave length of 540 nm and 640 nm. Alkaline and Acid phosphatase

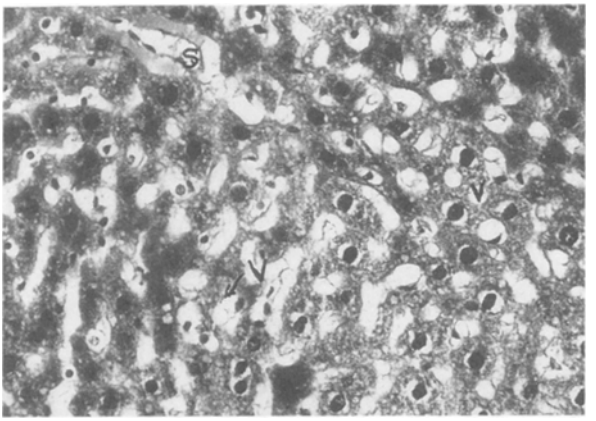

Figure 1. Photomicrograph showing wide sinusoidal space(s) Vacuolization of hepatocytes (V) (foamy appearance) 30 days treatment x 100.

were estimated by the method suggested by Fiske and Subbarow (1925), Glucose-6-phosphatase (G-6-P) activity was determined by Swanson (1955) method. Protein and glycogen were estimated by the method given by Lowery et al. (1951) and Montogomery (1957) respectively. Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) were estimated by the method given by Mohun and Cook (1957).

RESULTS AND DISCUSSION

No significant changes in the body weight and liver weight in relation to body weight were observed.

Definite histological changes were observed in liver at 30 and 60 days time intervals after the chronic administration of phosphamidon. Cloudy swelling and vacuolisation, which was histologically defined as that of fatty degeneration and not hydropic degeneration was detectable at 30 days treatment (Fig. 1). Enlargement and variation in the size of the nuclei and mild cytoplasmic degeneration were also observed at 30 days treatment (Fig. 2). Two months of chronic treatment of phosphamidon shows complete congestion of sinusoids and midzonal and periportal necrosis of the hepatocytes. The nuclei of hepatocytes revealed karyorrhexis (Figs. 3 & 4).

Significant increase in the activity of Alkaline phosphatase was observed after 30 days of treatment while no change was recorded after 60 days of treatment. Significant inhibition in the activity of Acid phosphatase was recorded after 30 days of treatment while a significant rise in the activity of the same enzyme was noticed after 60 days of treatment. Similarly, G-6-P also inhibited significantly by phosphamidon after 60 days of treatment. No change in the values of prtein and glycogen were observed after 30 days of treatment. Significant increase in the

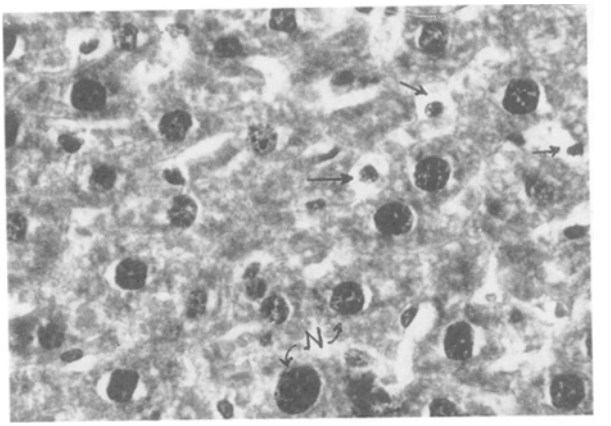

Figure 2 Photomicrograph showing enlargement of nucleus (N) and necrotic cells (arrow). 30 days after treatment X 200.

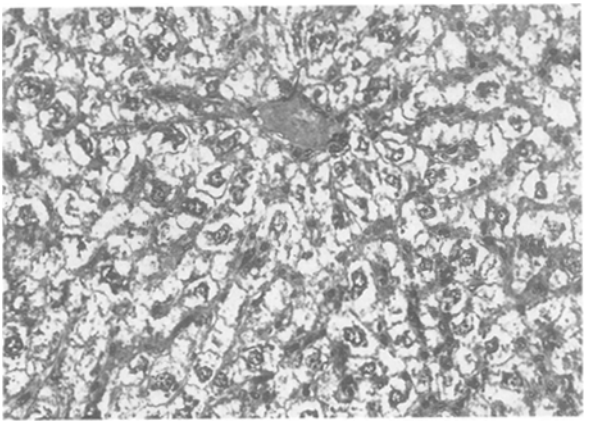

Figure 3 Photomicrograph showing cytoplasmic vacuolization and midzonal necrosis. 60 days after treatment X 100.

amount of protein was observed after 60 days of treatment while the value of glycogen remains unchanged (Table 1).

A significant increase in the values of SGOT, SGPT, and bilirubin were recorded after 30 as well as 60 days treatment (Table 2)

Present study indicates that the chronic exposure of the phosphamidon caused pathological and functional changes in the liver of mice. Though no significant change in the body weight and body weight in relation to liver weight has been noticed. Similarly no signs of intoxication were observed. The changes in the liver as observed in present investigation were midzonal necrosis, enlargement of nuclei of hepatocytes, and a mild fatty degeneration. Similar degenerative changes have also been reported in rodents by other investigators with other insecticides

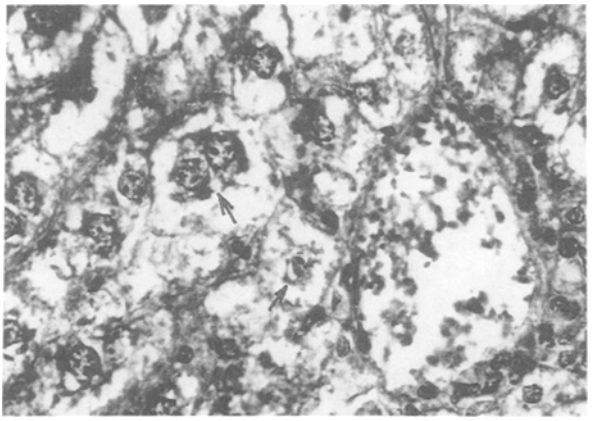

Figure 4 Photomicrograph showing disturbed architecture of hepatocytes. Note the severe cytoplasmic vacuolization and karyorrhexis. After 60 days of treatment X 200.

(**Dikshit et al.** 1975, Kruase and Homolo 1974, Sarin and Saxena 1978, Saxena and Sarin 1980). Enlargement of the size, and variation in the size of the nuclei are seen in the hepatic cells of the animals fed with carcinogenic aminozo dyes. This may be due to continuous synthesis of DNA by nuclei, which are unable to divide (Christie and Lepage 1961).

Fatty degeneration, histologically recognized as vacuolization, and not hydropic degeneration was observed by Morgan and Smith (1974). The absence of any definitive membrane around the vacuole suggested that they consisted of fat globules. In the present investigation mild fatty degeneration of the liver cell was frequently seen.

Phosphamidon caused increase in the activity of Alkaline phosphatase while it inhibited the activity of acid phosphatase. **Sarin and Saxena** (1978) also observed increase in the alkaline **phosphatase** in the gerbils greated with quinalphos but they observed an increase also in the activity of acid phosphatase which is in contradiction to the present investigation. The decrease in the activity of hepatic G-6-P which was observed in the present observation is with the accordance to Dinmann 1965, Murphy and Malley 1969 and Saxena and Sarin 1978. Hepatic protein also increases after 2 months of phosphamidon intoxication. But surprisingly glycogen remains unchanged. This interference of phosphamidon in protein metabolism may be due to autolysis of the hepatic cells as suggested by Wahi et al.(1956) or due to hepatic detoxification activities which can involve increase in protein metabolism (Bovet 1961). Increase in the level of SGOT, SGPT and bilirubin in the serum, may be due to pathological changes such as necrosis of hepatocytes which cause increase in the permeability of the cell membranes, resulting in the release of transaminases in the blood stream. From the observations of

Table 1 Enzyme activity and estimation of protein & glycogen in liver after chronic treatment of phosphamidon (35 ppm)

Days		Enzyme activity in mg pi[a] g/h+S.E.			mg/g	
		Alkaline Phosphatase	Acid Phosphatase	Glucose-6-Phosphatase	Protein	Glycogen
30	Control	0.87±0.05	6.01±0.18	6.01±0.18	76.12±2.19	0.95±0.07
30	Experimental	2.12±0.15[y]	3.85±0.26[y]	4.26±0.15[x]	79.40±2.86*	1.06±0.03*
60	Control	0.81±0.04	6.36±0.25	7.18±0.15	78.35±2.4	0.98±0.18
60	Experimental	0.85±0.05*	7.23±0.20[x]	6.77±0.15[z]	94.33±1.89[z]	0.85±0.02*

Each mean represents 10 animals

x = $p < .05$, y = $p < .001$, z = $p < .002$, * = Non significant.

Table 2. Quantitative determinations of SGOT, SGPT and bilirubin in serum after chronic treatment with phosphamidon (35 ppm)

Days	SGOT (SF Units/ ml)	SGPT (SF Units/ ml)	Bilirubin (mg/dl)
30 Control	22.4±0.7	44.3±0.8	0.5±0.08
30 Experimental	27.7±0.6	52.6±2.3[a]	0.9±0.05[c]
60 Control	24.4±0.5	46.6±0.8	0.5±0.04
60 Experimental	35.9±0.3	76.6±1.7[c]	1.2±0.12[b]

a = $p < .05$, b = $p < .01$, c = $p < .002$

the present investigations it is indicated that the continuous exposure of even low dose (35 ppm) of phosphamidon causes hepatic damage.

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