

Metiram-induced histological and histochemical alterations in Liver and kidney of pregnant mice

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Abstract: Metiram is a non-systemically acting fungicide of dithiocarbamate group. It is used on food and ornamental crops to prevent crop damage in the field and to protect harvested crops from deterioration in storage or transport. The present work was conducted to evaluate the histological and histochemical effects of metiram in pregnant albino mice. Oral treatment of metiram to pregnant mice from the 2nd day to 19th day of gestation induced many histological and histochemical alterations in the hepatic tissue and kidney cortex. The liver tissue showed congestion of blood vessels, leucocytic infiltrations, cytoplasmic vacuolization of the hepatocytes and fatty degeneration. The kidney cortex showed degeneration of renal tubules, atrophy of glomeruli and intertubular leucocytic infiltrations. Moreover, histochemical observations revealed reduction of total carbohydrates and total proteins in the hepatocytes and renal tubules. It is suggested that the histological and histochemical alterations observed in liver and kidney of pregnant mice by metiram may be mediated by depletion of antioxidants and elevation of lipid peroxidation.

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Key words: Metiram, pregnant mice, Liver- kidney, Histology, Histochemistry.

1. Introduction

Fungicides are extensively used against a wide range of fungal diseases of many field crops fruits and ornamentals. On the other hand, some of fungicides showed toxicity to humans, animals, and useful plants, in addition to its persistence (long life) in the environment. Moreover, these chemicals were shown to be present in fruits products prepared for human consumption (Cabras and Angioni 2000). Dasgupta et al. (2011) reported that residues of buprofezin, chlorpyrifos, metalaxyl, and myclobutanil were detected in incurred grape and wine samples. Metiram (polyram) is a non-systemically acting fungicide of dithiocarbamate group. It is used on food and ornamental crops to prevent crop damage in the field and to protect harvested crops from deterioration in storage or transport (Charls et al., 2000). The toxicity of metiram was studied in mammalian animals. Kornuta et al., (1996) reported the genotoxicity of metiram using alkaline unwinding assay DNA. Dermal application of metiram resulted in minimal to moderate exfoliation and ulcerative dermatitis in the skin of rabbits treated at the high-dose level (Ullmann et al., 1987). Sortwell et al., (1977) observed follicular hyperplasia in thyroid of female rhesus monkeys treated with metiram. The effect of the fungicides (maneb, metiram, and ziram) on human natural killer (NK) cells cytotoxic function was studied by Whalen et al., (2003). The results provide evidence of relative toxic potential for these compounds and the immunomodulatory effects on

both T and NK lymphocyte function. Sakr et al., (2009) reported that metiram induced histopathological as well as biochemical alterations in the liver of albino mice. The present study was undertaken to evaluate the histological and histochemical effects of metiram in pregnant albino mice.

2. Materials and Methods

Animals and experimental protocol

Fertile virgin females and fertile males of albino mice weighing 22 ±5 g. were obtained from Hellwan Animal Breeding Farm, Ministry of Health, Cairo, Egypt and used for experimentation. Mice were housed in individual cages and maintained in a room with good ventilation at 23°C. The housing room was maintained on a 12:12 h light: dark cycle. Standard rodent diet composed of 20% casein, 15% corn oil, 55% corn starch, 5% salt mixture and 5% vitaminized starch was supplied. Free excess of water was provided *ad-libitum*. All the experiments were done in compliance with the Guide for the Care and Use of Laboratory animals. Females were made pregnant by keeping them at a ratio of 1 male: 3 females with males for 12 hour between 8 P.M till 8 A.M. During the next morning, the prospective pregnant were examined for the presence of vaginal plugs. Vaginal smears were carried out to give a precise determination of the onset of gestation. The pregnant mice were divided into two groups each was composed of 15 individuals as follows:

Group I: Control pregnant mice.

GroupII: These animals were administered orally with $1/10$ LD₅₀ (284 mg/kg b.w.) of metiram dissolved in distilled water. It consists of 80% active ingredients [zinc ammoniate ethylene-bis (dithiocarbamate)-poly (ethylenethiuram disulfide) and 20% inert ingredients. Animals given metiram from day 2 to day 19 of gestation.

Histological and histochemical examination

The treated animals and their controls were sacrificed by decapitation on the day 19th of gestation. Their livers and kidneys were removed and fixed in 10% neutral formalin. Fixed materials were embedded in paraffin wax and sections of 5 micrometer thickness were cut. Slides were stained with haematoxylin and eosin for histological examination. For histochemical examination. For histochemical study specimens were fixed in Carnoy's fluid. Periodic acid Schiff's reaction was used for demonstration of polysaccharides (Kiernan, 1981). Total proteins were detected using the mercury bromophenol blue method (Pearse, 1972).

3. Results

Liver

i. Histological results

Sections of control mice liver revealed that the hepatocytes arranged in strands with one or two spherical nuclei and eosinophilic cytoplasm. The sinusoids are occupied by Kupffer cells (Fig.1a) Examination of liver of metiram-treated mice displayed apparent signs of degenerative changes. The normal structural organization of the hepatic lobules was impaired and the characteristic cord-like arrangement of the normal liver cells was lost. In addition, severe inflammatory leucocytic infiltrations were abundant. Such inflammatory infiltration is spread over several liver areas and around the blood vessels (Fig.1b). Enlargement and congestion of blood vessels, especially veins were observed. The hepatocytes were clearly manifested by marked cytoplasmic vacuolization (Fig, 1c) and most cells showed nuclei with signs of karyolysis and pyknosis. Moreover an obvious fatty degeneration indicated by large number of fatty droplets with different size was observed (Fig. 1d).

ii. Histochemical results

A considerable amount of carbohydrates in the cytoplasm of liver cells of control animals was detected by PAS-technique. These carbohydrates give red or magenta colour with Schiff's reagent and is not uniformly distributed in the cytoplasm of the

hepatocytes, but occurred concentrated at one pole of the cells; this is termed glycogen flight (Fig.2a). The nuclei appeared entirely PAS-negative indicating absolute lack of glycogen. Examination of sections obtained from liver of animals treated with metiram exhibited diminution in their carbohydrates content (Fig.2b).

Total proteins contents of the liver cells of control mice are positively reflected by the appearance of blue colour after staining with bromophenol blue. Generally, the cytoplasm of the hepatocytes contains excessive amount of total proteins in the form of fine granules (Fig.2c). In addition, both chromatin bodies and nucleoli exhibiting deep colouration. Kupffer cells, and endothelial lining cells of sinusoids give moderate reactivity with bromophenol blue. Also, the walls of blood vessels exhibited strong stainability. Application of the fungicide induced noticeable reduction in the total protein contents in the liver cells (Fig.2d).

Kidney

i. Histological observations

Figure (3a) showed that kidney cortex consists of renal corpuscles and tubules. Renal corpuscles consist of Bowman's capsule with double membrane with urinary space inbetween. Also, a tuft of glomerular capillaries is enclosed in Bowman's capsule. Renal tubules are of two types, proximal and distal tubules. Proximal tubules lined with low columnar epithelium and have narrow lumen. Distal tubules possess wide lumen and lined by cuboidal epithelial. Animals treated with metiram showed degeneration and deterioration of the cortical constituents. The epithelial lining of the renal tubules appeared with cloudy swelling and vacuolated cytoplasm with pyknotic nuclei. Intertubular leucocytic infiltrations were observed (Fig.3b). A number of glomerular capillaries were suffering from severe signs of glomerular congestion, while others were completely damaged (Fig.3c).

ii. Histochemical observations

In control mice, total carbohydrates exist in brush borders of tubular epithelial cells of these cells and the basement membranes (Fig.4a). Renal tubules as well as glomeruli of animals treated with metiram showed noticeable decrease in PAS positive materials (Fig. 4b).

The protein materials in the cells of renal tubules of control mice were displayed in the cytoplasm in the form of small bluish irregular particles. The nuclear envelope, chromatin materials and nucleoli are positively stained (Fig.4c). Examination of kidney of mice after treatment with metiram showed that most

of the cells lining tubular epithelia appeared degenerated and showed a reduction of their protein

content (Fig.4d).The glomeruli appeared with less amount of proteins.

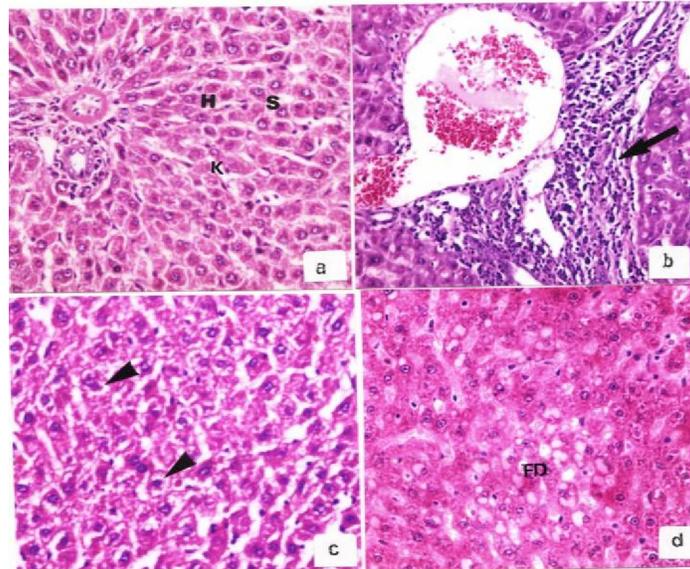


Fig.1. Sections in liver of (a): a control pregnant mouse showing hepatocytes (H), Kupffer cells (K) and Sinusoids (S),X120 (b): a pregnant mouse treated with metiram showing leucocytic infiltrations (arrow),X120 (c): a treated mouse showing cytoplasmic vacuolization of the hepatocytes (arrow heads), X120 (d): fatty degeneration (FD), X 120.

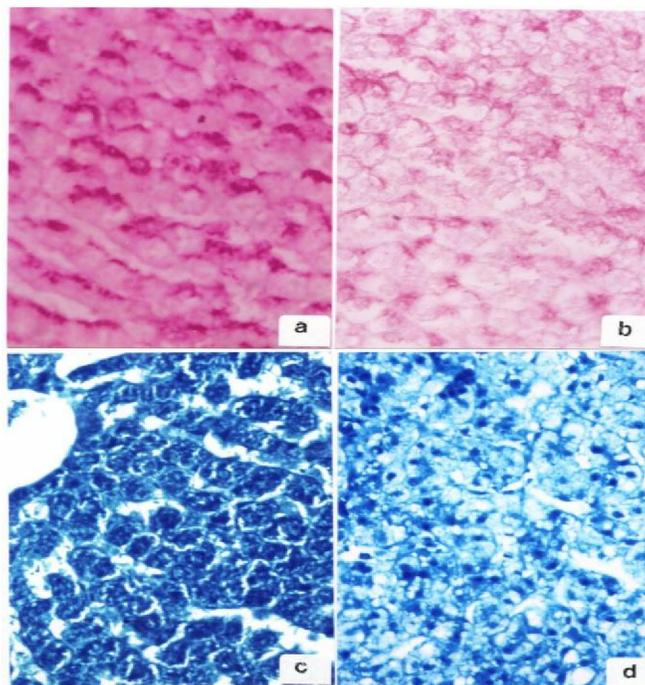


Fig.2. (a):Liver section of a control mouse showing distribution of carbohydrates in the cytoplasm of the hepatocytes,X300. (b): Noticeable decrease of carbohydrates in the hepatocytes of a pregnant mouse treated with metiram., X300(c): Normal proteinic content in the liver of a control animal,X300 (d): Marked reduction of proteins following treatment with metiram, X 300.

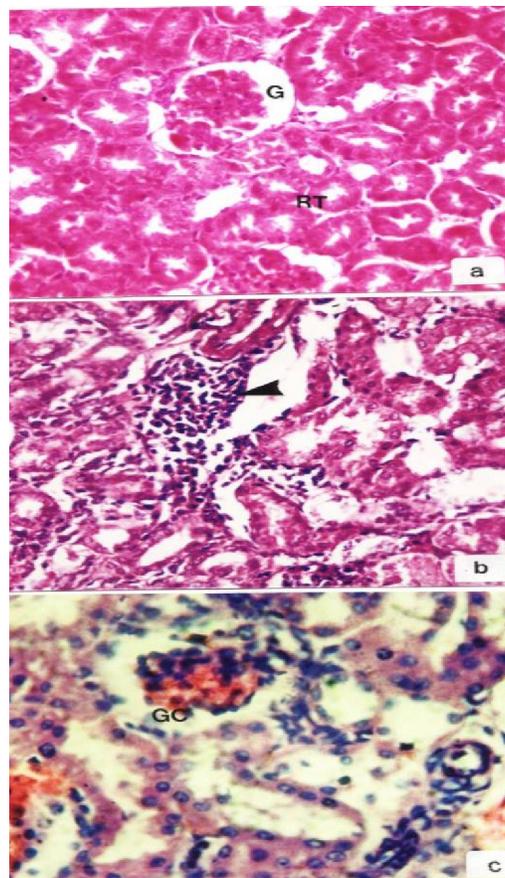


Fig.3. Sections in kidney cortex showing (a): control mouse with normal glomerulus (G) and renal tubules (RT), X 200 (b): a pregnant mouse treated with metiram showing leucocytic infiltrations (arrow head) and degenerated renal tubules, X 200 (c): congestion of glomerular capillaries (GC), X300.

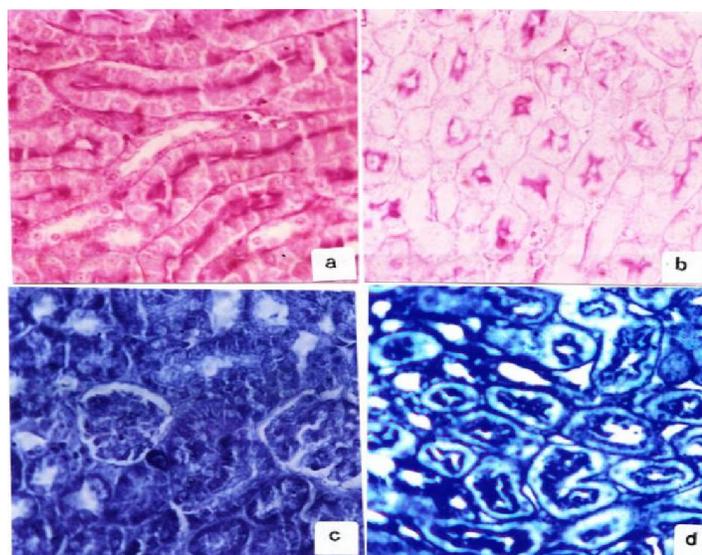


Fig.4. (a): T.S. in kidney cortex of a control pregnant mouse showing PAS-positive materials in the brush borders and basement membrane, X300 (b): T.S. of kidney cortex of a mouse treated with metiram showing weak PAS reaction, X300 (c): Normal protein content in renal tubules and glomeruli of a control mouse, X300 (d): Marked reduction of total proteins in a mouse treated with metiram, X 300.

4. Discussion

Results obtained in this work showed that treating pregnant mice with metiram induced many histopathological changes in the liver and kidney cortex. Similarly, Sakr et al. (2009) reported that metiram caused different histological and biochemical alterations in liver of mice. The effect of fungicides on mammalian tissues was investigated. Lamfon (2011) reported that metalaxyl induced hepatotoxicity in albino rats. Szepvolgyi et al., (1989) reported that when male and female rats were exposed to mancozeb, the liver showed centrilobular necrosis with extramedullary haemopoiesis and the kidney showed tubular dilation, necrosis and congestion of blood vessels. Özbay et al., (1991) reported that exposing mice to the fungicides, maneb and zineb caused blood congestion and mononuclear inflammatory cell infiltrations in the liver and kidney tissues. Ishiyama et al., (1990) found that diethyldithiocarbamate caused liver injury in the form of massive hepatic necrosis in the centrilobular region due to the suppression of the copper superoxide desmutase activity. Intratracheal instillation of diethyldithiocarbamate induced dystrophic changes in the lung and leads to fibrosing alveolitis (Tatrai et al., 1997). Selmanoglu et al. (2001) studied the effect of carbendazim fungicide on kidney of male rats. Their results revealed congestion, mononuclear cell infiltration and tubular degeneration. Dithiocarbamates (DTCs) fungicides have toxic effects on liver, kidney, testis and placenta, excessive exposure to the DTCs maneb and zineb caused acute renal failure and nephrotic syndrome in agricultural workers and led to kidney damage and reduced body weights in the offspring of exposed pregnant rat (Odermatt, 2004).

Treating mice with metiram induced reduction of total carbohydrates in hepatic cells and renal tubules. These results are similar to those reported in different organs under the effect of some fungicides. Mahadevaswami et al (2000) reported that mancozeb at a dose level of 600, 700 and 800 mg / kg day induced a significant decrease in the level of glycogen in the liver of albino rats. The decrease of carbohydrates by the metiram seems to be achieved through modifying the activities of the enzymes of glycolytic pathway, TCA cycle, gluconeogenesis and the oxidative phosphorylation (Shakoori et al. 1988). It was also reported that some pesticides may affect the carbohydrate metabolism through their effects on the endocrine system, especially by modifying the secretion of glucocorticoids and insulin (Pilo and Mehan, 1987). However, one or more of such factors could be considered as the causal agent of carbohydrate reduction observed in treated animals.

Results obtained in this work also revealed that pregnant mice treated with metiram showed a marked decrease in total proteins. Repeated administration of the fungicide, mancozeb induced a reduction in the protein content, in liver and ovary of rats (Baligar and Kaliwal, 2001). Sakr et al.(2004) observed reduction of total proteins in liver of benomyl-treated rats. El-Beih et al., (1991) found that the carbamate insecticide, lannate reduced total proteins in the hepatic cells of guinea pig. Abdeen et al., (1994) indicated that fenvalerate caused a decrease in protein content of mice hepatocytes. They added that protein decreased as a result of vacuolation and degeneration of the cells. The reduction in protein content observed in this work may be attributed partially to the decreased level of hepatic protein synthesis in the cells suffering from pathological changes due to the hyperactivity of hydrolytic enzymes (Sivaprasada *et al.*, 1983).

It is well known that overproduction of reactive oxygen species (ROS) metabolites can initiate lethal chain reactions, which involve oxidation and damage to structures that are crucial for cellular integrity and survival. These free radicals could then cause membrane and macromolecule damage by three basic mechanisms: lipid peroxidation; deoxyribonucleic acid (DNA) fragmentation and protein oxidation (Halliwell and Gutteridge, 1990). Oxidative damage is thought to be one of the main mechanisms involved in nearly all pesticides toxicity. In this concern, Calviello, et al. (2006) reported that fungicides-induced damage is closely associated with increase in lipid peroxidation and the decrease in the antioxidant enzymes. Sakr et al. (2007) found that mancozeb fungicide induced a significant decrease in the serum antioxidant superoxide dismutase and an increase in lipid peroxidation in albino rats. Muthuviveganadavel et al. (2008) reported that carbendazim administration caused significant decrease in lipid peroxidation in liver tissue of male rats. Therefore, it is suggested that the histological and histochemical alterations observed in liver and kidney of pregnant mice by metiram may be mediated by depletion of antioxidants and elevation of lipid peroxidation.

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