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Effect of Exopsure to Monocrotophos (an organophosphate) on Hepatotoxicity in female albino rat – Histochemical and Biochemical Studies

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Abstract
An evaluation of the toxic effects of the organophosphate, monocrotophos, was made by the histochemical and biochemical studies of general lipids in the rat liver after treatment with its low residual level doses (LD^{50} 1.4 mg/kg) to three groups TI, TII & R of six rats each for 15 days, 30 days and 30 days with recovery period of 15 days respectively. The treated groups TI & TII showed significant increase in lipids in hepatic cells and significant decrease in triglycerides in serum whereas in R group showed significant recovery. The observations are thus indicative of the hepatic toxicity caused by organophosphates at cellular level in the liver of rats.

Keywords: Moncrotophos, Organophosphate pesticide, General lipids, Triglycerides, Liver.

Introduction
Organophosphate compounds are one of the most frequently utilized class of pesticides throughout the world, because of their lesser persistence in the soil and also in fat stores of the body. These pesticides are essentially absorbed through the skin, gastro-intestinal tract and the respiratorty tract. Moncrotophos, an organophsphorous insecticide of low mammalian toxicity and selective insecticidal activity, has gained a wide application in the field of agriculture. A natural consequence of more extensive use of monocrotophos is that more people and other non-target organisms would have the opportunity to become exposed to it, which in turn would be expected to result in an increase in the frequency of toxic sequelae attributed to this compound [1].
Liver is known to be the major target organ for pesticides. According to Casarett and Bruce (1980) [2], the liver has a high capacity to bind chemicals and this organ probably concenterates more toxicants than any other organ. Various processes of metabolism and detoxification are catalyzed by hepatic enzymes [3]. 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. The toxic effects of organophsophorous insecticides on the neuronal functions and structure have been investigated by many workers, [4-6] but a very little attention has been paid on the toxic stress laid on the intactness of these cell structures and cellular organelles after treatment with organophosphates [7-9]. The present investigations were, therefore, made to throw light on the histochemical as well as biochemical changes in the general lipids of the liver of female albino rat after exposure to monocrotophos for various durations.

Material and Methods
Healthy adult female albino rats of Wistar strain in protestors 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). LD^{50} of Monocrotophos was standardized on the basis of the dose calculated by Janardhan *et al.* [10] and was found to be 14 mg/kg body weight. 1/5th 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 15 days. Another three group CI, CII and CIII (8 rats in same phase of 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 were recorded weekly.
At the end of treatment period, blood of the female rat from each group in protestors phase of estrus cycle was collected from the retro-orbital plexus under the light anesthesia. Serum was

prepared for estimation of triglycerides [11]. 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. For histochemical studies, small pieces of liver were fixed in formaldehyde calcium for 24hrs and processed for gelatin embedding according to the standard technique [13]. The gelatin sections were cut by cryostat at 10 µ thickness and later on subjected to Sudan Black B (SBB) staining technique [12]. Student's 't' test was employed for statistical analysis.

Results and Discussion

The lipids were stained with SBB. In the hepatocytes, the triglycerides were present in the form of duplex / crescentic bodies. In SBB, their outer rims were stained darkly as compared to medullary parts and were located near the membrane of the cells or near the nuclei (Pmgs. 1&2). In treated groups TI & TII, there was marked increase in lipids in necrotic hepatocytes, especially in duplex bodies (Pmgs. 3-5) showing an increase in general lipids and triglycerides. The increase in lipids might be due to accumulation of abnormal amounts of fat in the parenchymal cell [13]. The lipids that accumulated were predominately triglycerides. Whereas in blood serum, the level of triglycerides showed a reduction of 7.28% and 12.53% ($p<0.05$) in TI and TII groups as compared to their controls respectively. (Table). It had been shown by a number of investigators that a block of the secretion of hepatic triglycerides into the plasma was the basic mechanism underlying the fatty liver induced in the rat by various organophsophorous pesticides [14-15]. The decrease in concentration of plasma triglycerides paralled by an increase in triglycerides' in the hepatic cells observed during present studies are in strict agreement with the earlier studies made by various workers [16].

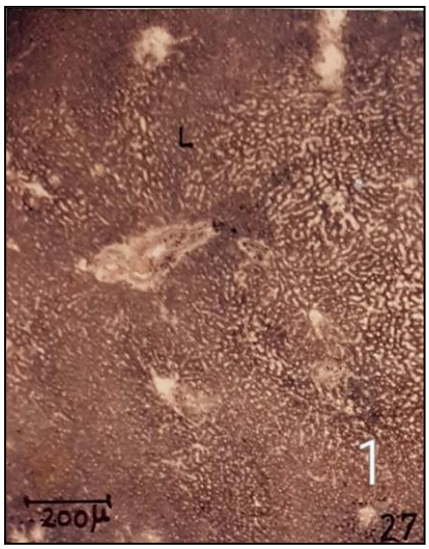
Table: Effect of Monocrotophos on triglycerides in serum of female albino rats in proestrus phase of estrous cycle

Parameters	2.8 mg/kg body weight monocrotophos/day					
	15 days treatment (TI)		30 days treatment (TII)		15 days recovery (R)	
	Contl	Exptl	Contl	Exptl	Contl	Exptl
Triglycerides % Change	72.43 ± 8.67 67.15 ±7.93 (-) 7.28%		74.67±6.82 65.31±9.14* (-) 12.53%		73.26±10.34 71.98 ±8.79* (-) 1.74%	

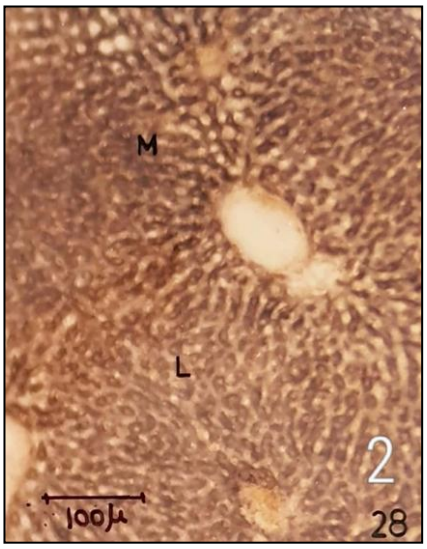
The values are expressed as Mean ± S.D. (n=5)
* P<0.05; ** p<0.01, when the values are compared with respective controls.

Organophsophorous pesticides also result in formation of reactive oxygen species which can cause hepatotoxicity [17]. They being lipophilic interact with cells through lipid-rich bio-membranes and damage membranes through lipid peroxidation. The synthesis of free radicals results in oxidative stress leading to disruption of membrane lipids and degradation of membrane lipids and hence cellular deterioration. They also impair enzymatic anti-oxidant defences like superoxide dismutase, catalase etc. [18-19].

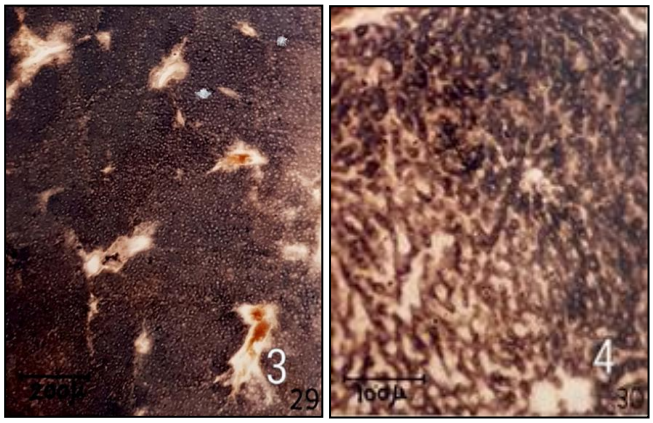
In R group, general lipids and triglycerides were moderately stained showing a lot of recovery in hepatocytes. (Pmg. 6). The serum level of triglycerides estimated was also recovered (Table). The recovery might be due to revival of reduced enzymatic activity responsible for detoxification of toxic agents in the liver of treated rats. Hence the workers who get exposed to organophsophorous sprays are required to take a brief period of rest to cope up with the any kind of abnormality and to minimize the danger of intoxication from organophsophorous pesticides including monocrotophos intoxication.



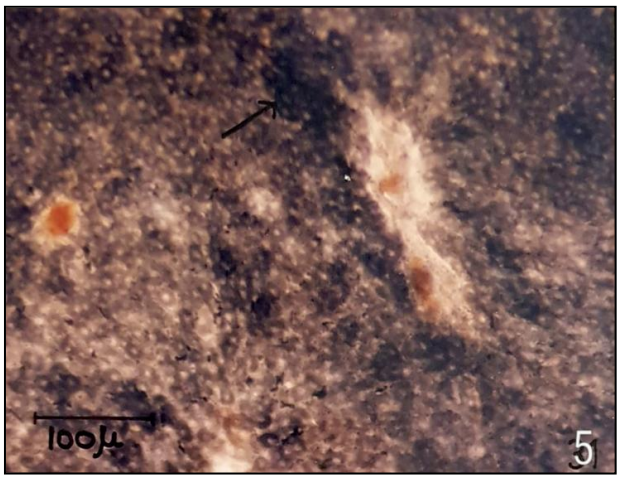
Pmg 1: T.S. control liver showing lipid bodies (L) in the hepatic celsl. FCa-PC/SBB



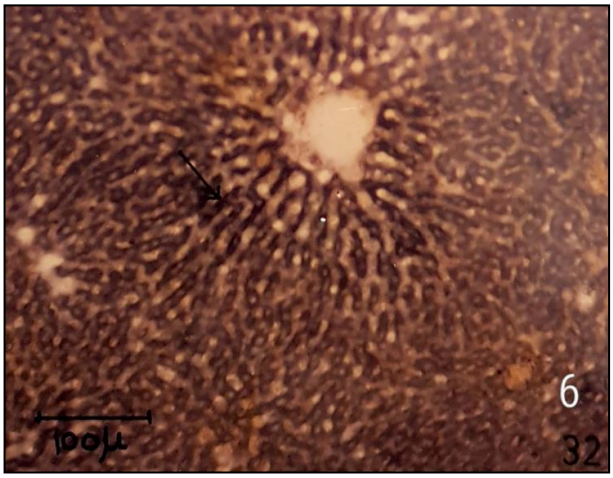
Pmg 2: T.S. control liver showing lipid bodies (L) in the hepatic cell. Some lipids are present in sinusoids and intralobular vein. FCa-PC/SBB



Pmgs 3 & 4: T.S. liver of TI group showing an increase in lipids in general lipids in hepatic cells (arrow). FCa-PC/SBB



Pmg 5: T.S. liver of TII group showing an abundant increase in lipid bodies in hepatic cells. The increase is more in some hepatic cells (arrow) and less in other hepatic cells. FCa-PC/SBB



Pmg 6: T.S. liver of R group showing some decrease in lipids in hepatic cells. FCa-PC/SBB

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