Pathological and Pharmacological Studies on Dichlorvos Pesticide

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SUMMARY

Dichlorvos diluted in paraffin oil absorbed and released into the bloodstream slowly especially if injected subcutaneously. This work provides and clarifies the dangers of exposure to one of the widely used pesticides and provides new methods that can apply for preparation of pellets from natural products like bees wax. This work highlighting the most important pathologic changes caused by dichlorvos in rabbits especially; brain, kidneys, myocardium, spleen, adrenal glands, uterus and gonads, in addition to providing advanced and new method for determination of dichlorvos and the use of bees wax instead of resin for preparation of pellets of dichlorvos. The carcinogenic effect of dichlorvos needs more investigation but there are some evidence appeared as abnormal cellular changes in the choroids plexus, subcutaneous interscapular gland and stomach.

INTRODUCTION

Dichlorvos (2, 2- dichlorovinyl phosphate, DDVP) is a highly volatile insecticide of the organophosphate group. It is classified by the WHO as a class IB (highly hazardous). The US Environmental Protection Agency (EPA) classifies it in category 2B (possibly carcinogenic to humans). It is used against a wide range of mite and insect pests of plants, farm animals and humans and as an anthelminthic. It has agricultural, public health and domestic uses. It is also used to control parasites in fish farming (WHO, 2007). In Egypt; there is a great confusion about its uses. However, it is evidenced that Faba beans have been treating with 14C-dichlorvos at recommended dose of 12 mg/kg seeds for storage. (Soli- man, 2005). The aims of this study are determination of dichlorvos, preparation of pellets of dichlorvos and investigation of its pathological changes in rabbits.

MATERIALS AND METHODS

Dichlorvos was obtained in its original formula and dissolved in paraffin oil. Fifteen rabbits of both sexes were divided into three groups each of five. The dichlorvos was determined spectrophotometrically. The pellets were prepared by using bees wax. The route of injection was tested which revealed that the
subcutaneous injection is the most suitable one. The rabbits of first group were injected subcutaneously by 15 mg/kg B. Wt. of dichlorvos dissolved in paraffin oil for three times with three days intervals. The rabbits of the second group were treated by the same manner but with a dose of 25 mg/kg B. Wt. The animals were sacrificed 3 days after the last injection. The third group was kept as control. Specimens from liver, kidneys, adrenals, myocardium, brain, uterus, ovaries, testicles, epididymides, stomach, intestine, vermiform process, and subcutaneous interscapular gland were collected for histopathological examinations.

RESULTS

1-Pharmacological studies:

A- Determination of dichlorvos (Fig. 1):

Alcoholic alkali is added to 2, 4-dinitrophenyl hydrazone a blue color is produced, probably as result of resonance delocalization of the negative charge resulting from removal of a proton presumably because of the formation of resonating quinoidal:

\[
\begin{align*}
\text{C} &= \text{O} + \text{H}_2\text{NHN} \quad \rightarrow \quad \text{C} = \text{NNH} \\
\text{NO}_2 &+ \text{H}_2\text{O}
\end{align*}
\]
The developed color provides very sensitive spectrophotometer assay for carbonyl compounds. An attempt to apply this reaction to dichlorovos was successful and a blue color was obtained and measured spectrophotometrically at 565 nm. Accordingly, trace quantities of dichlorovos was determined using this method considering the similarity between dichlorovos:

\[
\text{CH}_3\text{O} - \text{P} - \text{O} - \text{CH} = \text{CCl}_2
\]

And carbonyl compound conjugated to, carbon-to-carbon double bond.

**B- Calibration curve of dichlorovos (Fig., 2):**

1- 0.5 % w/v solution of dichlorovos was prepared as a standard solution in distilled water. 0.1, 0.2, 0.3, 0.4 and 0.5 ml samples were pipetted in 10 ml volumetric flasks. 2 ml of freshly prepared saturated ethanolic solution of 2, 4 – dinitrophenyl hydrazone and 0.1 ml conc. HCl were added to each flask. The flasks were stoppered loosely and heated on water bath (80°C) for 5 min. After cooling rapidly, 2 ml potassium hydroxide solutions were added and the developed color was left for 1 hr. The volumes were then completed using 50% v/v ethanol solution and the absorbance was measured spectrophotometrically at 565 nm. Blank determination was made using 1 ml of ethanol instead of sample.

2- Potassium hydroxide solution: 10 g of potassium hydroxide were dissolved in 20 ml distilled water and the solution was made up to 100 ml with ethanol.

3- A straight line calibration curve as a mean of triple determinations \((r \approx 0.99)\) was obtained as shown in Figure (2).

**C- Preparation of dichlorvos pellets (Fig.3):**

To prepare 50% coated pellets, 10 g of dichlorvos were accurately weighed and dissolved in 10g molten white beeswax. The solution was rapidly added drop wise to 500 ml distilled water with continuous stirring using magnetic stirrer. After adding all the dichlorvos solution, the stirring was continued for \(\frac{1}{2}\) hr. The pellets were filtered under vacuum and left for 24 hrs in open air for complete drying.

25% and 66% coated pellets were prepared by the same procedure.

**1-Size distribution of the pellets**

The prepared pellets were fractional into three size ranges using
1000 Mm, 500 Mm and 355 Mm sieves on a moving sieve shaker for 5 min.

2- Pathological changes:

A- Rabbits subcutaneously injected with dichlorvos (15 mg/kg B.Wt.);

Spleen: showed extensive depletion of the lymphocytes from the white pulp. The majority of the central arteries showed thickened walls with obliterated Lumina.

Liver: showed centrolobular hydropic degeneration, hyaline droplet degeneration and coagulative necrosis. The epithelial lining of some bile ducts was columnar with elongated nuclei almost occupying the majority of the cell. Such bile ducts were surrounded with numerous plasma cells and lymphocytes followed by a thick zone of fibrous connective tissue.

Kidneys: showed cystic dilatation of some renal tubules which caused pressure atrophy of the adjacent renal parenchyma with focal fibrosis infiltrated with lymphocytes which extended to involve the renal medulla.

Ovaries: showed ova disappearance from the graafian follicles. The follicular cells were detached, disintegrated and scattered in the follicular fluid, particularly in the mature graafian follicles. The majority of the primary follicles, under the tunica albuginea were replaced by empty cavities. The follicular cells, in the growing follicles, were progressively proliferating; however, they suffered necrosis in the majority of the mature follicles.

2- Determination of pellets content

To determine the dichlorvos content in the prepared pellets, four samples 100 mg each were crushed and the dichlorvos was dissolved in 100 ml distilled water in volumetric flasks.

1 ml samples were with drawn from each flask and the concentration of dichlorvos was determined spectrophotometrically as before.

Bartlett test was used to compare the dichlorvos contents in the pellets. The results showed significant difference in the drug content between the samples.

3- In vitro dissolution studies

500 mg of 50% coated pellets (500-1000 Mm fraction) were placed in a basket of aperture 425 Mm which was then immersed in 1000 ml of distilled water at 37 ± 1°C. A stirring speed of 100 r.p.m. was used. 1 ml samples were removed at time intervals up to 200 min. The samples were measured spectrophotometrically at 565 nm.

-The dissolution study was repeated for 25% and 66% coated pellets and the results are shown in Figure (3).
**Uterus:** showed extensive atrophy of the uterine wall. The epithelial lining of the endometrium was low columnar with dark oval nuclei, occupying almost the whole cytoplasm. The endometrium was thrown into branched papillary folds which occupied the majority of the uterine lumen.

**Testes:** showed dilated seminiferous tubules with minimum interstitial tissue. The seminiferous tubules exhibited extensive destruction of its germinal epithelial lining where the majority of such tubules were lined by a single layer of germinal epithelium and Sertoli cells and their lumina contained necrotic debris and desquamated germinal epithelium beside some giant cells. A peculiar type of dissociation of the germ cells was noticed. Such enlargements appeared as rods of the germ cells which were similar to the stage of prophase in the process of cellular division (Fig., 4).

**Epididymides:** was completely empty of sperms and showed numerous rounded germ cells.

**Brain:** showed encephalomalacia and demyelination of some nerve tracts. The cerebral Virchow-Robin spaces were over distended with cerebrospinal fluid. Some large neurons, in the cerebral cortex exhibited coagulative necrosis. Some necrotic neurons disappeared and were replaced by empty spaces. Proliferated glial cells were seen particularly around the lateral ventricles.

The epithelial covering of the choroids plexuses was vacuolated. The Purkinje cell layer was edematous and showed coagulative necrosis of some Purkinje cells which frequently disappeared. Necrosis affected the majority of the neurons in the granular layer (Fig. 5). The Virchow-Robin spaces, in the molecular layer, were widely dilated. Demyelination of the cerebellar white matter and peduncles was encountered. Some neurons in the cerebellar peduncles were necrotic. Vacuolation of the epithelial covering of the choroids plexus of the fourth ventricle was observed. The ependymal lining of the fourth ventricle was mostly flattened with subependymal congestion.

**Stomach:** showed cystic dilatation of the gastric glands with papillary hyperplastic projections of its mucosa was encountered. The smooth muscle bundles, among the pyloric gastric glands, were hypertrophic and partially replaced the gastric glands which showed cuboidal epithelial lining. Other gastric glands were completely obliterated. Desquamated epithelial covering of the fundic mucosa was seen. The majority of the epithelial lining of the gastric glands seemed to change to parietal cells.

**Duodenum:** showed hyperplastic histiocytes and necrotic tips of some villi. The lumens of the intestinal glands were almost completely obliterated with its swollen
epithelial lining. The latter was represented by tall columnar epithelium with oval vesicular nuclei occupying the lower half of their cytoplasm. The duodenal lumen contained necrotic debris.

**Myocardium:** showed extensive congestion and hemorrhages, particularly in the papillary muscles. Moreover, Zenker's necrosis and myomalacia with focal proliferation of the sarcolemmal nuclei and focal replacement of the necrotic muscle fibers with macrophages and lymphocytes were encountered.

**B- Rabbits subcutaneously injected with dichlorvos (25 mg/kg B.wt),**

**Spleen:** showed extensive depletion of the lymphocytes from the white pulp. The red pulp was highly congested. The splenic capsule was thickened with fibrous connective tissue.

**Liver:** showed centrolobular hydropic degeneration and coagulative necrosis. The epithelial lining of some bile ducts showed abnormal cellular growth surrounded by numerous lymphocytes (Fig., 6).

**Kidneys:** showed severe congestion and focal cortical hemorrhages. There was focal coagulative necrosis of the renal tubules, adjacent to the hemorrhagic areas, beside cloudy swelling and fine granular eosinophilic material inside the glomerular cavities, proximal and distal convoluted tubules showed similar granular eosinophilic material inside their cystic lumens (Fig., 7). The epithelial lining of such tubules was flattened or frequently necrotic. The glomeruli showed moderate hypercellularity and were partially distorted by the swollen renal tubules.

**Ovaries:** showed atretic graafian follicles, such follicles were represented by cavities containing follicular fluid, degenerated oocytes and scattered follicular cells the wall of the follicles were represented by three rows of follicular cells followed by two thecae. The primary and the growing follicles showed necrotic oocytes with intact progressively growing follicular cells. Numerous congested capillaries were seen in the ovarian stroma, particularly in the areas adjacent to the atretic follicles.

**Fallopian tubes:** showed numerous congested capillaries in their lamina propria besides being edematous. Its epithelial lining was ciliated, tall and pseudostratified columnar.

**Uterus:** showed edematous myometrium and endometrium. The endometrium was thrown into papillary projections inside the lumen of the uterus where it showed numerous secondary branching and rebranching papillae. The lamina propria showed a moderate number of uterine glands. The epithelial lining of both the endometrium and the uter-
ine glands was represented by low columnar epithelium having rounded vesicular nuclei occupying the basal half of the cytoplasm. The lamina propria showed numerous dilated capillaries. The congested capillaries showed the widest lumens towards the myometrium and gradually decreased in diameter towards the epithelial lining of the endometrium.

**Brain:** showed dilated Virchow-Robin spaces and vacuolations around some neurons which indicate brain edema, leuko-encephalomalacia and demyelination of some nerve tracts were frequently seen. Satelliteosis and neuronophagia were outstanding particularly in the thalamus as the microglia was extraordinary surrounding and invading the neurons. Such microglia was represented by large rounded and vesicular nuclei which frequently replaced the invaded neurons to form Babe's nodules. The majority of normal structure of the choroids plexus, in the lateral ventricles, was replaced by hypercellular a vascular structure which resembles the adenoma of the choroids plexus. Many nuclei of such structure were pyknotic. Such structure was focally replaced by cavities causing the surrounding cells to appear crescentic. The cytoplasm of some choroids plexus epithelium was vacuolated. The ependymal lining, adjacent to the adenomatous growths, was flattened.

**Stomach:** showed the surface of the pyloric mucosa covered with mucus mixed with desquamated epithelium. The gastric glands were cystic as they were over distended with mucus. Such glandular cysts disfigured the normal structure of the gastric mucosa. The fundic mucosa showed focal atrophy and necrosis. The majority of the epithelial lining of the gastric glands were of the parietal cell type-The epithelial lining of some gastric pits showed basophilic cytoplasm with round to oval nuclei occupying the basal half of the cytoplasm.

**Vermiform process:** showed extensive depletion of lymphocytes from its lymphoid follicles with moderate proliferation of histiocytes. The epithelial covering the dome-like structure of the lymphoid tissue showed a gradual decrease in height till it became flat of represented by a thin eosinophilic band just lying on the lymphocytes. Meanwhile, the epithelial lining of the chamber, where the lymphoid structure lies, was simple columnar epithelium similar to that of the luminal epithelium.

**Myocardium:** showed cloudy swelling and myomalacia. The necrotic muscle fibers were replaced by reticulin framework with some Anitschkow myocytes and proliferated sarcolemmal nuclei. Moderate focal congestion was encountered.

**Interscapular subcutaneous gland:** showed diffuse replacement of...
the glandular tissue with adipose tissue which was focally infiltrated with round cells (lymphocytes and macrophages) and giant cells of the Langerhan's type which were adjacent to some cystic ducts. The latter were lined by cuboidal to flattened epithelium. The epithelial lining of some acini formed syncetial giant cells.

DISCUSSION

Pharmacological studies

Determination of dichlorvos:

An accurate gravimetric analysis of carbonyl compounds is based on their conversion to the corresponding 2, 4-dinitrophenyl hydrazones which are insoluble, colored crystalline derivatives. They are easily isolated and weighted and the high molecular weight of the hydrazones permits the analysis of relatively small samples. A sample containing about \(4 \times 10^{-4}\) moles of aldehyde is adequate for analysis (Siggia, 1979). The hydrazones formation is carried out in acid solution and the mixture filtered through a tarred filter, which is then weighted. This method is quite specific with most classes of compounds presenting no interference.

The quantitative determination of traces of dichlorvos can be carried out in water or organic solvents. Absorption spectra are run in alkaline alcoholic solution of 2, 4-dinitrophenyl hydrazones. The position of absorption as well as the value of \(E_{\text{max}}\) is nearly independent of the concentration of the base as long as a sufficient excess is present (Lippin and Clark, 1951). In the assay method, it is unnecessary to isolate the hydrazones. It is prepared in solution using an excess of 2, 4-dinitrophenyl hydrazones, the addition of base converts the excess reagent to a very light yellow substance. The absorption of which is corrected by using a blank determination. Calibration curve relating the absorption of color to the amount of the hydrazones formed is a reliable and sensitive measure for the parent compound (dichlorvos).

Preparation of dichlorvos pellets

- It is obvious that the dissolution of dichlorvos is significantly delayed in the case of pellets when compared to its direct dissolution without coating. Moreover, the percentage of coating considerably affects the retardation of dissolution, i.e. as the percentage of coating increases, the retardation affect increases although the difference in dissolution rate between 50% and 66% coating is not that much. Therefore, it was decided to continue the study with 50% coated pellets.

Pathological changes:

The use of dichlorvos dissolved in paraffin oil was appropriate methods because other evidences
indicated that dichlorvos appears to be rapidly absorbed by dermal exposure. Monkeys exposed dermally to dichlorvos in xylene solution at doses of 50, 75, or 100 mg/kg exhibited signs of neurotoxicity within 15–20 minutes of administration, indicating rapid absorption Durham et al. (1957). Also, Blair et al. (1975) mentioned that dichlorovos is a small, lipid-soluble molecule that can be absorbed by passive diffusion through the lungs, gastrointestinal tract, or skin. This is due to the rapid degradation of dichlorvos by tissue esterases, particularly in the liver and the serum. The half-life of dichlorvos in human blood in vitro is about 10 minutes.

The pathologic changes that have been observed in different organs indicated the pathogenic effects of dichlorvos. Which probably due to its cholinesterase inhibition in one part and in other part by its cytotoxic effects. Wright et al. (1979) described that the toxicity of dichlorvos results from its inhibition of neural acetylcholinesterase. This enzyme is necessary to hydrolyze acetylcholine and terminate its action at synapses and neuromuscular junctions. The dichlorovinyloxy group of this molecule withdraws electrons from the phosphorus atom, leaving it susceptible to nucleophilic attack.

Our results indicated that dichlorvos can cause cancer. The mechanism of dichlorvos-induced carcinogenicity is not known. However, Benford et al. (1994) by a study of B6C3F1 mouse forestomach from mice treated with dichlorvos by gavages in corn oil showed increases in replicative DNA synthesis (associated with increased cell proliferation). Unscheduled DNA synthesis (associated with DNA repair) was not increased by dichlorvos treatment, but was increased by 1-methyl-3-nitro-1-nitrosoguanidine, a known genotoxic forestomach carcinogen. The authors concluded that the forestomach tumors seen in the 2-year carcinogenicity study (NTP, 1989) may have been mediated by enforced cellular proliferation rather than by a genotoxic mechanism. Two organizations have reviewed the evidence for dichlorvos carcinogenicity in humans from the results obtained in test systems. The EPA has classified dichlorvos as a probable human carcinogen (Category B2) on the basis of significant increases of forestomach tumors in mice and leukemias and pancreatic acinar adenomas in rats. Supporting evidence included observation of tumors at other sites in the rat and the observation that dichlorvos and a major metabolite, dichloroacetaldehyde, are mutagenic in vitro test systems. The International Agency for Research on Cancer (IARC) has classified dichlorvos as possibly carcinogenic to humans (Group
2B) based on inadequate evidence in humans for the carcinogenicity of dichlorvos and sufficient evidence in experimental animals for the carcinogenicity of dichlorvos (IARC 1991).
Fig. (2) Calibration curve of dichlorvos
Fig. (3) Dissolution rate of dichlorvos pellets prepared with different percentages of coating.

- Pure dichlorvos
- 25% coated dichlorvos
- 50% coated dichlorvos
- 66% coated dichlorvos

% of dichlorvos dissolved vs. time (minute)
Fig. (4)-Testes- seminiferous tubules exhibiting extensive destruction of its germinal epithelial lining, H&E., X 1200.

Fig. (5)-Brain- large neuron showing karyolysis in the presence of nissl granules in their cytoplasm, H&E., X 1200.
Fig. (6) - Liver: bile duct exhibiting abnormal cellular growth which is surrounded by lymphocytes, H&E., X 800.

Fig. (7) - Kidneys: the proximal and distal convoluted tubules showing fine granular eosinophilic material inside their cystic lumens. H&E., X 300.
REFERENCES


