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Effects of monocrotophos (an organophosphate) on ultra-structure and SDH of liver; AChE, glucose and bilirubin in blood of rat

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Abstract
Four groups of Wistar rats were taken for experimental work. 1/5th of LD_{50} dose (14 mg/kg body weight) of monocrotophos was administered by intragastric intubation to groups TI, TII and R for 15 days, 30 days, and 30 days with recovery of 30 days respectively. Corresponding controls for all the three groups were fed on normal diet. Ultrastructural studies were made with liver, whereas hepatic succinate dehydrogenase and serum level of glucose and bilirubin were studied biochemically. In control group rats, the liver tissue revealed normal histoarchitecture showing well defined nucleus with nucleolus, mitochondria with intact cristae, parallel pleats of rough endoplasmic reticulum with ribosomes studded on them, glycogen rosettes and smooth component of the endoplasmic reticulum. In groups TI and TII, marked alterations in histoarchitecture as compared to control were observed. Most prominent effect was the loss of nuclear membrane resulting in the exculsion of nuclear material in the cytoplasm. Proliferation of rough endoplasmic reticulum with a concomitant decrease in the smooth component was a discernible effect. Glycogen deposits also decreased whereas increase in the serum level of glucose was observed. Marked mitochondrial swelling with a moderate loss of cristae was one of the most important features of the necrotic hepatic cells which are also correlated with the observed decrease in the activity of hepatic succinate dehydrogenases. Enormous infiltration of the cytoplasm with certain electron transparent vacuoles of unknown origin was also observed. The serum activity of acetylcholinesterase was found to be significantly inhibited whereas serum level of bilirubin was found to be increased. Group R showed a lot of recovery at ultrastructure as well as at biochemical levels as compared to TI and TII groups.

Keywords: Monocrotophos, Organophosphate pesticide, Toxicity, Electron microscopy

1. Introduction
Monocrotophos [3 hydroxy-N-methyI-cis-crotonamide dimethylphosphate], an organophosphorous insecticide is widely used as an effective crop protectant. It has both systemic and contact properties and has been used against a wide range of insects including mites, Boll worms, sucking insects, leaf eating beetles and other larvae on variety of crops [1]. The toxicity of the insecticidally active organophosphorous compounds to mammals and insects is primarily attributed to their ability to inhibit acetyl cholinesterase (AChE) [2, 3]. A few workers [4, 5] reported effect of some pesticides on activity through phosphorylation of the active serine hydroxyl group situated in the active centre of acetyl cholinesterase into acetic acid and thus making the enzyme non-available to hydrolyze acetylcholine (ACh) into acetic acid and choline. This results in the accumulation of acetylcholine at all sites of cholinergic transmission, hereby causing continuous stimulation of the muscle or nerve fiber, resulting eventually in the exhaustion and tetany [6].
The liver is the major organ for detoxification and so the changes in its histoarchitecture as well as serum biochemistry related to this organ is used as a major indicator of the stress factor imposed by any pesticide. The toxic effects of organophosphorous insecticides on the neuronal functions and structure have been investigated by many workers, but the cytotoxicity of these insecticides on the liver has been assessed to a very little extent [4, 7-9]. Various processes of metabolism and detoxification are catalyzed by hepatic enzymes [10]. These are located in various membranous compartments of liver cells and integrity of these membranes play a vital role in the metabolism of these insecticides, but a very little attention has been paid to the toxic stress laid on the intactness of these cell structures and cellular organelles at ultra-structural level after treatment with organophosphates [11-13]. The present investigations were, therefore, made to throw light on the ultra-structural changes in the liver of female albino rat after exposure to monocrotophos for various durations.

Materials and Methods

LD_{50} of monocrotophos was standardized on the basis of the dose calculated by Janardhan *et al.* [14] and was found to be 14 mg/kg body weight. Adult female albino rats of Wistar strain in proestrous phase of estrus cycle weighing 100-150 gm were obtained and divided into three groups TI, TII and R groups (8 rats in each group). $1/5^{th}$Of LD_{50} value of monocrotophos i.e. 2.8 mg/kg body weight was administered for 15 days to TI group and for 30 days to TII group. To the rats of R group, the same dose was given for 30 days and then the rats were kept on normal conditions i.e. without monocrotophos for 30 days. Another three group CI, CII and CIII (8 rats in the same phase of the estrus cycle in each group) were kept as corresponding controls for all the treatment groups. All the animals were kept on the commercial standard diet and tap water *ad libitum*. The weight of animals was recorded weekly.

At the end of the treatment period, for serum preparation, blood of the overnight fasted rats of each group was collected from the retro- orbital plexus by means of fine sterilized glass capillary under light ether anesthesia. Serum was used for the estimation of acetyl cholinesterase (AChE) [15], glucose [16] and bilirubin [16]. The rats were sacrificed by cervical dislocation. The thoracic cavity was cut opened to take out the liver in all the groups. The extraneous material was removed and liver was washed in saline. Liver (1kg) was perfused and homogenized in cold 0.25 M sucrose solution to estimate the activity of hepatic succinate dehydrogenase. The homogenate was centrifuged at 150g for 10 min. and the clear supernatant fluid adjusted to 10% (w/v) strength with sucrose solution, were used for the source of the enzyme. Extraction was done at 0-5 ^{0}C; the activity of the enzyme was determined by the method of Kun and Abood [17].

For electron microscopic studies small pieces of control and treated groups were fixed overnight in 2.5% glutaraldehyde buffered at pH 7.2 with 0.2M phosphate buffered at 4^{0}C followed by post fixation in 1% osmic acid solution for 2hr. The samples were then dehydrated with graded concentrations of acetone followed by toluene and embedded in araldite embedding medium. Ultra-thin sections were cut and stained with uranyl acetate and lead citrate and examined in CM-10 Philips electron microscope.

Results and Discussion

In monocrotophos treated rats, significant inhibition in serum acetyl cholinesterase (AChE) was observed in both TI and TII groups. Moreover, the rats of R group also showed statistically significant depression in the acetyl cholinesterase (AChE) activity, but the degree of inhibition was far less than that of TI and TII, thus apparently indicating the recovery potential of the enzyme on the withdrawal of treatment (Table1). Serum bilirubin as well as glucose level showed an increase in TI and TII groups, which recovered back in R groups as compared to their controls respectively (Table1). A statistically significant inhibited levels of succinate dehydrogenase (SDH) were reported in the liver of rats of TI and TII groups (Table 2). The withdrawal of monocrotophos treatment in the R group normalized the level of SDH.

Table 1: Effect of monocrotophos on some biochemical variables in blood serum of Female albino rats of proestrus phase of estrous cycle

Parameters	2.8mg/kg body weight moncrotophos/day					
	15 days treatment(TI)		30 days treatment(TII)		30 days recovery(R)	
	Contl	Exptl	Contl	Exptl	Contl	Exptl
Acetylcholinesteras (µmole min^{-1} ml^{-1}) %change	0.527±0.05	.359±0.03***	0.523±0.06	0.297±0.04***	0.529±0.03	0.469±0.05
	(-)31.87%		(-)43.21%		(-)11.34%	
Glucose(mg /dI) %change	142.21±5.92	149.42±7.25*	140.60±4.92	150.81±6.96**	145.36±10.32	146.23±8
	(+)5.06%		(+)7.26%		(+)0.598%	
Bilirubin(mg/dI) %change	0.119±0.02	0.18±0.05	0.12 ±0.03	0.19±0.06*	0.12±0.0	0.13±0.04
	(+)51.26%		(+)58.33%		(+)8.33%	

Values are expressed as mean ±S.D (n=6)

*P<0.05; **P<0.01; ***P<0.001, when the values are compared with respective controls.

Table 2: Effect of monocrotophos on succinate dehydrogenase in liver of Female albino rats of proestrus phase of estrous cycle

Parameters	2.8mg/kg body weight monocrotophos/day					
	15 days treatment(TI)		30 days treatment(TII)		30 days recovery(R)	
	Contl	Exptl	Contl	Exptl	Contl	Exptl
Succinate Dehydrogenase (mg/g protein/h) % Change	3.92±0.55	2.89±0.98*	3.96±1.05	2.68±0.87**	3.89±1.12	3.60±0.92
	(-)26.67%		(-)32.32%		(-)7.45%	

Values are expressed as mean ± S.D. (n=6)

*P<0.05; **P<0.01, when the values are compared with respective controls.

The electron microscopic observations revealed marked effects on the nucleus, endoplasmic reticulum, mitochondria, glycogen content and biliary pathways in liver cells of both TI and TII groups. So far, there is no reference available on the studies done on the effects of monocrotophos on the ultra-structure of liver to the best of knowledge of present workers. Control rats, which remained without any treatment showed a clear and fine hepatic cellular structure (Fig.1). Nuclear structures were well intact, with fine nuclear chromatin and well intact nuclear membrane (fig.1a). Mitochondria were round to oval in shape and uniform in size with well intact cristae. Rough endoplasmic reticulum (RER) in the form of parallel pleats was found in close association with the mitochondria. Fine ribosomes were studded onto the lamellae of RER, which is indicative of normal protein synthesis (fig.1a). Smooth endoplasmic reticulum (SER) was also discernible at many places. Golgi complex was well developed with a typically smooth sac like structures on both the ends of its flattened cisternae (fig. 1b). Abundant star – shaped rosettes of glycogen were present in the cytoplasm (Fig. 1b). A well-defined bile canaliculi, with microvilli of variable size projecting as small protuberances in the lumen were observed (Fig 1c). Moreover, desmosomes were also evident in the close proximity of these bile canaculi representing the cellular integrity of the adjacent hepatocytes

Liver of rats exposed to moncrotophos for 15 days (TI) and 30 days (TII) showed many pathological changes in both necrotic as well as less affected hepatic cells. The prominent

effects were evident on the membranous integrity of various organelles. In TI group, the nucleus was reduced in size in necrotic (Fig. 2a) as well as less affected (Fig. 2b) hepatic cells. This may cause decreased rate of DNA synthesis and hence of protein synthesis.

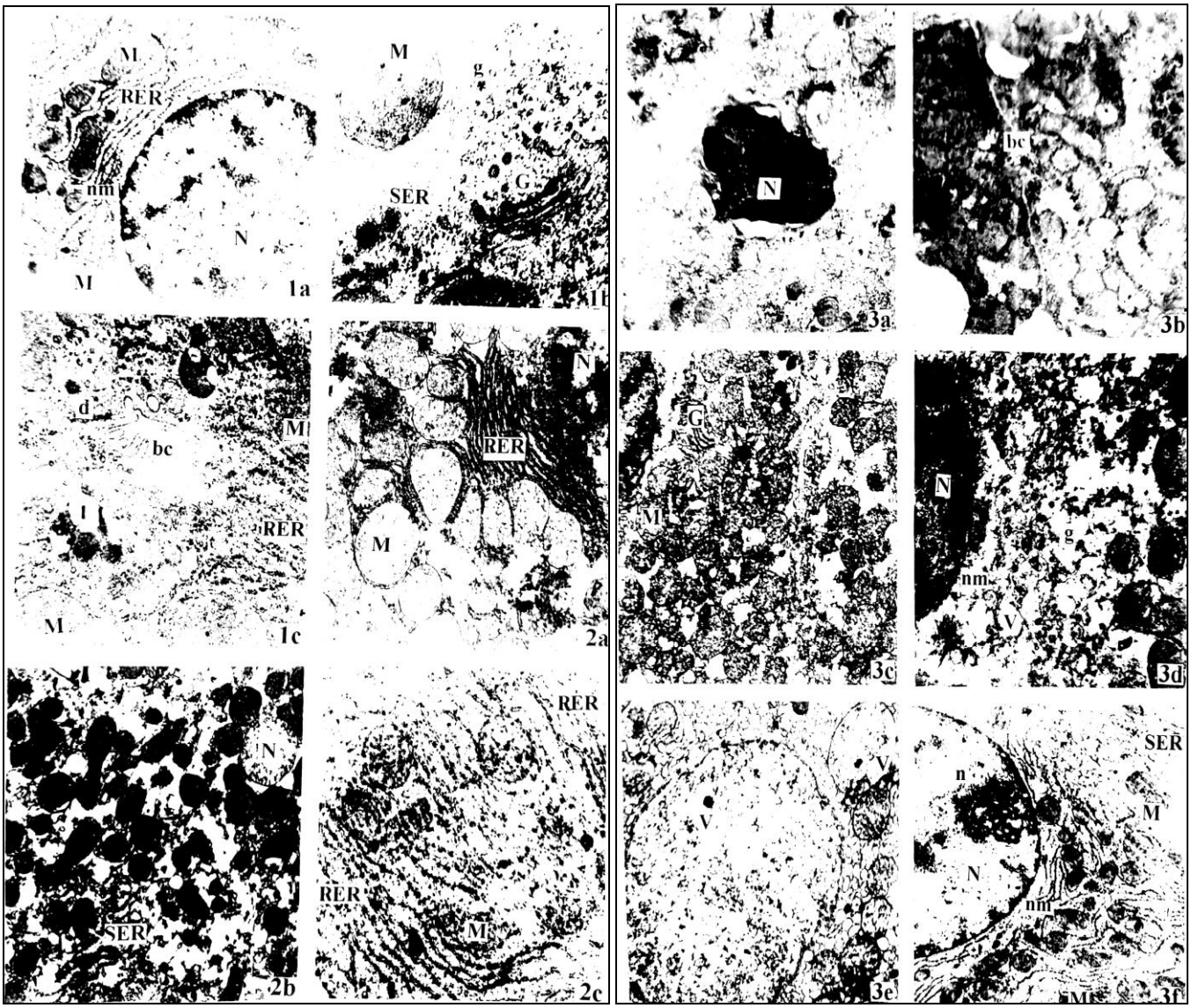
Furthermore, marked mitochondria swelling with a moderates loss of cristae in necrotic hepatocytes was one of the most important features in these rats (Fig. 2a) whereas an increase in number of mitochondria was observed in less affected hepatic cells (Fig. 2b) which may be indicative of increased energy requirements of the cells in an effort to overcome the noxious effects of this organophosphate pesticide. An increased number of mitochondria in any pathological condition is documented to reflect the process of uncoupling of oxidative phosphorylation. This process of disturbed oxidative phosphorylation was marked; and the disturbances were observed in succinate dehydrogenase activities in the liver of the rats. Analogous observations of marked swelling and an increase in the number of mitochondria were also reported by EI-Elamy *et al*. [18] during a study on the effect of pyrethroid insecticide neopybuthrin. Manring and Moreland [19] reported swollen mitochondria in rats treated with chlordecone (an organophosphate).

Marked proliferation of SER in less affected hepatocytes (Fig. 2b) with a concomitant increase in rough component i.e. RER in necrotic hepatic cells (Fig. 2c) were discernible effects. Proliferation of SER observed during present studies might be correlated with induction of microsomal enzyme activity and is indicative of cellular detoxification. Similar observations have been made for a wide variety of aromatic chlorinated hydrocarbons in rats by Fowler *et al*. [20] A marked increase in the amount of RER might be due to the fact that RER is associated with the ribosomes responsible for synthesis of proteins and, therefore, increased requirement of protein synthesis can be the only plausible reason for the proliferation of RER in less affected hepatic cells.

There was a significant decrease in glycogen content observed in the necrotic hepatic cells (Fig 2c), which was in agreement with the biochemical studies of increased blood glucose content and so was attributed to the enhanced process of glycogenolysis. This hyperglycemia induced by monocrotophos might be related to the increased phenomenon of glycogenolysis in liver due to possible anoxic conditions established by OP compounds. Similar hyperglycemia due to monocrotophos treatment has been demonstrated by many other workers in fish [21] and rats [22] and due to organophosphate pesticide in fish [23-25]. It was postulated by a few workers that gluconeogenesis plays a pivotal role in the glucose homeostasis in order to maintain a constant level of glucose in blood which is being compromised due to excessive utilization in the energy production due to possible anoxic condition established by organophosphorous compounds.

Increase in duration of pesticide treatment for 30 days produced more marked alterations. Nuclear membrane was significantly lost in the necrotic hepatic cells resulting in the exclusion of nuclear material in the cytoplasm (Fig 3a). The biliary channels were observed to be blocked due to the increase in size and thickness of the microvilli into the lumen of bile canaliculi (fig3b). The Golgi complex was also found to be contracted (fig. 3c). Blockage of canaliculi and biochemically observed increase in serum bilirubin levels are suggestive of cholestatic symptoms produced due to toxicity of monocrotophos. These observations in present studies are further supported by many other workers [26, 27] who documented that higher level of bilirubin has been associated with hepatocellular dysfunction leading to high rate of hemolytic breakdown of red cells. Bhatnager and Jain [28] also reported an increase in bilirubin in serum of mice treated with phosphamidon. They suggested that an increase may be due to pathological changes such as necrosis of hepatocytes, which cause increase in the permeability of cell membranes and hence of release of bilirubin in the blood stream. Javitt [29] has demonstrated the cholestatic symptoms in liver due to conjugation of Taurme with lithocholic acid. He reported that the dose dependent cessation of bile flow and blockage of bile channels occur when different hepatic changes were produced by Taurme. Enormous infiltration of nuclear cytoplasm as well as of mitochondria with certain electron transparent vacuoles of unknown origin was also observed in necrotic hepatic cells (Figs 3d/ 3e). Several histopathological studies done on liver of fish by different workers also revealed nuclear pycnosis, mild to severe necrosis, disrupted hepatocytes, disorganized hepatic canaliculi, disintegrated blood vessels, ruptured central vein and vacuolation on exposure to monocrotophos [30] and other organophosphates [31-37].

Keeping the monocrotophos treated rats on recovery for 30 days in R group resulted in quite appreciable normalization of the hepatic histoarchitecture. Some extent of mitochondrial swelling and proliferation of RER, were still evident (Fig. 3f). The recovery might be due to revival of reduced enzymatic activity responsible for detoxification of toxic agents in the liver of treated rats. Moreover, cessation of exposure to monocrotophos in R group resulted in significant normalization of the inhibited levels of acetylcholinesterase. This could be due to non-persistent nature and reversible inhibition of the organophosphates which by undergoing hydrolysis might lead to the restoration of active acetyl cholinesterase and hence might release the inhibitory effect on the various receptors from accumulated acetylcholine in the synapses [38] and resulted in restoration of normal metabolic activities and histoarchitecture of liver. Therefore, it is concluded that histoarchitectural as well as biochemical changes due to exposure to monocrotphos were dose and duration dependent and cessation of exposure resulted in significant recovery. Hence the workers who get exposed to organophosphorous sprays are required to take a brief period of rest to cope up with the any kind of abnormality with the severe inhibition of AChE activity and to minimize the danger of intoxication from organophosphorous pesticides including moncrotophos intoxication.



Explanation to figures.

TEM of liver

Fig 1: In control group showing (a) well defined nucleus (N), intact nuclear membrane (nm). Mitochondria (M), parallel pleats of rough endoplasmic reticulum (RER) and blebs of smooth endoplasmic reticulum SER); (b) well developed Golgi complex (G) and abundant rosettes of glycogen (g); (C) bile canaliculi (bc) and lysosomes (I).

Fig 2: In TI group (a) increased number of swollen mitochondria (b) increased number of mitochondria, a few dividing mitochondria and proliferated smooth endoplasmic reticulum (c) markedly proliferated rough endoplasmic reticulum and reduced level if glycogen.

Fig 3: In TII group (a) badly contacted nucleus and absence of nuclear membrane (b) complete blockage of bile canaliculi (c) badly contracted Golgi complex (d) contracted nucleus with disrupted nuclear membrane, reduced level of glycogen rosettes and vacuolaton of cytoplasm.

Fig 3f: R group showing intact nucleus, nucleolus. Moderately swollen mitochondria rosettes of glycogen, moderate amount of SER as well as RER.

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