Int J Pharm Bio Sci 2015 Oct; 6(4): (B) 1 - 8

Research Article **Pathology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

ALTERATIONS IN LIPID CONTENT IN MANCOZEB INDUCED ALBINO MICE

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ABSTRACT

The histochemical effects of the lipid and the biochemical analysis of cholesterol content induced by mancozeb, a widely used fungicide in India and in Madhya Pradesh were observed on Swiss albino mice (*Mus musculus albinicus*). Three groups were used for the present experiment, where Group I was taken as control and was administered distilled water. Group II and III were the test groups receiving mancozeb at a dose of 4.2 mg/kg bw and 6.7 mg/kg bw respectively for 4 weeks. Thereafter, the histochemical analysis of lipid using Sudan Black III stain in liver and kidney tissues was performed which revealed normal lipid content in the control (Group I), while increase in the lipid content was noted in both Group II and Group III. The biochemical analysis of the total serum cholesterol level also showed increase in the treated groups as compared to the control group. This study indicates marked alteration in the lipid content in mancozeb exposed albino mice.

Keywords*: lipid, cholesterol, histochemical, mice, mancozeb*

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INTRODUCTION

The increasing human population demands more food production for which we have to depend more on fertilizers and pesticides. The indiscriminate use of pesticides, insecticides, fungicides, etc. poses threat to non-target animals and even humans who are exposed to these chemicals. One such fungicide of concern is mancozeb, a polymeric complex of Zinc and Manganese salts of ethylene bis dithio carbamate group. It is commonly used for foliar application and seed-treatment in agriculture[1]. Mancozeb is listed to have low acute toxicity however; several researchers have shown its toxicological effects on thyroid, gonads and chromosomes of bone marrow cells in mice [2a,2b,3] and pathological changes in liver, kidney, spleen and heart[3,4]. Reproductive toxicity in animals was also documented by various workers[5,6]. Treatment with endosulfan (10 mg/ kg/ day) in rats revealed kidney damage, vacuolisation and necrosis [7].Since kidneys and liver are associated with metabolism and elimination of toxicants from the body they are considered as key points to elucidate toxicity of chemicals. In view of the pivotal role performed by the liver and kidney in detoxification, they were chosen as the focal organ of study in the current study to examine the histological changes in response to the toxic effects of mancozeb. Very few reports were available regarding the histochemical and biochemical effects of mancozeb with regards to lipid and cholesterol content. Hence, the present investigation concentrates on the histochemical and biochemical alteration in the lipid content and total serum cholesterol of albino mice induced by mancozeb.

MATERIALS AND METHODS

Experimental Animals

Mice weighing between (25-30 gm) were selected randomly and kept in individual cages with 12:12 hour light and dark cycle and at room temperature of 26 ± 10^0 C. Synthetic pellet diets and water were provided *ad libitum*. They were obtained from College of Veterinary Science and Animal Husbandry, Rasalpura, Mhow, Indore (M.P.) after obtaining the approval from Institutional Animal Ethical Committee (IAEC) vide proposal no. CPCSEA/118/2014 dated 20/12/012 and were kept in the animal house of IPS Academy, Indore (CPCSEA Reg. No.465/01/ab dated 24th August, 2001). Animals were divided into three groups with 6 mice in each group. All experiments were performed in compliance with as per guidance for care and use of laboratory animals[9].

Experimental Design

Mancozeb (commercial grade 75% wettable powder) was made available from Indofil Chemical Company, Mumbai and dissolved in distilled water for oral administration.

Group I **:** Animals in this group were the controls and were given orally distilled water consecutively for 6 days a week for 30 days.

Group II **:** Animals in this group were given mancozeb orally in dose of 4.2 mg/kg/day (MCZ D1) in equivalent volume in the same procedure.

Group III **:** Animals in this group were given mancozeb orally in dose of 6.7 mg/kg/day (MCZ D2) in equivalent volume in the same procedure.

Biochemical Assays

For the biochemical studies blood samples were collected from the animals of all the groups on 7th, 15th, 21st and 30th days by cardiac puncture after which the animals were sacrificed following the guidelines. The serum was prepared and total serum cholesterol estimation was performed using Sackett, (1925)[10] method. The data was statistically analysed using ANOVA by MCTAC software.

Histochemical Assays

For the histochemical study, tissue samples were obtained from Group I, II and III on 15th and 30th day of treatment. The animals were dissected to remove the liver and kidney tissues and preserved in 10% formalin after washing in saline. The tissue samples were subjected to routine histological procedures and paraffin blocks were made. Sections were made from the blocks and routine procedure was followed for slide preparation. The lipid

content was detected using Sudan Black III stain method [10] for lipid detection and were analysed on the basis of intensity[11].

RESULTS

Biochemical changes

The serum analyses for total serum cholesterol are listed in Table 1. The results show significant increase of cholesterol level in mancozeb exposed groups as compared to that of control group during the treatment period. A highly significant positive correlation was seen in the results. After 4 weeks of treatment with mancozeb, a marked increase in total serum cholesterol was noted in Group III than Group II. The results are expressed in figure 1 which reveals the gradual increase in the total serum cholesterol content from day 7 to day 30.

Table 1

Biochemical content of total serum cholesterol levels in mancozeb exposed albino mice

TREATMENT	GROUP NO.	TOTAL SERUM CHOLESTEROL			
		Day 7	Day 15	Day 21	Day 30
GROUPS		Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
CONTROL UNTREATED	I	92.6±2.60	92.4±2.51	91.82±3.04	90.5±4.50
MCZ D1 (4.2 mg/kg/day)	II	109.7±3.06**	117.5±2.29**	129±2.52**	146±4.0**
MCZ D2 (6.7mg/kg/day)	III	112±2.0**	121±2.91**	132±3.50**	155±3.10**

p<0.01 statistically highly significant,*p between 0.05 to 0.01 significant, p>0.05 non- significant.

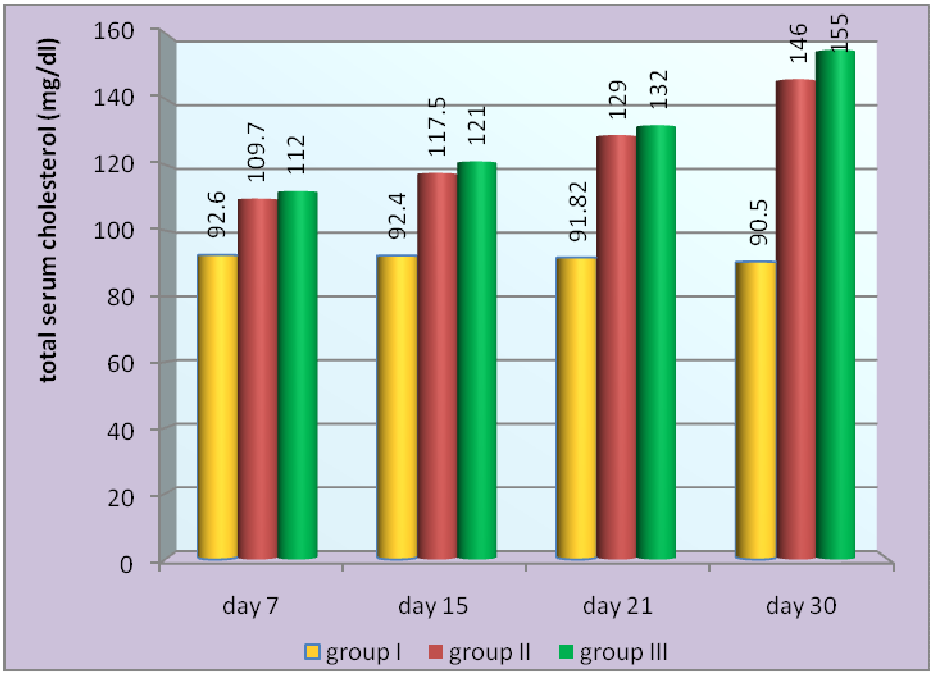

Figure 1

Total serum cholesterol levels in mancozeb exposed albino mice

Histochemical changes

The sections from all groups of mice in control and mancozeb treated groups were examined under light microscope. The intensities of stain were recorded in Table 2. The intensity of staining was assessed to analyse the lipid content present in the liver and kidney cells of the normal mice with treated mice at different concentrations of mancozeb. The sections from the liver and kidney tissues of control mice showed less lipid content as compared to that of the treated groups. Group II treated with 4.2 mg/kg/bw revealed moderate intensity while Group III treated with 6.7 mg/kg/bw showed highest intensity. The histochemical analysis thus revealed an increase in the lipid content in the treated mice.

TABLE 2

The intensities of lipid content in liver and kidney of control and experimental albino mice exposed to mancozeb

Group No.	Groups	Doses mg/kgbw	Day 15	Day 30
I	Control	-	++	++
II	MCZ-D1	4.156	++	+++
III	MCZ-D2	6.650	+++	+++

+++ = Very strong
++ = Strong
+ = Positive

The Sudan Black III stain used for lipid analysis showed blackish to brown granules of phospholipids with blue nuclei in the cells. Fatty accumulations were seen as flocculations long with increased vacuolisations due to the administration of mancozeb in albino mice. The lipid inclusions were uniformly distributed throughout the cytoplasm, perinuclear and peripheral in position and particularly accumulated in the cytoplasm adjacent to sinusoid in the liver cells. Lipid droplets were extensively distributed in most areas of the liver and kidney sections.

Liver tissue

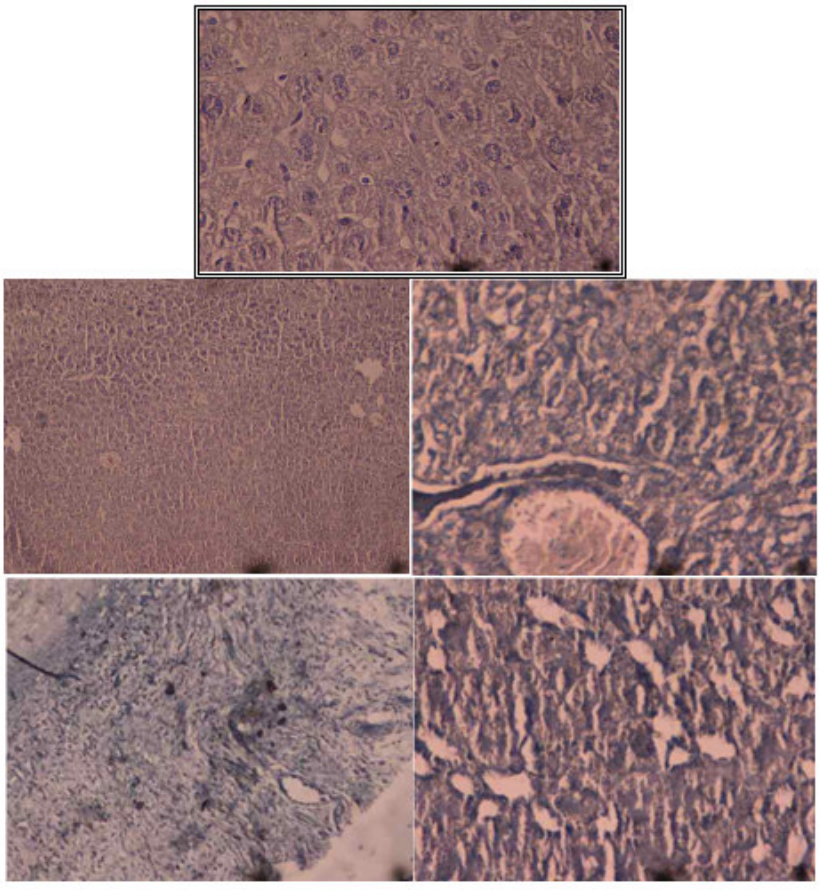

Figure 2

Photomicrographs of sections from Sudan Black III stained liver of (A): Control showing normal radially arranged hepatocytes and lipid content and blue nuclei and brownish to blackish granules., X 10; (B): Group II of 15 day showing loss of radial arrangement of hepatocytes and increased stain, X10; (C); Group II of 30 days showing cytoplasmic vacuolisation, lipid inclusions; (D): Group III of 15 days showing loss of normal architecture and increases intensity of stain; (E): Group III of 30 days showing intense staining and vacuolisations.

Kidney Tissue

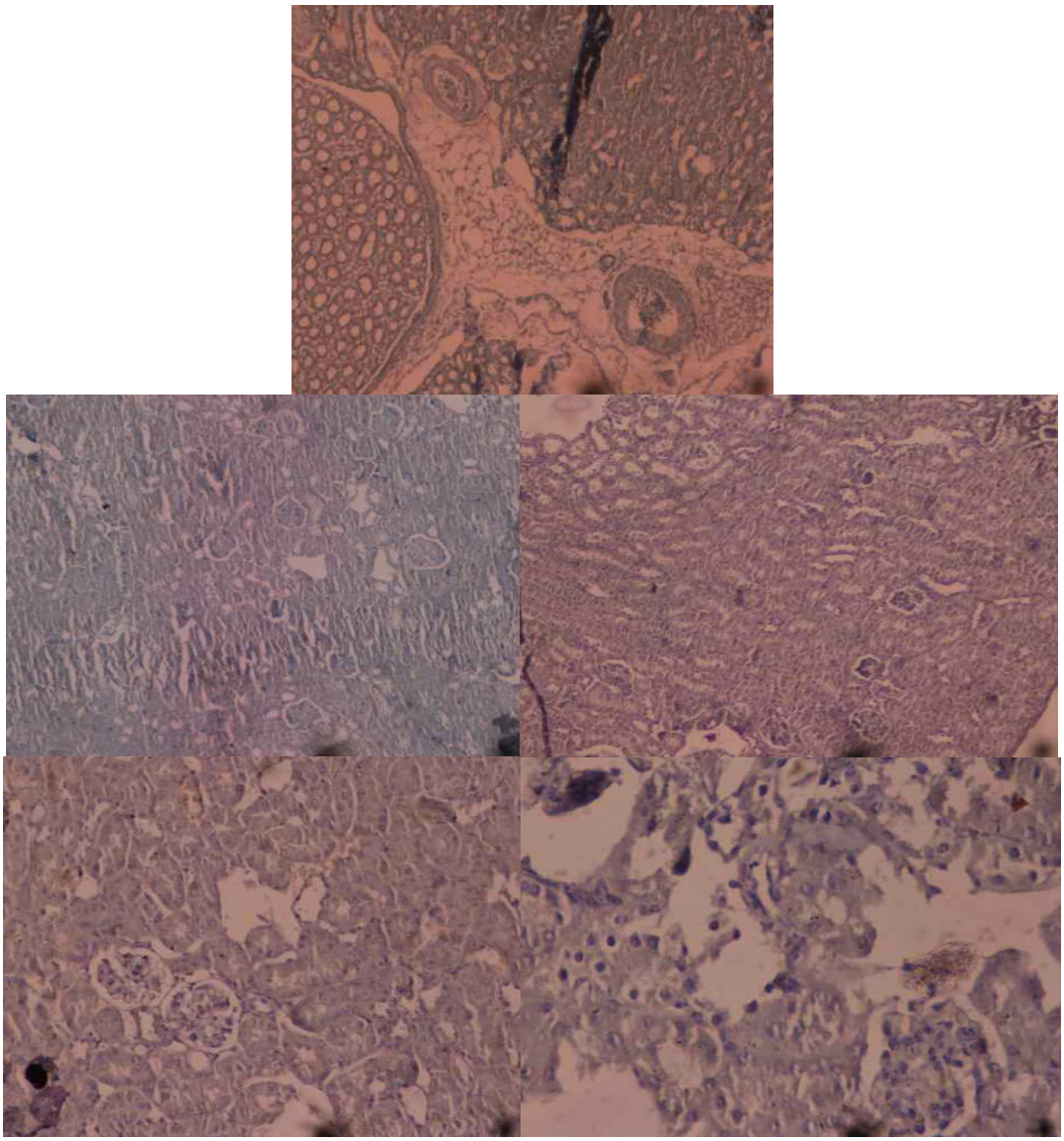

Figure 3

Photomicrographs of sections from Sudan Black III stained kidney of (A): Control showing normal glomerular network and lipid content with blue nuclei and brownish to blackish granules., X 10; (B): Group II of 15 day showing increased stain and little disorganised tubules, X10; (C); Group II of 30 days showing lipid inclusions, vacuolisations; (D): Group III of 15 days showing loss of normal architecture and increased intensity of stain and flocculations with degenerated tubules; (E): Group III of 30 days showing intense staining and vacuolisations and loss of normal architecture.

DISCUSSION

The dithiocarbamate group of pesticides are known for their biological effects through their metabolites like ethylene thiourea[12,13,14]. Proteins, carbohydrates and lipids are essential constituents of the food of animals. The lipids are the sources of energy. Cholesterol is the precursor for steroid hormones and also for vitamin D, which is essential for regulation of calcium and phosphorus metabolism and bone growth. Cholesterol is essential for membrane synthesis. In the present study, total serum cholesterol content significantly increased after treating the mice with mancozeb. Other authors reported that mancozeb at 600,700 and 800 mg/kg/day induced significant increase in serum cholesterol level in rats[15,16,17]. A significant increase was noticed in the total lipid content in hepatopancreas of crab during exposure to endosulfan[18]. Aldrin

also increased lipid and cholesterol levels in the liver of *Rana hexadactyla* [19]. A significant increase in cholesterol in carbosulfan induced kidney of male and female mice was also noted[20]. A similar report of increase in serum cholesterol level in rats exposed to Benzenhexachloride was also recorded[21]. The increased cholesterol level could be attributed partially to disorder of metabolic pathways[22,23]. Such changes suggest increased catabolism of bio molecules or reduced anabolism due to impaired tissue function in order to meet the energy demand of animal. The plasma cholesterol levels are considered valuable indicator of drug-induced disruption of lipid metabolism and development of fatty liver and altered cholesterol levels are implicated in impaired biliary excretion. Marked dose-dependent increase of serum cholesterol in mancozeb induced mice suggests increased synthesis and accumulation of cholesterol in the liver and kidney with probably impaired biliary function. The increase in cholesterol level indicates inhibitory action of pesticide on Cyt-p-450 enzymes[24,25] or might be due to high affinity binding[26].Histochemical techniques help to analyze not only the localization of protein, lipid and glycogen etc. but also molecular changes of at cellular level. The histochemical tests reveal the localization of chemical product of cellular activity. The liver is the most important multifaceted active target organ in a vertebrate body, as it is the chief metabolic and detoxification centre [27,28]. It synthesizes bile which contains bile salts, bile pigments, cholesterol and lecithin. Many reports indicate that it is the organ with the highest concentration of the pesticide since all toxins pass through liver during detoxification and hence manifest the highest toxin subjecting it with greatest damage or impairment due to pesticide treatment[29,30]. Kidney another active organ also plays a very important role in removing toxins from our body. Most toxicants are excreted through the kidney when exposed to pesticides and heavy metals. The pathological effects of heavy metals and pesticides on kidney of various animals have been studied by several workers[31,32,33]. Earlier studies reported that the dose exposure of carbosulfan affects the kidney functions leading to physiological impairment affecting the cellular defence mechanism and detoxification system in kidney. Chemicals tend to cause toxicity in these organs as they accumulate in the cells of kidney tubules and hepatocytes leading to changes in biochemical contents and histology[34]. The increase in the lipid content in these tissues suggests toxic effects of mancozeb and confirms with the biochemical findings. In the present study, mancozeb exposure leads to histochemical alterations in lipid content. The increase in lipid content in liver and kidneys might be to due to inhibition in the activity of enzymes involved in cholesterol break up results into deposition of cholesterol into the cell. Similar interpretation was confirmed by others authors on fungicides within the same class[35,36].

CONCLUSION

Results reported in the present study showed mancozeb exposure leads to abnormal and highly significant changes biochemical and histochemical activities during the four weeks exposure period. An increase in cholesterol and lipid content was found during the study. This interpretation was confirmed by other authors' on fungicides within the same class. Mancozeb administered at very low doses produced significant detrimental effects on Swiss albino mice. The toxicological and environmental problems resulting from the widespread use of fungicides in agriculture have raised concerns, particularly with respect to the potential toxic effects in human and animals leading to more scientific solutions to such problems.

Conflict of interest: none.

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