Oral Intoxication Of Balaldi Rabbits With Metalaxyl Fungicide.

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Abstract

Several pesticides are widely employed in agriculture. The purpose of this study was to asses the oral toxicity of commonly used systemic fungicide, metalaxyl , which used on a wide range of crops in several countries including Egypt.This study was carried out on 15 female baladi rabbits which divided into three groups, one of them kept as control administered corn oil alone , the second group orally administered with 1/10 LD_{50} of metalaxyl twice weekly for 4 months. The third group was fed lettuce, (previously sprayed with metalaxyl for 3 days) for 4 months concurrently with dry ration. A statistically significant decrease of body weight in both treated groups. Biochemical analysis revealed an increase in serum transaminases (AST & ALT), serum lactate dehydrogenase (LDH) ,creatinine phosphokinase (CPK), urea and creatinine in both metalaxyl treated rabbits. Metalaxyl residues were higher in the muscle and liver of orally treated group while wasn't detected in the rabbit muscle after feeding conversely their liver contained metalaxyl . liver ,kidney and heart showed histopathological lesions of both treated groups.

Thus, metalaxyl induced adverse health effects in baladi rabbits either after feeding or after oral administration.

Key words: fungicide, metalaxyl; oral intoxication; residues.

Introduction

Phenylamides fungicides have been commercially used since 1978. They are highly active specifically controlling plant pathogens of Oomycetes [1].Phenylamides fungicides are a class of systemic compounds including metalaxyl (Ridomil, Oxidixyl, Benalaxyl and Ofurace) that show excellent protective and curative eradication antifungal activity used in mixtures as foliar spray for tropical and subtropical crops, controlling of soil-born pathogenes as soil treatment or as seed treatment for downy

mildewes control [2].Metalaxyl applied on some food crops, tobacco, ornamentals, conifer and turf in several countries,products containing metalaxyl must bear the signal word " caution" [3] Metalaxyl is highly soluble in water (7.1g/l) and it is photolytically stable in water and soil during exposure to direct sun light with a half life of 400 days **[4-6]** More over plant uptake, microbial degradation, photodecomposition and leaching are the major routes of metalaxyl dissipation. Commercial products either contain metalaxyl alone or more likely are mixtures with different rates of residual protective fungicides as mancozeb, copper, folpet or carbendazim **[7].**

Hypertrophy of the hepatocytes was recorded in both sexes of Sprague-Dawely rats either after oral administration or after given diet containing 50,250 and 1250 ppm of metalaxyl for 3 months **[8,9].** More over focal hepatic fibrosis and brown pigmentation were observed in the liver of Beagle dogs orally administered metalaxyl in gelatine capsule (80 mg/kg bwt.) daily for two years **[10]**

The residues of metalaxyl in rats orally administered doses of 0.5 and 25 mg/kg b.wt. were low except in the liver and blood which were 0.002- 0.004 ppm for 0.5 mg/kg b.wt. dose rate. The liver, fat and blood residues for dose level 25 mg/kg b.wt. were 0.146 - 0.225 ppm.and less than 0.02% of the dose appeared in expired air **[11].**

Metalaxyl was administered in gelatine capsule at a dose of 0,0.8, 8 and 80 mg/kg body weight daily for 2 years to groups of six male and six female Beagle dogs. He found higher dose group significantly increased serum alkaline phosphatase and alanine amino transferase levels.**[10]**

The metalaxyl has central nervous system depression and cardiotoxic effects in treated rats **[12].** Metalaxyl was not irritating to the skin of rabbits but slightly irritating to their eyes **[13].**

Pregnant Chinchilla rabbits orally treated with metalaxyl at dose level 0,5,10 and 20 mg/kg b.wt. on 6 -18 days of gestation showed reduced food consumption in treated groups with 10 and 20 mg/kg b.wt. accompanied by slight weight loss on day 28 of gestation. All rabbits were

sacrificed and the exposed foeti were examined for visceral and skeletal malformations. Metalaxyl did not adversely influence embryonic foetal development in Chinchilla rabbits **[14].**

Previous study applied 0,10,100 or 1000mg powdered metalaxyl (purity, 92%)/ kg b.wt./ day to the clipped skin of male and female Newzealand white rabbits under semi-occlusive dressing for 6 hrs/day, 5 days/week for 3 weeks. Multifocal dermatitis in treated and untreated areas of the skin in both treated and control rabbits. Haematological and clinical chemical analysis and post-mortem examinations for organ weight, gross and histopathological lesions in brain, pituitary, heart, thyroid, adrenals, genital organs, liver, kidney and skin revealed no treatment-related changes **[15].**

Metalaxyl has been studied in the rabbit for systemic toxicity. Orally and dermally, but the oral ingestion was more effective than dermal application from 1000 to 6000 mg/kg bwt. It has to be classified as harmful by ingestion. **[16]**

There is scanty available information on oral toxicity of metalaxyl in rabbits. This study was aimed to elucidate the toxic effects of metalaxyl either by oral intubation or by feeding in baladi rabbits.

Material and Methods

Animals and design of the experiment :

Total number of 15 healthy female baladi rabbits with body weight ranged from 1211.00 ± 8.03 to 1329 ± 100.13 g. obtained from Zagazig local market. The animals were kept under the same hygienic conditions for 2 weeks before the experiment, housed individually in a separate row galvanized wire mesh cages (70x 60 x50 cm)and placed in a well ventillated house. The cages were provided with feeders and automatic drinkers. The manure was removed through a central trough with automatic flushing. Rabbits were fed ad-libitum with a commercial Pelleted ration from San El-Hager feeding company. The ration consisted of 34% soybean meal, 26.4%

yellow corn, 35 molasses, 4% cotton seed cake, 1% limestone, 0.255 common salt, 0.30% vitamins and mineral premix and 0.05 % DL Methionine. Rabbits were vaccinated against haemorrhagic septicemia. The animals were divided into 3 groups (5 in each) the first group kept as control orally administered corn oil alone. The second group fed green lettuce sprayed with the field dose (200 g./100 litre water) after 3 days of spraying.The third group orally administered metalaxyl 1/10 LD_{50} (69.7 mg/kg b.wt) twice weekly for 4 months after dissolving in corn oil according to **[17].** Along the experimental period all groups fed commercial pellets ad-libitum concurrently with lettuce.

The fungicide:

- Common name: metalaxyl
-Registered trade name: Ridomil, Ridomil plus,WS, Fubol, Acylon Super F, CGA 48988 and Folpet, Apron 35, Apron 70 SD.
- The formula used: Ridomil gold plus.
-Empirical formula: $C_{15}H_{21}NO_4$
-Chemical name (IUPAC): Methyl *N- (2-methoxyacetyl)-N- (2, 6-xylyl)-DL-alalinate*
- The compound was supplied by: Syngenta Co., Egypt.
-Metalaxyl standard : supplied by Wako Pure Chemical Industeries, ltd. (Japan).
- LD50 of metalaxyl in rabbits was (697 mg/kg b.wt.) according to ***[16]***

Sampling:

At the end of the experiment all animals were slaughtered and blood samples were collected for sera separation which kept at -20°C till biochemical analysis for measuring AST and ALT according to **[18].** LDH according to **[19]**, CPK according to **[20].** , Creatinine according to **[21]** and Urea according to **[22].**

Specimens of heart, lung, liver and kidney kept in formalin 10% for histopathological examination according to **[23].**

Residual analysis:

Samples for residual analysis were taken from muscles and livers of treated rabbits and kept deep freezed until analysis using high performance chromatography (HPLC) according to the method described by **[24]** Then partitioning carried out two times using 15, 10 ml acetonitril saturated with n-hexane in 100 ml separating funnel and filtration through anhydrous

sodium sulphate. Collected fraction evaporated till dryness through rotatory evaporator (35°). The samples were retained from the flasks by acetonitril then collected in tubes to be ready for injection into HPLC.Working conditions of the analyzed fungicide (metalaxyl) presented were as following (Retention time:2.345 min , wave length :220 nm and detection limit 20 ng). Suitable aliquots (20µl) from extracts and standard were injected **[24].**

Statistical analysis

The obtained data were analyzed using statistical package for social science **[25].**

Results

No clinical signs were observed on the rabbits of both treated groups along the experimental period.

The final body weight of rabbits in the feeding and oral treated groups were significantly decreased 1060.00 ± 45.50 and 1095.80 ± 29.87 g. respectively compared with the control group 1716.66 ± 73.33 g. .

Table (1): Showing change in the body weight of baladi rabbits either fed green lettuce sprayed with the field dose (200 g./100 liter water) after 3 days of spraying or orally treated with 1/10 LD_{50} of metalaxyl (69.7 mg / kg b.wt.) for 4 months compared with the control group. (Means ± S.E.).

	control group	Feeding group	Oral group
Initial b.wt. (g)	1329.00±100.13[a]	1211.00±8.03[a]	1312.00±84.41[a]
Final b.wt. weight(g)	1716.66±73.33[a]	1060.00±45.50[b]	1095.80±29.87[b]

Means within the same row have different superscripts are significantly different ($p \leq 0.05$).

The biochemical analysis revealed a significant increase in the level of ALT enzyme (75.4±1.60, 204.80±2.59) and AST enzyme (94.60±1.07 , 271.8±0.96) in both feeding and oral groups respectively when compared to the control group (16.4±1.43, 26.80±0.58).There was also a significant increase in the levels of CPK and LDH in feeding group and oral treated groups (5330.40±374.24 and 921.60±1.077 , 5772.4±224.9 and 970.60±5.016) respectively comparing with the control group

(2310.00±2.89 and 771.40±2.31). Urea level in the feeding (52.80±0.58) and oral (64.80±0.01) which were significantly increased than the control group (42.60±0.92, 0.72±0.00) respectively. Creatinine in both feeding (1.39±0.006) and oral (1.84±0.01) groups significantly increased the control group (0.72±0.007)

Table (2): showing some biochemical changes in baladi rabbits either fed green lettuce sprayed with the field dose (200 g. / 100 liter water) after 3 days of spraying or orally treated with 1/10 LD_{50} of metalaxyl (69.7 mg / kg b.wt.) for 4 months compared with the control group. (Means ± S.E.).

Parameter	Control	Feeding group	Oral group
ALT(I/U)	16.4 ± 1.43^{c}	75.4 ± 1.60^{b}	204.80 ± 2.59^{a}
AST(I/U)	26.80 ± 0.58^{c}	94.60 ± 1.07^{b}	271.8 ± 0.96^{a}
CPK(U/I)	2310.00 ± 2.89^{b}	5330.40 ± 374.24^{a}	5772.4 ± 224.96^{a}
LDH(U/I)	771.40 ± 2.31^{c}	92160 ± 1.077^{b}	970.60 ± 5.016^{a}
Urea (mg/dl)	42.60 ± 0.92^{c}	52.80 ± 0.58^{b}	64.80 ± 0.01^{a}
Creatinine (mg/dl)	0.72 ± 0.007^{c}	1.39 ± 0.006^{b}	1.84 ± 0.01^{a}

Means within the same row have different superscripts are significantly different ($p\leq 0.05$).

Histopathological changes in liver of baladi rabbits of the feeding group were hydropic degenerative changes, centrilobular coagulative necrosis. Thick fibrous tissue containing lymphocytes were seen in portal area and interlobular tissue **(Fig.2).** Proliferative epithelium of bile duct forming numerous bile ductules with lymphocytosis was common in the portal areas. While the hepatic cells of baladi rabbits administered 1/10 LD_{50} metalaxyl orally for 4 months suffered from vascular and hydropic degeneration with portal leukocytic aggregations mainly lymphocytes, thickened interlobular tissues by proliferative bile ductules and fibrous tissue infiltrated with leukocytes named mononuclear cells with congested blood vessels **(Fig.3)** other portal areas, the bile duct epithelium showed hyperplasia in the form of papillary projections with edema. Renal tubules

of rabbits in the feeding group revealed diffuse coagulative necrosis and hydropic degeneration **(Fig.5)**, some glomeruli showed hypercellularity in the renal cortex. Nephrotic changes varied from cloudy swelling, hydropic degeneration or coagulative necrosis with multiple hyaline casts inside some tubular luminae **(Fig.6),** more over renal arteriole had endotheliosis and hyalinized media with perivascular edema. Mild interstial round cell aggregations with fibroblasts proliferation were encountered in the kidneys of rabbits administered 1/10 LD_{50} metalaxyl orally for 4 months. Heart of rabbits in feeding group revealed inter and intra muscular edema with pyknotic nuclei and diffuse hyalinization of the majority of the myocardial muscles **(Fig.8)** some muscle fibers exhibited myolysis with congestion of intermascular capillaries.While Intermuscular edema and partial hyaline degeneration were common lesion in the myocardial muscles of rabbits orally administered 1/10 LD_{50} metalaxyl for 4 months **(Fig.9)** moreover, some muscle fibers showed myomalacia cordis with congested intermascular blood vessels. Lungs of rabbit in feeding group appeared thickened pulmonary and interalveolar septae with interstitial lymphocytic aggregations were common **(Fig.11)** parabronchial lymphoid hyperplasia with perivascular edema could be seen. While Mild peribronchial leukocytic infiltration, congested pulmonary blood vessels with perivascular and interstatial leukocytic aggregations mainly lymphocytes and haemorrhages were seen **(Fig.12)** portal ateletcasis of some alveoli alternating with focal alveolar emphysema were be noticed in the lungs of rabbits of second group.

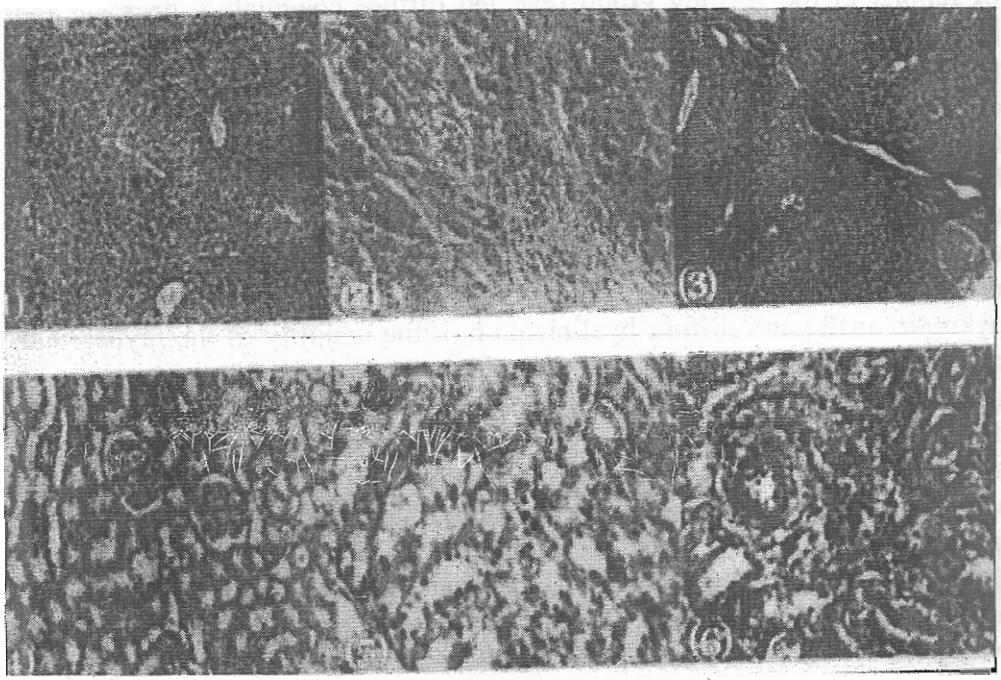


Fig. (1). Section in the Liver of control baladi rabbits showing normal hepatic parenchyma (H&E X150).

Fig. (2). Section in the liver of female baladi rabbits fed green lettuce sprayed with field dose (200 g./100 liter water) after 3 days of spraying showing portal interlobular fibrosis (H&E X300)

Fig. (3). Section in the liver of female baladi rabbits orally administered 1/10 LD_{50} of metalaxyl (697mg/ kg.bwt) twice weekly for 4 months showing thickened interlobular tissues and congested blood vessels (H&E X150)..

Fig. (4). Section in the Kidney of control showing normal parenchyma (H&E X150).

Fig. (5). Section in the Kidney of female baladi rabbits fed green lettuce sprayed with field dose (200 g./100 liter water) after 3 days of spraying showing diffuse coagulative necrosis of tubular epithelium (H&E X300).

Fig. (6). Section in the Kidney of female baladi rabbits orally administered 1/10 LD_{50} of metalaxyl (697mg/ kg.bwt) twice weekly for 4 months showing hyaline casts , nephrosis and hyalinized wall of renal arteriole(H&E X300).

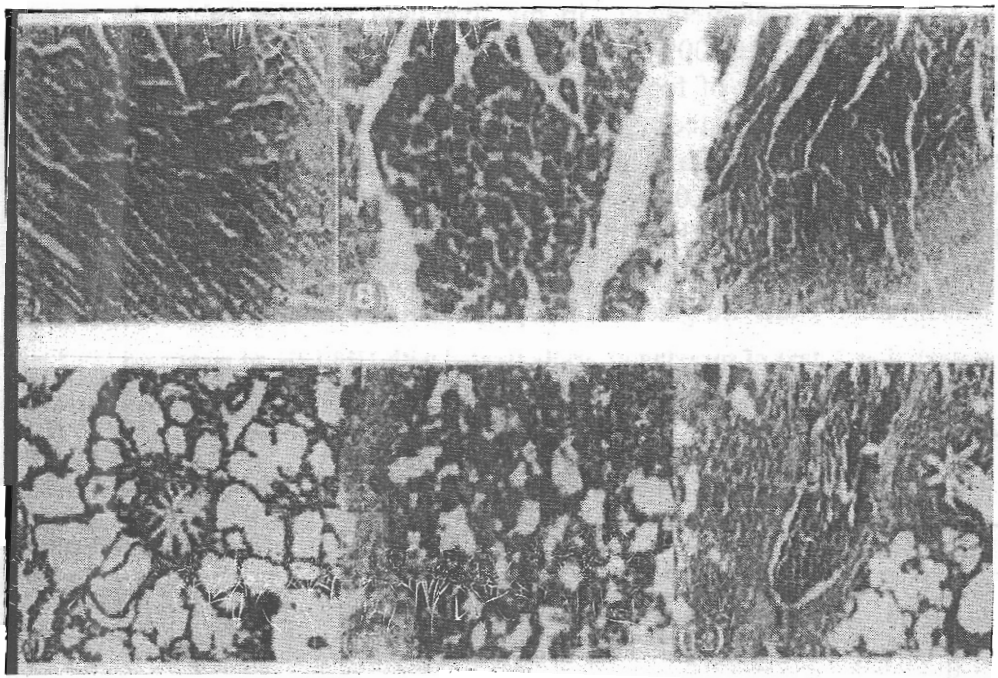


Fig. (7). Section in the heart of control baladi rabbits showing normal cardiac muscle (H&E X150).

Fig. (8). Section in the heart of female baladi rabbits fed green lettuce sprayed with field dose (200 g./100 liter water) after 3 days of spraying showing inter and intramuscular oedema with pyknotic nuclei and diffuse hyalinization of muscle fibers (H&E X300)

Fig. (9). Section in the heart of female baladi rabbits orally administered 1/10 LD_{50} of metalaxyl (697mg/ kg.bwt) twice weekly for 4 months showing Inter muscular edema and partial hyaline degeneration of some myocardial muscles (H&E X150).

Fig. (10). Section in the Lungs of control showing normal pulmonary tissue. (H&E X150).

Fig. (11). Section in the Lungs of female baladi rabbits fed green lettuce sprayed with field dose (200 g./100 liter water) after 3 days of spraying showing thickened pulmonary and interlobular septae (H&E X150).

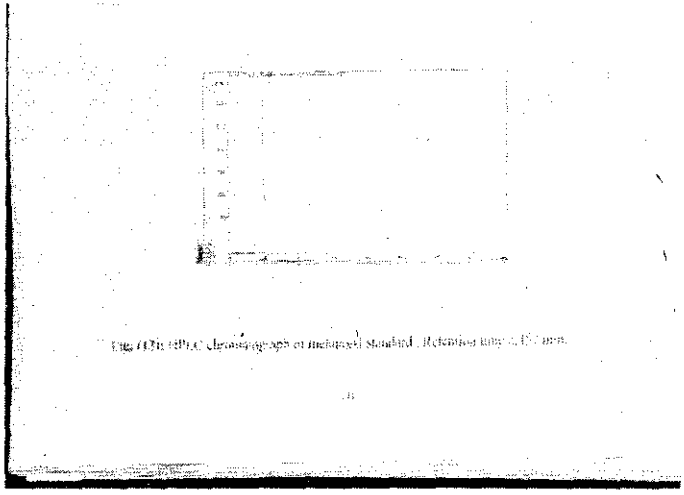
Fig. (12). Section in the Lungs of female baladi rabbits orally administered 1/10 LD50 of metalaxyl (697mg/ kg.bwt) twice weekly for 4 months showing congested pulmonary blood vessels perivascular lymphocytic aggregations and hemorrhage (H&E X300).

Residues of metalaxyl in the liver and muscles of orally treated rabbits were 0.619±0.009 ppm and 0. 86±0. ppm respectively. Metalaxyl residues in the liver of feeding group were 0.0.026±0. 001 ppm but there were no residues detected in the muscles of these rabbits. Statistical analysis showing that the metalaxyl residues in the muscles of oral treated group were significantly high 0.86± 0.006 ppm than that of the liver 0.619±0.009 ppm as shown in table (3).

Table (3): Quantitative estimation of metalaxyl residues in the liver and muscles of baladi rabbits either fed green lettuce sprayed with the field dose (200 g. /100 liter water) after 3 days of spraying or orally treated with 1/10 LD_{50} of metalaxyl (69.7 mg / kg b.wt.) for 4 months (Means ± S.E.).

Organ / Treated group	liver	Muscle
Feeding	0.026± 0.001^{c} ppm	0.00±0.00^{c} ppm
Oral	0.619±0.009^{b} ppm	0.86± 0.006^{a} ppm

Means within the same column have different superscripts are significantly different ($p \leq 0.05$).



. Fig.(13): HPLC chromatograph of metalaxyl standard .

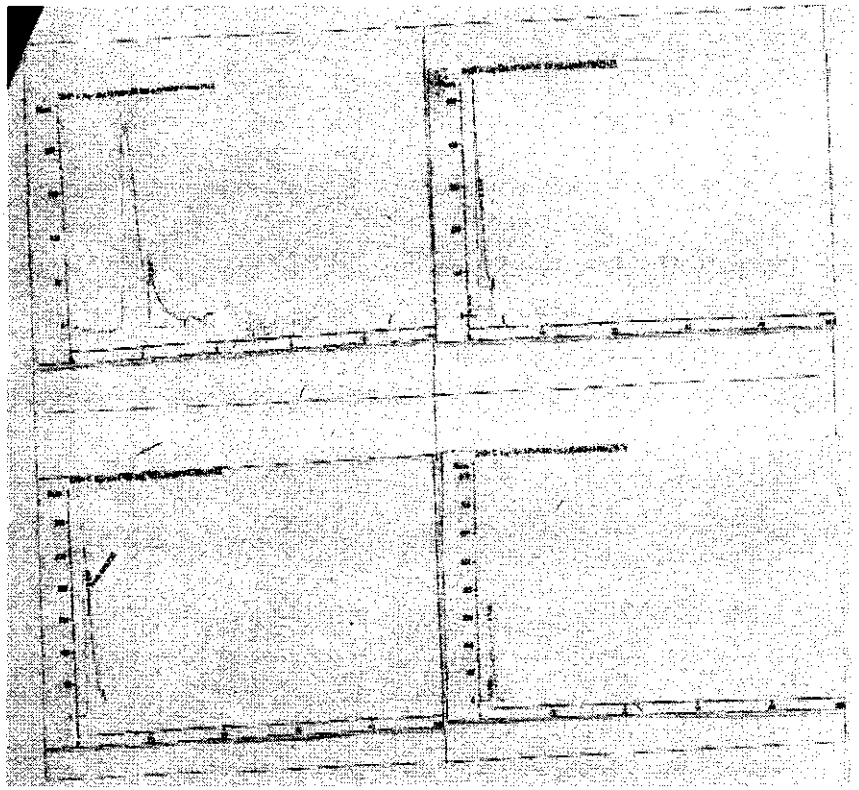


Fig. (14): HPLC chromatograph of metalaxyl residue in liver of baladi rabbits orally administered 1/10 LD_{50} (697mg/ kg.bwt) twice weekly for 4 months.

Fig .(15): HPLC chromatograph of metalaxyl residue in liver of baladi rabbits fed green lettuce sprayed with field dose (200 g./100 liter water) after 3 days of spraying .

Fig .(16): HPLC chromatograph of metalaxyl residue in muscles of baladi rabbits orally administered 1/10 LD_{50} (697mg/ kg.bwt) twice weekly for 4 months.

Fig .(17): HPLC chromatograph of metalaxyl residue in muscles of baladi rabbits fed green lettuce sprayed with field dose (200 g./100 liter water) after 3 days of spraying .

Discussion

Fungicides are chemical agents used to prevent or eradicate fungal infections in plants and seeds. The types of fungicides used in agriculture and food processing range from those relatively low toxicity to mammals to those which are highly lethal and tend to persist for long periods. In general the compounds designed to eradicate a fungus are inherently more toxic and more hazardous to plants and livestock than are the protectants **[26].**

This work revealed a significant final body weight loss of both orally administered or fed metalaxyl rabbits compared with the control group. (Table 1). This agrees with the results obtained by **[14, 27]** in albino rats orally treated with 1/10 LD_{50} for two months and pregnant Chinchilla rabbits respectively. This may be attributed to loss of appetite after treatment with metalxyl **[28]** or due to its effect on protein synthesis and amines metabolism **[29]** and accompanied by reduced food consumption **[14].**

Regarding to the effect of Metalaxyl on some sera biochemical parameters and histopathological lesions. Oral administration of metalaxyl 1/10 LD_{50} found to be highly effective than fed green lettuce sprayed with field dose after 3 days with spraying. This study revealed a significant increase in the level of transaminases (AST & ALT) table (2) in both

metalaxyl treated group than control group. Similar results was recorded by **[27]** in albino rats after their treatment with 1/10 LD_{50} of metalaxyl for two months and by **[10]** in beagle dogs orally administered 0.8, 8.0 and 80 mg metalaxyl / kg b.wt. for two years. This may be attributed to metalalxyl had a mild enzyme inducing effect which considered as a consequences of its hepatotoxicity **[30]** or aminotransferses are sensitive index of hepatic impairment **[31]**. This confirmed by our histopathological study whereas the liver of rabbits of both treated groups showed degenerative changes and coagulative necrosis (fig 2 and 3). These results in agreement with the results obtained by **[27]** in metalaxyl treated rats. Liver is considered the primary target for long term of metalaxyl treatment in rats **[32].**

Female baladi rabbit sera CPK and LDH showed a significant increase after oral treatment and feeding with metalaxyl for 4 months this may be attributed to that they are considered strongly indicative to myocardial damage which is confirmed by our histopathological results (fig. 8 and 9).

Increased CPK and LDH usually related to damage of cardiac muscle and liver **[33]** which confirmed by histopathological results (Figs 8 & 9) and confirmed by **[12]** who recorded that metalaxyl has cardiotoxic effect in treated albino rats. Our results disagree with **[27]** who found decrease in CPK and LDH of metalaxyl treated rats this may be attributed to the differences in dose, species or duration.

Sera urea and creatinine levels increased after metalaxyl oral administration and feeding which may be owed to its nephrotoixc effect as clearified in our histopathological results (Figs 5 and 6) due to the urine is the major route of metalaxyl elimination in the female **[4],** also its metabolites are generally excreted in urine and faces in treated rats **[34].** Metalaxyl is considered as carcinogen by its induction of Cyp3A isoformes in kidneys, liver and lungs of Swisss albino CD1 mice after single i.p injection of 200 mg or 400 mg / kg b.wt. Conversely our results disagree with the results obtained by **[10, 32]** who found that decreased urea in rats and decreased creatinine in beagle dogs respectively. Lungs of rabbits either treated or fed metalaxyl showed congested pulmonary blood vessels with interstitial leuckocytic aggregations and haemorrhage and thickened

pulmonary and interalveolar septa (Figs. 11&12).This agrees with the results obtained by **[27]** This may be attributed to excretion of metalaxyl in the expired air as reported by **[11].**

Regarding to the residue of metalxyl in the muscles and liver of baladi rabbit after either oral treatment with 1/10 LD_{50} of metalaxyl or feeding of metalaxyl sprayed lettuce using HPLC analysis. Metalaxyl residues were detected in muscle , liver of orally treated baladi rabbits while it was identified only in the liver but not in the muscles of baladi rabbit fed metalaxyl sprayed lettuce (Table, 3) .Muscles accumulate more metalaxyl than liver of orally treated rabbits which is similar to that detected by **[27]** after long term metalaxyl treatment . Liver of baladi rabbit fed lettuce sprayed with metalaxyl contained (0.026 ± 0.001) that may be due to the presence of metalaxyl in lettuce green parts which confirmed by **[35]** who detected unchanged metalaxyl in lettuce green parts (21.5 %) the rest is considered of several polar products. Similarly lactating Holestien cows dosed daily at a rate of 75 ppm metalaxyl in the diet per cow / day for 28 days revealed the absence of metalaxyl residues in liver, muscle and kidneys and even milk **[36].** This may be attributed to the differences in duration, species or due to its rapid absorption and excretion **[11].**

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الملخص العربي

تسمم الأرانب البلدية بمبيد الفطريات الميتالاكسيل عن طريق الفم

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نظرا لشيوع استخدام المبيدات الفطرية لأغراض متعددة كالقضاء علي الفطريات التي تصيب النباتات في الحقول وكذلك بذور هذه النباتات وبذور المحاصيل المخزنة مما يؤدي إلي حدوث بعض حالات التسمم في الإنسان والحيوان والدواجن نتيجة تناول الأغذية الخضراء والعلائق الجافة التي قد سبق رشها بهذه المبيدات أو شرب المياه الملوثة وكذلك قد يحدث التسمم نتيجة التعرض المتوالي لرزاز هذه المبيدات واستنشاقه مما يؤدي إلي امتصاصه من خلال الأغشية المبطنة وكذلك عن طريق الجلد.

تهدف هذه الدراسة إلي تقييم التأثير السام للمبيد الفطري الميتالاكسيل عند استخدامه عن طريق الفم لما له من انتشار واسع في مصروا لعديد من البلدان .

تم استخدام 15من إناث الأرانب البلدية مقسمة إلي 3 مجموعات متساوية ، استخدمت المجموعة الأولي كمجموعة ضابطة وتم تجريع المجموعة الثانية بجرعة تعادل عشر الجرعة نصف المميتة للميتالاكسيل (697 مجم / كجم من وزن الجسم) مرتين أسبوعيا بينما تم تغذية المجموعة الثالثة علي نبات الخس الذي سبق رشه بالجرعة الحقلية (200جم / 100 لتر ماء) المستخدمة لرش الخضروات بعد 3 أيام من الرش إلي جانب العليقة الجافة لمدة 4 اشهر.

أظهرت النتائج نقصا معنويا في وزن الجسم في كلا المجموعتين المعاملتين كما اظهر التحليل الكيميائي زيادة في معدلات الالانين ترانس امينيز والاسبرتيت ترانس امينيز و لاكتيت ديهيدروجينيز وكرياتين فسفوكينيز في مصل كلا المجموعتين المجرعتين وكذلك وجدت زادة معنوية في معدلات كلا من اليوريا و الكرياتينين .كما أظهرت النتائج وجود متبقيات الميتالاكسيل باعلي النسب في عضلات وكبد المجموعة المجرعة عن طريق الفم و لم تظهر في عضلات المجموعة التي تم تغذيتها علي الخس المرشوش بينما وجدت المتبقيات في كبد أرانب تلك المجموعة. كما أظهرت النتائج وجود تغيرات مجهرية في كل من الكبد ,الكلي ,الرئة والقلب للأرانب المجرعة في كلا المجموعتين.

دلت هذه الدراسة علي التأثير الضار لمبيد الميتالاكسيل علي صحة الأرانب البلدية التي تم تغذيتها علي النباتات المرشوشة بهذا المبيد وكذلك المجرعة به ووجود متبقياته في لحوم وكبد هذه الأرانب مما يؤدي إلي خطورته علي صحة الإنسان .