HISTOLOGICAL AND HISTOCHEMICAL STUDIES ON THE EFFECT OF THE INSECTICIDE, MONOCROTOPHOS, ON THE LIVER AND KIDNEYS OF ALBINO MICE (*Mus musculus*)

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ABSTRACT

The effect of the insecticide, monocrotophos on the albino mice was evaluated by studying the histological and histochemical changes in the liver and kidneys tissues of mice treated orally with different doses of such insecticide.

The toxicity symptoms were monitored. The median lethal dose (LD50) was determined as 7.5 mg/kg body weight. The doses applied in the study were 1/3, 1/6, 1/20 and 1/40 LD50, the former three doses were used for the acute treatments, where the animals were treated daily for seven successive days, while the daily treatment with the lowest dose extended to 20 successive days for studying the cumulative effect. In addition, possibility of recovery was monitored in case of giving 1/6 LD50 one and two weeks after the last dose.

In the liver, in case of all the used doses, the liver cells appeared necrotic and structureless with ill-defined cellular boundaries and showed pyknotic and chromatolytic nuclei. Activated Kupffer cells and dilation of blood vessels with eroded walls were seen. In addition, great number of inflammatory leucocytes invaded the degenerated areas were seen.

In the kidney, in all the used doses, obvious signs of injury were observed, such as dissociation of brush borders of the proximal tubule cells, pyknotic nuclei, leucocytic infiltration and hemorrhagic foci. The parietal layer of the Bowman's capsule was irregular and mostly degenerated and the urinary spaces were diminuted or demolished.

Examination of both liver and kidney tissues obtained one and two weeks after giving 1/6 LD50 for seven successive days showed partial recovery after one week; such recovery became more pronounced after two weeks.

Electron microscope examination of both liver and kidney tissues revealed variable degrees of pathological changes. The cytoplasm of such cells showed numerous small vesicles. Profiles of dense RER, degenerated mitochondria, cytosegrosomes and many lipid droplets were observed in the degenerated cells of such tissues. Pyknotic and chromatolytic nuclei were pronounced in such cells. Dead and deteriorated cells were also described. In the kidney tissue congested glomeruli, narrowing urinary spaces, increased mesangial matrix, proliferation of mesangial cells, atrophied podocytes and fusion of their secondary foot process were illustrated. In addition, degenerated mesangial cells and podocytes were observed with very electron dense and highly indented nuclei.

Histochemical changes in the liver and kidney tissues due to the effect of monocrotophos proceeded almost parallel observations to those of the histopathological alterations. Determination of polysaccharides, proteins and DNA contents in both liver and kidney tissues obtained from mice treated with monocrotophos indicated marked depletion of such materials, in comparison with control treatments – in a dose-dependent manner – especially in liver tissues. In addition, results obtained indicated positive correlation between proteins and DNA contents, *i.e.* loss of DNA goes hand in hand with the decline of protein inclusions in both liver and kidney tissues.

SUMMARY

SUMMARY

Nowadays the organophosphorous pesticides are widely used for controlling several pests including insects. However, they are responsible for creating serious environmental problems, such as toxication for man and his domestic animals. Therefore, the present work was concerned to throw light on the effect of the organophosphorous insecticide "monocrotophos" on the albino mice *Mus musculus*.

The median lethal dose (LD_{50}) was determined 7.5 mg/kg mouse body weight. The doses applied in the study were, 1/3, 1/6, 1/20 and 1/40 LD_{50} (2.53, 1.25, 0.375 and 0.19 mg/kg, respectively). The former three doses were used for the acute treatments, where the mice were treated daily for seven successive days. The lowest dose (1/40 LD_{50}) was used for studying the cumulative effect; the treatment period extended to 20 successive days.

Toxicity symptoms on such animals were described. By using the light microscope, histological and histochemical changes were evaluated in the liver and kidney tissues of mice treated orally with the aforementioned doses of monocrotophos and compared with the untreated control mice. In addition, the ultrastructural observations and possibility of recovery of mice treated with (1/6 LD_{50}) were evaluated 7 and 14 days after the last treatment. The following results were obtained:

Microscopical Examinations

The Liver

I. Histological Observations:

a. The Acute Treatments:

Noticeable histopathological alterations were recorded in liver tissues of monocrotophos-treated mice, compared with the control. The magnitudes of these changes were dose-dependent.

By using the low dose (1/20 LD_{50}), the stainability of the hepatocytes was developed and vacuolar degeneration in the cytoplasm was determined. Nuclear degeneration was represented by the appearance of pyknotic and hypertrophied nuclei with irregular nuclear envelopes. In addition, dilation of some blood sinusoids, congestion of hepatic vessels, an increase in number of the binucleate hepatocytes, hypertrophied hepatic and Kupffer cells and abundant lymphocytic infiltration were the most marked tissue impairments.

By using the median dose (1/6 LD_{50}), the above-mentioned histopathological changes were more prominent. The cytoplasmic vacuoles increased in number and size. Some of these vacuoles were observed containing basophilic bodies of different sizes. Furthermore, pyknotic, karyorrhexic and chromatolytic nuclei were demonstrated in the degenerated hepatocytes.

The high dose (1/3 LD_{50}) produced severe damage to the liver tissues such as dissociation of the hepatic strands and increase of the necrotic hepatocytes. The degenerated liver cells exhibited ill-defined appearance with disturbed cellular boundaries and pyknotic and chromatolytic nuclei. In addition, hallow spaces usually appeared around the degenerated nuclei. Dilation of blood sinusoids and congestion of blood vessels were demonstrated. Also, high amount of inflammatory leucocytes invaded the degenerated areas.

b. <u>The Cumulative Treatment:</u>

Histopathological examination of mice liver tissues after 20 days of treatment with monocrotophos at the level of 1/40 LD_{50}, showed various

degenerative changes. The hepatocytes were less eosinophilic with indefinite cellular boundaries indicating some cytoplasmic alterations. The observed cytoplasmic vacuoles were more prominent than those observed in the liver tissues of untreated mice; however, they were less than those recorded in case of the acute treatment. Pyknotic and chromatolytic nuclei of the hepatocytes, dilated blood vessels, eroded walls of blood vessels and activated Kupffer cells were also seen.

c. The Recovery Treatment:

Microscopical examination of liver tissues of mice treated with the median dose (1/6 LD_{50}) for seven successive days and examined one week after the last dose revealed partial recovery of the hepatic tissues. Moreover, examination after two weeks indicated more recovery; this may indicate progressive withdrawal of the insecticide from the animal body after stopping the treatment with the insecticide.

The Kidney

I. Histological Observations

a. The Acute Treatments:

The recorded histopathological changes in kidney tissues of mice treated with monocrotophos indicated dose-dependence. However, approximately severe effects were recorded at the median and the high doses, 1/6 and 1/3 LD_{50}, respectively.

The low dose (1/20 LD_{50}) caused shrinkage of some glomeruli; some were congested and the cellularity in some others were increased. Severe congestion of interstitial blood vessels and diminution or disappearance of the urinary space were observed. The renal tubule cells showed many vacuoles and pyknotic nuclei and disappearance of the microvilli of the proximal tubule cells.

In case of the median and high doses, the Malpighian corpuscles revealed pronounced signs of injury represented by cellular abnormalities, necrotic, and shrunken and deeply clefted glomeruli. The urinary spaces were diminuted or demolished. Obvious signs of injury, such as dissociation of brush borders of the proximal tubule cells, coagulative degeneration of the cytoplasm, highly vacuolated and deteriorated renal cells with pyknotic nuclei. Leucocytic infiltration and hemorrhagic foci were also observed.

b. The Cumulative Treatment:

Examination of kidney tissues treated daily with an oral dose of 1/40 LD_{50} of monocrotophos for twenty successive days showed cellular abnormalities, widening of the urinary spaces, increased cellularity of the glomeruli and adhesion with the parietal layers of Bowman's capsules. The cells of the renal tubules displayed degeneration, vacuolated cytoplasm and pyknotic nuclei were. Dissociated cells and cell debris were found in the tubule lumina.

c. The Recovery Treatment:

The pathological alterations observed in the kidney tissues obtained from mice treated with the median dose for one week showed partial recovery when inspected on the seventh day after the last dose and the recovery became more pronounced on the fourteenth day.

II. Ultrastructural Observations:

Liver and kidney tissues obtained from mice treated with the median dose of monocrotophos (1/6 LD_{50}) were examined by using the electron microscope. Pathological alterations were monitored. The cytoplasm of such cells revealed numerous small vesicles. Profiles of dense RER, degenerated mitochondrial, cytosegrosomes and many lipid droplets were observed in the degenerated cells of such tissues. Pyknotic and

chromatolytic nuclei were pronounced in such cells. Dead and deteriorated cells were also described. In the kidney tissue, congested glomeruli, narrowing urinary spaces, increased mesangial matrix, proliferation of mesangial cells, atrophied podocytes and fusion of their secondary foot processes were illustrated. In addition, degenerated mesangial cells and podocytes with very electron dense and highly indented nuclei were observed.

III. Histochemical Observations:

The recorded histochemical changes in liver and kidney tissues obtained from mice treated with different doses of monocrotophos preceded almost parallel observations to those of the histopathological ones. *i.e.* changes in liver tissues indicated apparent dose-dependence, being more pronounced on using the high dose. On the other hand, examination of kidney tissues showed slight dose-dependence.

III.1. The Polysaccharide Contents

In liver tissues, at each of acute and cumulative treatments, marked depletions of glycogen were recorded. These reductions were correlated with the dose level, *i.e.* the low and high reductions were observed on using the low and high doses of the insecticide, respectively.

In kidney tissues, different doses of acute and cumulative treatments caused almost constant and remarkable effects. Marked depletion of PAS positive materials from the nephron units was recorded.

III.2. Protein and DNA Contents:

Recorded observations indicated simultaneous alterations of proteins and deoxyribonucleic acid (DNA) in both of liver and kidney tissues obtained of monocrotophos-treated mice. Gradual depletions were recorded with the gradual increase of the used dose, where the highest reductions were associated with the highest dose.

In the cumulative treatment, conspicuous reductions in contents of both materials were also recorded in liver and kidney tissues.

These results indicated positive correspondence between both tissue components, *i.e.* the loss of DNA goes hand in hand with the decline of protein inclusions in both liver and kidney tissues obtained from mice treated with any of the used doses.