Cytogenetic, Biochemical And Ultrastructural Studies On Female Baladi Rabbits Intoxicated With Metalaxyl Fungicide

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ABSTRACT

The current experiment was carried out on fifteen female baladi rabbits to elucidate the toxic effects from chronic exposure to the commonly used fungicide metalaxyl. The rabbits were randomly classified into three equal groups; the first group was orally administered $^{1}/_{10}$ LD_{50} of metalaxyl (69.7 mg/kg B.wt) twice weekly for 4 months, the second group was fed green lettuce after spraying with metalaxyl field dose (200 gm/100 litre water), the third group was orally administered corn oil and kept as control. The experimental period extended for 4 months from first exposure where all animals were sacrificed . The results indicated significant decrease in serum levels of total protein and albumin in both treated groups. Moreover, the fungicide metalaxyl increased significantly the levels of gamma- glutamyltransferase (GGT) and alkaline phosphatase of the treated groups besides, significant decrease of the serum level of the follicular stimulating hormone (FSH). Cytogenetic examination of bone marrow revealed that chromosomes of rabbits intoxicated with metalaxyl reflected various types of abnormalities; structural and numerical. Histopathological investigation of the liver of orally administered metalaxyl group showed necrotic and apoptotic changes in the hepatocytes. Some central veins were congested and the blood sinusoids were ill- defined in between hepatocyte cords, the ducts of some portal tracts appeared with thickend wall and surrounded by cellular infiltrations. The liver sections of metalaxyl feeding group appeared more preserved histological structure except presence of slightly congested central veins, and few hepatocytes with apoptotic nuclei.

INTRODUCTION

Pesticides play an important role in modern agriculture. Many new chemicals formulations are unable to control various types of newly emerging pests, microorganisms and weeds *(1-3)*. Interest on pesticide toxicity has particularly increased over the past years due to increasing evidence of their carcinogenic, mutagenic and teratogenic effects in experimental animals and exposed humans. The current procedures for hazard assessment of pesticides are based essentially on information from toxicological studies. However, available information is sometimes scanty; this introduces considerable uncertainty in risk assessment procedures *(4)*. The phenylamides are a highly active class of fungicides *(5)*.

Metalaxyl is a new and highly effective benzenoid fungicide. It is residual fungicide with systematic properties suitable for preventive and curative control of diseases caused by air – and soil borne oomycetes. On account of these properties and its broad-spectrum activity, this compound has been registered for use on a wide range of crops in both temperate and tropical regions. In the accessible literature there are scanty data of toxic characteristics of methyl *(6)*. Experimental-use permits are in effect authorizing the application of metalaxyl on some food crops, although tobacco, ornamentals, conifer, and turf applications are the major uses. Smokers could be exposed through inhalation *(7)*.

Itterly (8) showed that metalaxyl undergoes extenstive phase II reactions, namely conjugation with sulfuric acid and glucuronic acid. Sulfonation and glucuronidation are competing pathways for hydroxylated metalaxyl metabolites.

Under field conditions, metalaxyl has a half-life of one to eight weeks in soil, its average half- life in soil is about 70 days *(9)*. In a large scale, national survey, metalaxyl was detected in the groundwater of several states at

concentrations of 0.27ppb to 2.3 ppb ***(10). EPA, (11)*** found that Metalaxyl is moderately stable under normal environmental conditions. It is photolytically stable in water when exposed to sunlight , with a half life of 400 days and is stable to photodegradation in soil. In aquatic systems, metalaxyl degrades moderately rapidly, very little of the chemical is lost to volatilization. Metalaxyl is persistent and mobile, and both metalaxyl and its major degradate readily leach in many soils. Monitoring data demonstrate that metalaxyl and its primary degradate have the potential to reach groundwater.

Orally administered metalaxyl is readily absorbed from the gut into the general circulation and rapidly excreted in rats ***(12,13).*** The preferred route of excretion is via the urine for females and the faeces for males. Because of the rapid elimination of the compound, the residual radioactivity in tissues was generally low.

Gfeller et al., (14) carried out a follow – up 90 day study to evaluate the effects of 0,10,50,250 and 1250ppm of metalaxyl incorporated in the diet of rats, they found a dose related decrease in total leucocyte count, and a significant increase in relative liver weights for females. Administration of metalaxyl in rats at 40 or 80 mg/kg B.wt/ day orally for 3-7 days significantly increased the concentration and / or the activity of several hepatic enzymes ***(15).*** Large ovarian cysts were reported in treated female rats at dose of 1250 ppm of metataxyl ***(16).***

Metalaxyl was tested for mutagenic effects on L5178Y/ TK+/- mouse lymphoma cells *in vitro*, no, evidence of mutagenic effects of metalaxyl was observed in this mammalian forward mutation system ***(17).*** Metalaxyl did not increase unscheduled DNA synthesis in rat primary hepatocytes or in human fibroblasts, these results suggest that metalaxyl is not genotoxic ***(18).***

The cytogenetic effects *in vitro* that quantified by cultured human peripheral blood lymphocytes, revealed significant induction of chromosomal aberrations with 300 and 1000 micrograms/ ml metalaxyl in the absence of microsomal activation ***(19).***

Scanty and often inconclusive results are available on genetic activity, mutagenicity of metalaxyl indicates a general tendency to give negative results ***(4).*** However, studies on metalaxyl capacity to induce cytogenetic effects in living systems, including human chromosomes, have not been documented and because of chromosomal aberrations are direct indicators of genetic damage by xenobiotics ***(19).*** In this study we investigate the genetic toxicity of metalaxyl, via studying chromosomal aberrations in chronic intoxicated female rabbit. Also, recording some biochemical responses as well as histopathological investigations of the liver via electron microscopic technique.

MATERIAL AND METHODS

Chemical compound:

Common name: Metalaxyl, produced by Ciba- Geigy Corporation, Basel, Switzerland.

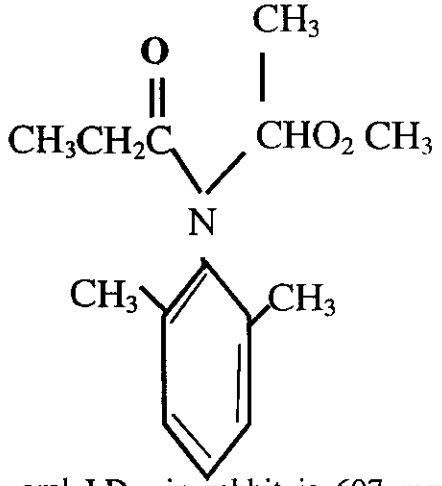
Synonyms : Ridomil, Ridomil plus, Apron 35 Ws , Apron 70 SD, Fubal, Acylon super F.

Chemical class: Benzenoid fungicide

Chemical formula: methyl-N-(2,6-dimethylphenyl)-N-(2-methoxyacetyl) -DL-alaninate

Empirical formula: $C_{15}H_{21}NO_4$

Structural formula



The oral LD_{50} in rabbit is 697 mg/kg B.wt. ***ISPRA, (20).***

Animal and treatment

The study was carried out on fifteen female Baladi rabbits with an average body weight from 1200 to 1750 gm obtained from a local market of Zagzaig city, Sharkia Governorate. Rabbits were kept in individual metal cages that provided with feeders and automatic drinkers. The cages were placed in a well vintillated building provided with electric fans during the whole experimental period under hygienic conditions, fed on commercial pelleted ration with green lettuce and provided with water *ad-libitum*, through the experiment. The experiment was ended after four months of first exposure to the fungicide where all rabbits were sacrificed. Animals were classified randomly into three equal groups (n=5). Corn oil was used for dissolving the fungicide *(21)*. Table 1 show groups and dosing.

Table 1. Classification and dosing of metalaxyl in female Baladi rabbits

Groups	No.	Treatment (dose, duration)
G1 (orally administered metalaxyl group)	5	$^{1}/_{10}$ LD_{50} of metalaxyl (69.7 mg /kg B.wt), orally administered twice weekly for 4 months
G2 (metalaxyl feeding group)	5	Fed green lettuce sprayed with field dose of metalaxyl (200 gm/100 liter water) for 4 months
G3 (control)	5	Orally administered corn oil twice weekly for 4 months

Sampling and analysis

Serum biochemical parameters

Blood samples were collected at the end of the experimental period for serum separation which kept at -20°C till used serum GGT *(22)*, alkaline phosphase *(23)*, total protein *(24)* and albumin *(25)* were estimated.

Hormonal estimation

Follicular stimulating hormone (FSH) was estimated using the electrochemiluminescence immunoassay *(26)*.

Chromosomal investigation

It was carried out after obtaining chromosomes from the bone marrow cells at metaphase spreads *(27)* and chromosomal aberrations were recorded *(28)*.

Histopathological examination

Specimens were collected from livers of all groups and fixed in neutral buffer formalin then processed for histopathological investigation using light microscope *(29)*. Another liver samples were fixed by immersion in 3% glutaraldehyde solution in 0.1M phosphate buffer solution (pH 7.4) for 2 h followed by post – fixation in 1% osmium tetraoxide (O_2O_4) in 0.1 M phosphate buffer (pH7.3) for 2h at 4°C . Then the tissues were dehydrated in up – graded ethanol and finally embedded in Quetol 812. Semi – thin sections (1μm) were stained with 1% methylene blue. Ultra – thin sections were double stained with uranium acetate and lead citrate and photographed with a transmission electron microscope.

Statistical analysis

Data of the present study was statistically analyzed for variance of ANOVA *(30)*. Difference among treatment means were compared using Duncan's multiple range test *(31)*. Data were presented as ± SE using SAS *(32)*

RESULTS

1. Effect of metalaxyl on some biochemical parameters

Table 2 demonstrates that female rabbits orally administered the fungicide metalaxyl twice weekly at dose level of $^{1}/_{10}$ LD_{50} (69.7mg/kg B.wt) and those fed green lettuce sprayed with metalaxyl field dose (200 g /100 liter water) for 4 months showed significant decrease ($P<0.05$) in the serum levels of total protein and albumin in both treated groups compared to control one, while the difference between the treated groups was non significant.

On the contrary Table 2 displayed significant increase ($P<0.05$) in the levels of GGT and alkaline phosphatase of the treated groups compared to control rabbits with non significant difference between the treated groups. In rabbits of both treated groups the level of FSH revealed significant decrease ($P<0.05$) comparing with the control group while the difference was non significant when comparing the treated groups to each other.

2. Cytogenetic effect of metalaxyl on bone marrow cells of female Baladi rabbits

The results of cytogeneticl examination of bone marrow cells of the female baladi rabbits after oral administration with metalaxyl twice weekly with a dose of $^1/_{10}$ LD_{50} (69.7 mg/kg B.wt) or after feeding with green lettuce spayed with the fungicide for 4 months comparing with the control rabbits is listed in table 3 which shows that metalaxyl inflected several types of chromosomal abnormalities either structural or numerical aberrations. The normal diploid number in metaphase spread of rabbit is 2 N= 44 (Fig. 1). The main types of abnormalities in bone marrow cells of both treated groups were ring chromosome, chromatid gap, chromatid break, centromeric attenuation, pulverization, end to end associations and hypoploidy (Fig. 2-4). It can be seen from the results that chromosomal aberration were more frequent in rabbits of the group that orally administered with metalaxyl than those of the which fed with green lettuce sprayed with the fungicide. The examined animals showed no, consistent pattern of the frequency of chromosomal aberrations but was different even with the same group.

3.Histopathological results

Light microscopical examination of the liver sections of control rabbits showed normal structure of hepatic parenchyma; liver lobules consisted of branching and anastomizing cords of hepatocytes radiating from central vein. The hepatocytes were acidophilic and contained central pale stained nuclei and some were binucleated. The hepatocyte cords were separated by blood sinusoids (Fig. 5). Examination of liver sections of rabbits orally administered with metalaxyl twice weekly at dose level of $^1/_{10}$ LD_{50} for 4 months showed enlarged hepatocytes and their cytoplasm were pale stained . Some hepatocytes contained darkly stained nuclei. Some central veins were congested and the blood sinusoids were ill defined in between hepatocyte cords. The bile ducts of some portal tracts appeared with thickened wall surrounded by cellular infiltrations (Figs. 6, 7). Histopathological findings of liver sections of rabbits fed green lettuce sprayed with metalaxyl field dose (200 gm/100 litre water) revealed preserved structure of hepatic tissue except the presence of slightly congested central veins, branches of portal vein and few hepatocytes appeared with stained nuclei (Fig. 8).

Ultrastructural examination of liver sections of control group showed hepatocytes contained euchromatic nuclei, rough endoplasmic reticutun and mitochondia with electron dense matrix. Some hepatocytes were bincucleated . Bile canaleculi formed by the plasma membranes of adjacent hepatocytes and lightly bounded by junctional coplexes. Short mirovilli were projecting into the bile canaliculi and long microvilli were seen projecting toward the blood sinusoids (Fig. 9). Ultrastructural findings of liver sections of orally administered metalaxyl group revealed the presence of hepatocytes contained shrunken nuclei with more heterochromatin. Some mitochondria with less electron dense matrix, cytoplasmic vaculations and short irrigular rough endoplasmic reticulum were observed in the hepatocytes cytoplasm. Some bile canaliculi were narrow with irregular microvilli (Fig. 10). Hepatocytes with electron lucent cytoplasm, few organelles , lysosomes, and phagosomes were also delected. Irrigaular short microvilli toward blood sinusoids were noticed . Ultra structural sections of the livers of rabbits of feeding metalaxyl group showed hepatocytes with preserved structure except the presence of some cytoplasmic fat globules and lysosomes. The tight junction between the hepatocytes were observed between some hepatocytes and blood sinusoids (Fig. 11).

Table 2. Effect of treatment of female baladi rabbits with metalaxyl for 4 months on some serum biochemical parameters (Means ± SE) (n= 5)

Parameters / Group & treatment	Total protein (gm/dl)	Albumin (gm/dl)	GGT (µ/l)	Alkaline phosphatase (IU/L)	FSH (IU/L)
G1 Orally administred twice weekly metalaxyl at dose level of $^{1}/_{10}$ LD_{50} (69.7mg /kg B.wt)	6.93 ± 0.18^{b}	3.36 ± 0.12^{b}	38.13 ± 1.77^{a}	85.10 ± 1.43^{a}	3.08 ± 0.02^{a}
G2 Fed green lettuce sprayed with metalaxyl field dose (200 g/100 liter water	6.70 ± 0.20^{b}	3.43 ± 0.23^{b}	37.86 ± 1.27^{a}	83.26 ± 0.61^{a}	3.20 ± 0.11^{a}
G3 (control) Orally administered corn oil	9.13 ± 0.20^{a}	4.60 ± 0.11^{a}	25.13 ± 0.76^{b}	75.20 ± 1.28^{b}	4.32 ± 0.19^{a}

Means within the same column carrying different superscripts are significantly different at ($P<0.05$)

Table 3. Mean value of different chromosomal aberrations induced by metalaxyl treatment in female Baladi rabbits for 4 months (Mean ± SE) (n=5).

Parameter / Group treatment	Total aberrated cells	Ring	Gap	Break	CA	Deletion	Pulverization	EE	Total structural aberration	Hypoploidy	Hyperploidy	Total numerical aberration	Total chromosomal aberration
G1 orally administred twice weekly metalaxyl at dose of $^1/_{10}$ LD_{50} (69.7mg /kg B.wt)	11.40 ± 0.50^a	1.20 ± 0.80^a	1.20 ± 0.98^a	0.40 ± 0.40^a	10.40 ± 0.46^a	2.00 ± 0.089^b	12.40 ± 1.72^a	0.00 ± 0.00^b	27.60 ± 0.74^a	1.20 ± 0.80^a	0.00 ± 0.00^a	1.20 ± 0.80^a	28.80 ± 1.01^a
G2 fed green lettuce sprayed with metalaxyl field dose (200 g/100 liter water)	7.20 ± 0.37^b	0.80 ± 0.8^a	1.60 ± 0.97^a	0.00 ± 0.00^a	4.80 ± 1.01^b	9.20 ± 0.80^a	0.40 ± 0.40^b	6.40 ± 0.79^a	23.20 ± 0.48^b	2.00 ± 0.89^a	0.00 ± 0.00^a	2.00 ± 0.89^a	25.20 ± 1.01^b
G3 (control) orally administered corn oil	1.0 ± 0.31^c	0.00 ± 0.00^a	0.40 ± 0.40^a	0.00 ± 0.00^a	1.20 ± 0.48^c	0.00 ± 0.00^b	0.00 ± 0.00^b	0.00 ± 0.00^b	1.60 ± 0.40^c	0.80 ± 0.48^a	0.00 ± 0.00^a	0.80 ± 0.48^a	2.40 ± 0.79^c

Means within the same column carrying different superscripts are significantly different at ($P<0.05$)

CA: centromeric attenuation EE: end to end associations

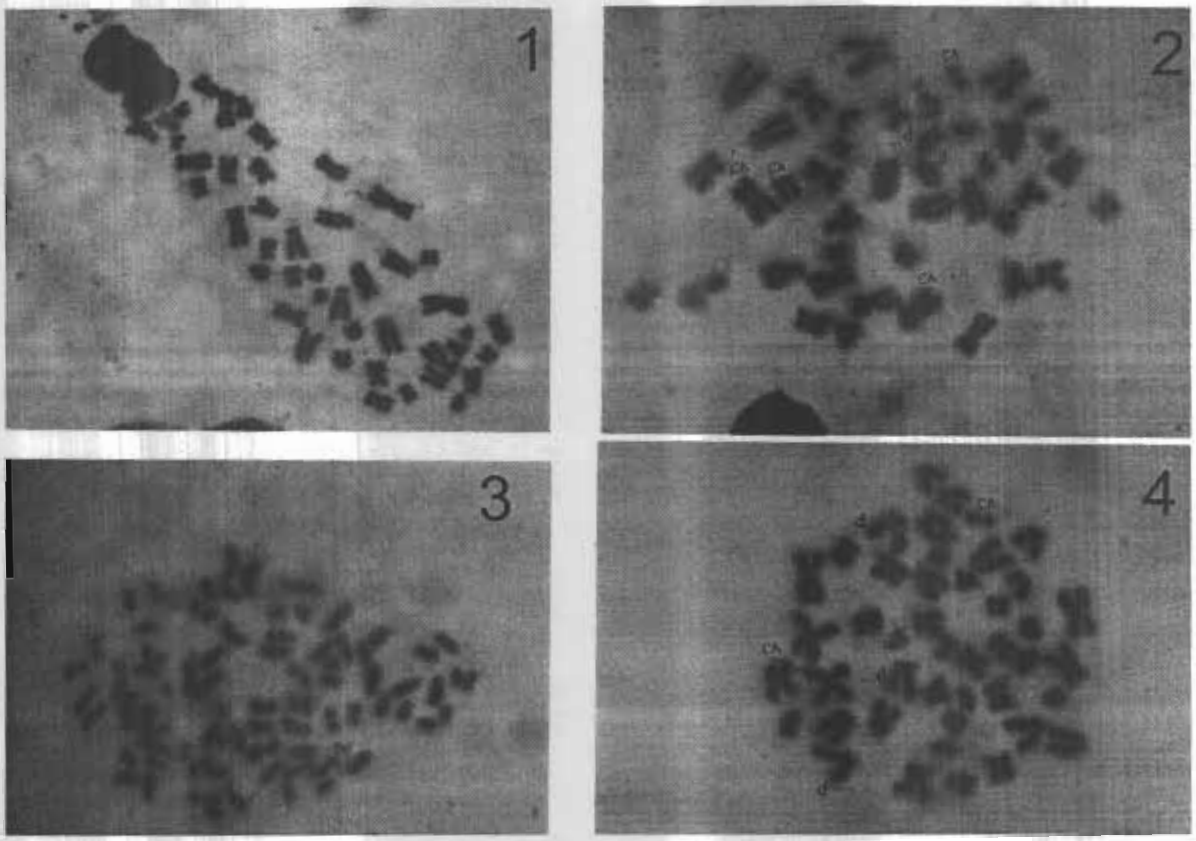


Fig. 1. Metaphase spread of control female Baladi rabbit (N=44) chromosomes (Giemsa x 1000).
Fig. 2. Metaphase spread of female Baladi rabbit orally administered metalaxyl ($^{1}/_{10}$ LD_{50}) twice weekly for 4 months showing centromeric attenuation (CA) and delation (d). Giemsa x 1000).
Fig. 3. Metaphase spread of female Baladi rabbit orally administered metalaxyl ($^{1}/_{10}LD_{50}$) twice weekly for 4 months showing pulverzation (Giemsa x 1000).
Fig. 4. Metaphase spread of female Baladi rabbit fed green lettuce sprayed with metalaxyl field dose (200 gm/100 litre water) showing centromeric attenuation (CA) and delation (d) (Giemsa x 1000).

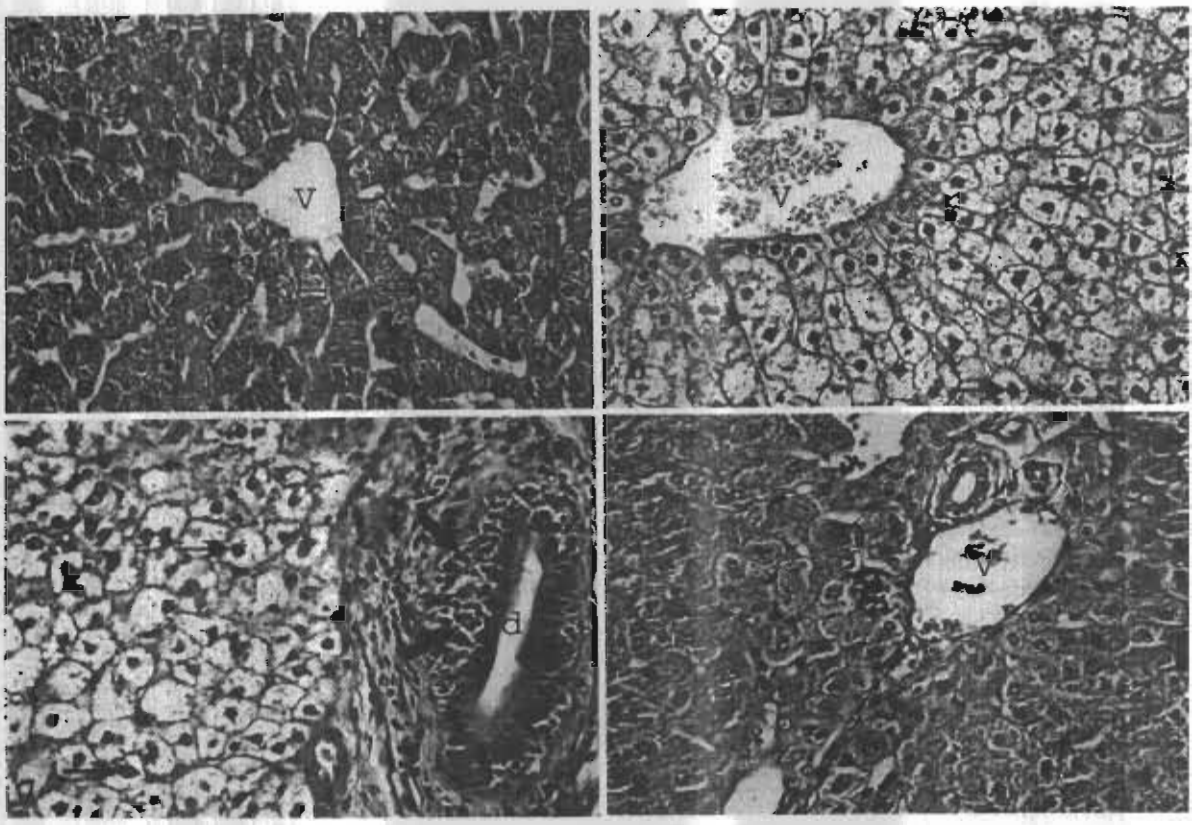


Fig. 5. A photomicrograph of a section in the liver of control group showing branching and anastomosing cords of hepatocytes radiating from central vein (V). The hepatocytes containing central pale stained nuclei (arrows) and some are binucleated (double arrow) . Blood sinusoids (S) are seen separating the hepatocytes cords (H & E x 400).

Fig. 6. A photomicrograph of a section in the liver of orally administered metalaxyl group (69.7mg/kg B.wt) twice weekly for 4 months group showing enlarged hepatocytes containing pale stained cytoplasm and darkly stained nuclei (arrow). The central vein is congested (V) and the blood sinusoids are ill – defined (H & E x 400).

Fig. 7. A photomicrograph of a section in liver of orally administered metalaxyl group (69.7mg/kg B.wt) twice weekly for 4 months group showing enlarged hepatocytes containing pale stained cytoplasm and darkly stained nuclei (arrows) Cellular infiltration (thick arrows) around the thickened bile duct (d) (H & E x 400).

Fig. 8. A photomicrograph of a section in liver of metalaxyl feeding group showing portal area containing congested portal vein branch (V) and some hepatocytes with darkly stained nuclei (arrows) are noticed (H & E x 400).

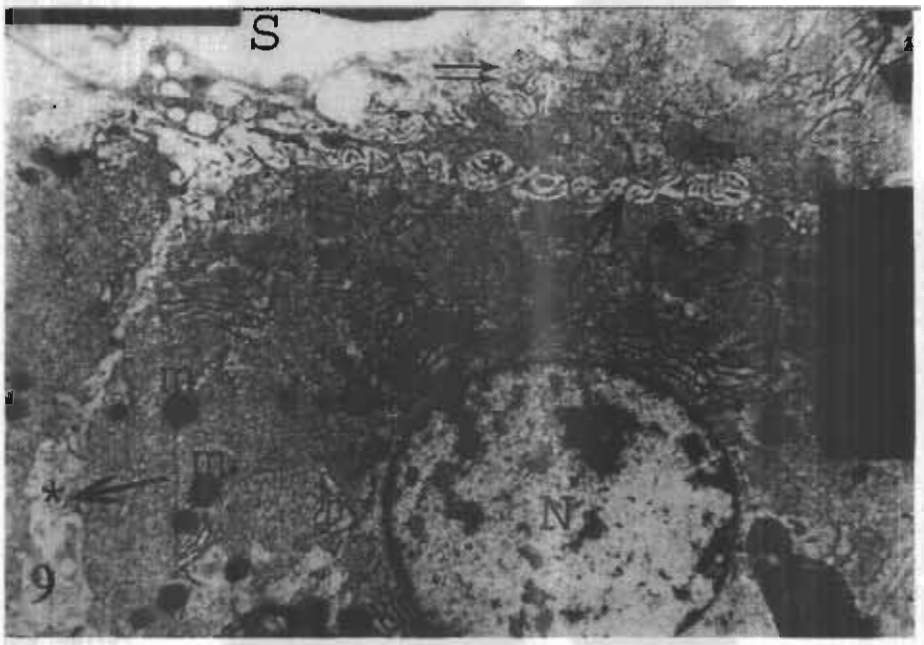


Fig. 9. An electron micrograph from a section in the liver of a control group showing hepatocyte with euchromatic nucleus (N), rough endoplasmic reticulum (r) and mitochondria with electron dense matrix (m). Long microvilli (double arrows) are seen projecting toward blood sinusoid (S). Short microvilli (arrows) projecting into the bile canaleculi (*) are also noticed (TEM x 11000).

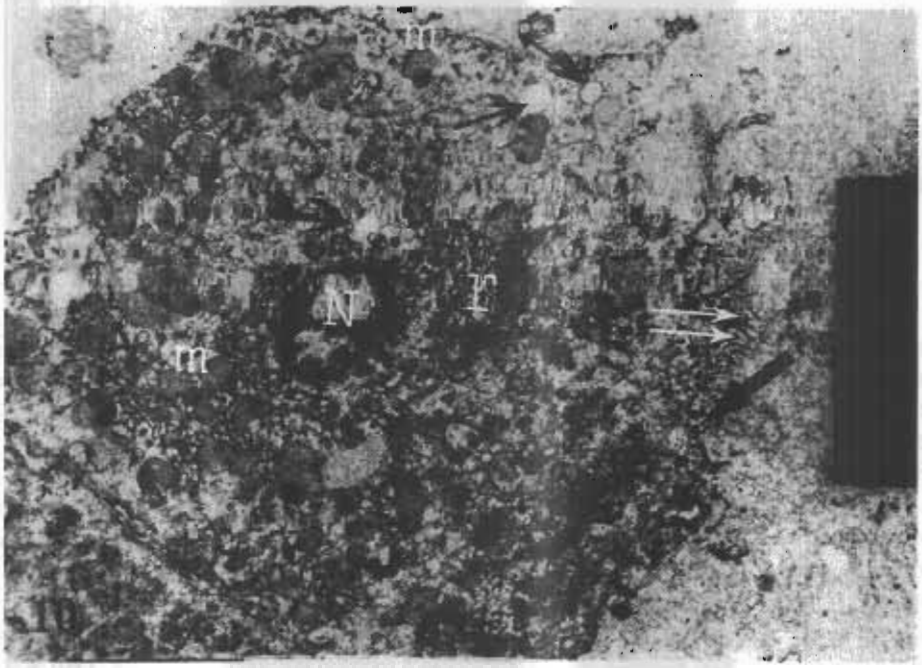


Fig. 10. An electron micrograph from a section in the liver of orally administered metalaxyl group (69.7mg/kg B.wt) twice weekly for 4 months showing hepatocyte containing shrunken nucleus with more heterochromatin (N). The cytoplasm contains mitochondria with less electron dense matrix (m), vaculations (arrows) and short irregular rough endoplasmic reticulum ®. Narrow bile canaliculi (thick arrow) and destructed microvilli (double arrow) are seen (TEM x 9000).

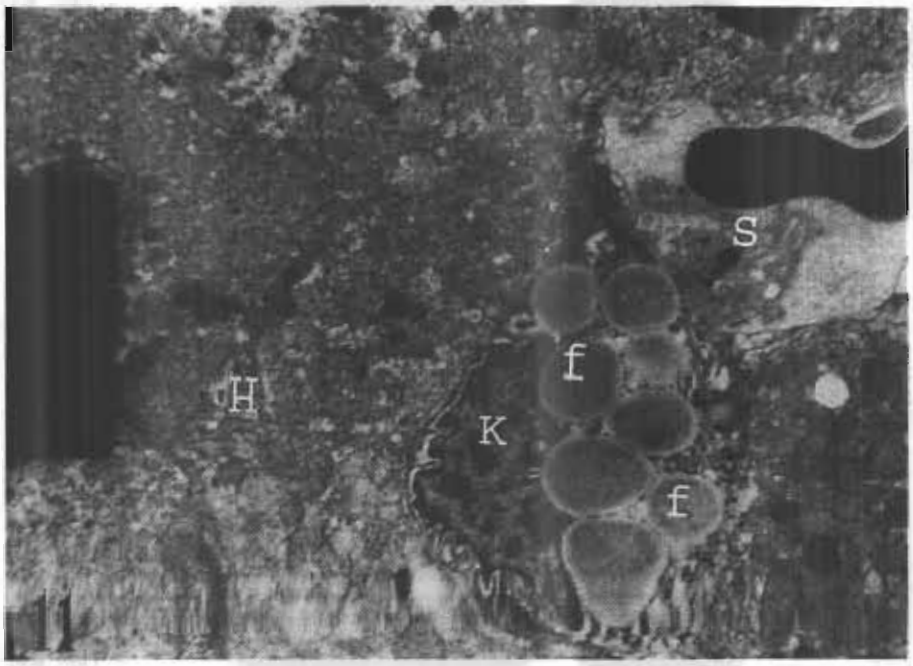


Fig. 11. An electron micrograph from a section in liver of metalaxyl feeding group showing ito cell (K) containing fat globules (f) between hepatocyte (H) and blood sinusoid (S) (TEM X 11000).

DISCUSSION

Metalaxyl is an economically important systemic fungicide with high activity against fungal pathogens of the order of Peronosporales. It is registered for use on a wide range of crops and in several countries *(19)*.

The current experimental work had been shown that metalaxyl significantly decreased serum levels of total protein and albumin in both treated groups comparing with the control one. The pronounced reduction may be attributed to the mode of action of metalaxyl which is act by inhibition of protein synthesis *(33)*. On the same context those observations recorded by *(18)* which reported that metalaxyl did not increase unscheduled DNA synthesis in rat primary hepatocytes or in human fibroblasts. The reduction in both levels of total protein and albumin could reflects the hepatotoxic and the immunotoxic effects of metataxyl chronicity in rabbits which manifested and confirmed by our histopathological findings which revealed an obvious aggregation of the lymphocytes inside the hepatic tissues as a sign of an immune response resulted from the repeated exposure of the hepatic tissues to the fungicide as these inflammatory cells play an important role to overcome the toxic compounds *(34)* . Also, our histopathological results manifested fat infiltration; cytoptasmic fat globules which postulated to be a defense mechanism by which hepatocyte attempt to collect all toxic compounds invading the cell in these vacuoles prior to excretion *(35)*.

In alike manner those findings previously reported that rats fed diets containing technical – grade metalaxyl at a concentration of 0,50,250 and 1250 ppm for 2 years revealed centriacinar, periacinar hepatocytic vacuolation in the liver of various degrees of severity indicating fatty changes *(36)*. Two

year chronic feeding of rats with metalaxyl at dose level 12.5 mg/kg / day showed increased liver weight and periacinar vacuolization of hepatocytes *(37)*. Another study of 2 year oncogenic mice with 37.5mg/kg /day revealed fatty infiltration of liver besides another study on mice per sex per group received 0,50,250 and 1250 ppm of metalaxyl in the diet for 104 weeks revealed large hepatocytic fatty, vacuolation for high dose males and females *(18)*.

Moreover, the irregular short microvilli (MV) toward blood sinusoids which were noticed in the hepatocytes of the metalaxyl treated groups could be considered a sign of an immune response ; the action – based cytoskeletal core structure MV, acting as a diffusion barrier between membrane and cytoplasm to inhibit the entrance of hydrophilic and lipophilic xenobiotics via MV into the cytoplasm. Furthermore, the polarized organization of the epithetial secretory response to various external signals generates a basal- to- apical lipid flow that clears the plasma membrane from lipophilic xenobiotics *(38)*. Besides our results came in harmony with the findings recorded *(14)* carried out a follow–up 90 day study to evaluate the effects of 0,10,15,250 and 1250 ppm of metalaxyl incorporated in the diet of rats, where it has been found that a dose related decrease in total leucocyte count, also, there was a significant increase in relative liver weight for females in the 250 and 1250 ppm dose groups.

On the other hand, observations recorded by ***Harada*** *(39)* showed that dogs treated with metalaxyl at dose of 80 mg/kg B.wt./day had significantly increased total protein, albumin and calcium concentration, and the female rats treated with metalaxyl- M at 150 and 300 mg /kg B.wt./day showed increased plasma albumin and globulin concentrations and reduced bilirubin *(40)*. On the same context *(20)* recorded increased albumin level in a short term toxicity by metalaxyl which was administered orally in rats at dose from 0.6 to 86 mg/kg B.wt. / day and in dogs in doses from 0.8 to 80 mg/kg B.wt. / day . This variation from the results of our study may be attributed to the difference in dose, species and duration of exposure.

The data of the present study demonstrated an obvious significant increase in GGT and alkaline phosphatase levels in both treated groups with the fungicide. The liver is the most sensitive organ to biochemical changes therefore, its functions are greatly affected by pollutants resulting in an increase in serum enzymes levels *(41)*. The decreasing in blood concentrations of metalaxyl after 6h may be due to enterophepatic circulation of metalaxyl or its metabolites *(15)*. Dogs treated with metalaxyl at dose of 80 mg/kg B.wt/day had significantly increased serum activities of alkaline phosphatase and alanine aminotranferase *(39)*. ***IRIS*** *(37)* reported that alkaline phosphatase levels in the dogs given 1000 ppm of metalaxyl in the diet were significantly increased. The liver was the target organ of metalaxyl toxicity as indicated by increase in relative and absolute weights and in alkaline phosphatase activity at dose level of 7.3 mg/kg B.wt/ day in dogs *(42)*. The forementioned results about the elevation of the liver enzymes in the current experimental work could be supported by the histopathological findings which showed some hepatic cells contained shrunken nuclei with more heterochromatin which most probably indicating apoptosis. These observations is in consistence with previous several studies *(43-45)* which reported that environmental stressors (metals, particulate matter and pesticides) can induce apoptotic cell death. It is well known that apoptosis is an important and controlled form of cell death that occurs under a variety of physiological and pathological conditions *(46)*. Also, our study revealed some mitochondria with less electron dense matrix which were observed in metalaxyl treated groups. Apoptosis may be induced via the extrinsic pathway by the activation of death receptors or intrinsic pathway by intracellular stimuli that transmit a signal to the mitochondria, the pesticides have been shown to induce apoptosis by activation of intracellular caspase – 3 *(47)*. Aactivation of caspase -3 is associated with morphological and structural changes characteristic of

apoptosis *(48)*. Another possible alternative scenario of metalaxyl inducing apoptosis could be due to induction oxidative stress *(49)*. When intracellular reactive oxygen intermediate (ROI) exceeds the antioxidant defense capacity of the cell, increased ROI leads to lipid peroxidation and generalized oxidative damage to all mitochondrial components, which supported in the present work by presence of cytoplalsmic globules. The increased lipid peroxidation caused by ROS, which lead to mitochondrial vacuolization, structural lesions, and concurrent mitochondrial dysfunction, also, he added that mitochondria are often associated with fatty acid – containing oil droplets from which they derive raw materials for oxidation and lipid synthesis and metabolism occurs primarily in mitochondria *(50)*. Thus, mitochondrial structural lesions may have resulted in early liver disorders of lipid metabolism.

Regarding to the effect of metalaxyl on FSH; our data showed a significant decrease of serum levels in both treated groups. Female rats treated with metalaxyl at dose of 1250 ppm resulted in large ovarian cysts *(16)*. Moreover, the reduction in the level of FSH which was recorded in the present experiment could be confirmed by our ultra-histopathological findings which may support the impairment in metabolism of the hormone.

Concerning the cytogenetic examination of bone marrow cells of the treated rabbits with metalaxyl; the current study revealed various types of chromosomal aberrations (structural and numerical). It has been recorded complete cellular toxicity at 1.6 mg/ml and 5mg/ml of metalaxyl both with or without activation, where a significant increase in the percentage of chromosomally aberrant cells was observed only in the absence of activation at 1200 µg/ml *(51)*. Human peripheral blood lymphocytes cultured which revealed significant induction of chromosomal aberrations with 300 and 1000µg/ml metalaxyl in the absence of microsomal activation *(19)*. Metalaxyl was tested for its mutagenic effects on L51 78Y/TK+/- mouse lymphoma cells *in vitro* , no evidence of mutagenic effects was observed in this mammalian forward mutation system *(17)*. Another study on rat primary hepatocytes or in human fibroblasts, suggested that metalaxyl is not genotoxic *(18)* .

It could be concluded that metalaxyl induced chromosomal aberrations hepatic lesions and altered levels of some biochemical parameters which were more severe in group (1) than group (2).

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الملخص العربى

دراسات وراثية خلوية وبيوكيميائية وباثولوجية على إناث الأرانب البلدية المتسممة بالمبيد الفطرى الميتالاكسل

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أجريت هذه الدراسة لمعرفة التأثيرات المختلفة من التعرض المزمن للمبيد الفطرى واسع الانتشار الميتالاكسل فى إناث الارانب البلدية والتى تشمل دراسة تأثيره على بعض التغيرات البيوكيميائية ، وتأثيره على صورة الكروموسوات وكذلك تأثيرة الهستوباثولوجى على أنسجة الكبد.

أستخدم لهذا الغرض عدد خمسة عشر من إناث الأرانب البلدية والتى قسمت إلى ثلاث مجموعات متساوية ، المجموعة الاولى تم تجريعها عن طريق الفم بجرعة تمثل ١٠/١ من الجرعة النصف المميته للمبيد (٦٩,٧ ميلجم/ كجم من وزن الحيوان) مرتان أسبوعيا ، المجموعة الثانية تم تغذيتها على نبات الخس بعد رشه بالجرعة الحقلية للمبيد وهى (٢٠٠جم / ١٠٠ لتر ماء) وتركه ثلاثة أيام بعد الرش ثم تقديمة لكى تتناولة ارانب المجموعة الثانية كغذاء مع العليقة المركزة الجافة ، اما المجموعة الثالثة فقد تم تجريعها عن طريق الفم مرتان أسبوعيا بزيت الذرة (وهى المادة التى يتم إذابة المبيد باستخدامها) واستخدمت كمجموعة ضابطة وقد أستمرت المجموعات الثلاث فى التعرض لمدة أربعة أشهر (وهى مدة التجربة). وقد أظهرت النتائج أن مبيد الميتالاكسل أدى إلى حدوث نقص معنوى فى مستوى كل من البروتين الكلى والالبيومين فى المجموعات التى تم تعرضها للمبيد مقارنة بالمجموعة الضابطة . أيضا تسبب المبيد فى حدوث زيادة معنوية ملحوظة فى مستوى إنزيمى الجاما جلوتاميل ترانسفيريز (GGT) والفوسفاتيز الكلوى (Akaline phosphatase) إضافة إلى إحداث المبيد إلى نقص معنوى فى مستوى هرمون FSH فى المجموعات المعالجة بالمبيد الميتالاكسل مقارنة بالمجموعة الضابطة . كما أثبت الفحص الكروموسومى إلى وجود تشوهات للكروموسات شكلية وعددية فى كلتا المجموعتين التى تم تعرضها للمبيد . أما عن الفحص الباثولوجى لأنسجة الكبد باستخدام المجهر الضوئى والالكترونى فقد أظهرت الدراسة أن الميتالاكسل فى المجموعة التى تم علاجها عن طريق التجريع بالفم أدى إلى تحورات نخرية وموت مبرمج للخلايا الكبدية وظهرت بعض الأوردة الوسطى محقنة والحبيبات الدموية غير واضحة المعالم بين الخلايا الكبدية وكانت القناة الصفراوية سميكة الجدار ومحاطة بارتشاح خلوى ، بينما ظهر النسيج الكبدى فى المجموعة الثانية التى تم تعرضها للمبيد بعد رشه على نبات الخس بتركيب هستولوجى قريب من الطبيعى ماعدا وجود بعض الأوردة الوسطى المحتقنة مع وجود موت مبرمج لبعض الخلايا الكبدية . مم سبق ذكره من تأثيرات ضارة للمبيد فى هذه الدراسة لذا ننصح بأن يكون استخدام هذا المبيد الفطرى عند الضرورة البالغة وبحرص شديد .