BIOCHEMICAL STUDIES ON METALAXYL IN EXPERIMENTAL ANIMALS

By

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ABSTRACT

Tahany Abd Elghaffar Ahmed Ali: Biochemical Studies on Metalaxyl in Experimental Animals. Unpublished M.Sc. Thesis, Department of Agriculture Biochemistry, Faculty of Agriculture, Ain Shams University, 2011

The present study carried out to through light on toxicological effects of metalaxyl technical pesticides on some physiological and biochemical prameters ***(Rattus rattus*** **)** in albino rat was treated with metalaxyl orally by gavage at concentrations (0, 50, 100, 150 and 200 mg/kg of pesticide). For a period of 28 days .The blood samples were collected weekly from eye vein with cappillary tube and plasma was gained to investigate the hematological markers and biochemical parameters in plasma also segments of the livers and kidneys of treated animals treatment and control used for histopathological examination.

The main results of these studies indicated that:

RBC_S count, Hb conc, MCH, MCHC value, albumin value, creatinine level, γGT activity Total cholesterol, Triglycerides level, Glucose, phosphorous levels were significant decrease through the experimental periods, While WBC_S count , MCV, PCV value, HDL levels, ALP, ALT, AST enzymes activity, Urea, calcium concentration, Phospholipids level in generally were significant increase, Also, the results indicated that, Total Protein and globuline levels were slightly increase through the experimental periods in metalaxyl treated animals Pathological changes are observed when the pesticide dose increases in liver tissue and kidney tissue.

Key words: metalaxy, Subacute Toxicity, Haematotoxicity, Liver Function, Kidney Function, Lipid Profile, Electrolites,Histopathology.

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CONTENT

I-INTRODUCTION

Pesticides are chemical compounds that are used to kill pests, including insects, rodents, fungi and unwanted plants (weeds). Pesticides are used in public health to kill vectors of disease, such as mosquitoes, and in agriculture, to kill pests that damage crops. By their nature, pesticides are potentially toxic to other organisms, including humans, and need to be used safely and disposed of properly.

Metalaxyl is a systemic, benzenoid fungicide used in mixtures as a foliar spray for tropical and subtropical crops, as a soil treatment for control of soil-borne pathogens, and as a seed treatment to control downy mildews (**Kimmel et. al., 1986).** 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 **(FDA 1986**) Products containing metalaxyl must bear the signal word "Caution." It's biochemical action is to inhibit the protein synthesis in fungi by interference with the synthesis of ribosomal RNA. Many investigations were carried out to determine the acute, chronic, teratogenic and mutagenic toxicity of metalaxyl, when the EPA Cancer Assessment. Group decided that metalaxyl should not be classified as an oncogen because even through a study should significant parafollicular adenonas of the thyroid in female rats at low middle doses, this did not occure at the higher dose (**NRC 1987)**. In this study was carried out to through light on the toxicological effects of technical metalaxyl through determination of hematological, biochemical and histopathological alterations in adult male albino rats within and after subacute oral exposure to four different doses of technical grade of metalaxyl for 28 days.

II- REVIEW of LITREATURE

2-1: Biochemical Toxicity Aspects of Metalaxyl :

Groups of 20 male and 20 female Sprague-Dawley rats received diets containing metalaxyl (purity,99%)at a concentration of 0, 50, 250 and 1250 p.p.m. for 3 months in a study carried out by **Drake (1977).** The results were no deaths and no overt signs of toxicity were reported. Ophthalamic examination revealed no treatment-related changes. Males at the highest dose had minimally decreased body-weight gain (terminal weight, 97% of control) and food consumption (week 13, 94% of control). Haematology, blood biochemistry and urine analysis revealed no treatment- related changes.

Finn et al. (1977). Conducted a study to evaluate, the effect of metalaxyl on four group of beagle dogs, 6-9 months of age. They administration the metalaxyl through diets at levels of 0, 250 and 1250 mg/kg for 91 days. The obtained data showed that, biochemical parameters were normal in treated animals except, a significant increase in serum alkaline phosphatase activity was noticed in animals at the highest level. This increase in serum alkaline phosphatase activity was time related.

Groups of 60 male and 60 female Swiss mice were given diets containing metalaxyl (purity 93–94.6 %) at a concentration of 0, 50, 250 and 1250 p.p.m. for 104 weeks by **Mc Sheehy et. al., (1980).**The evaluation of the slides of liver have evidenced that, no carcinogenic response to treatment. But, the tumours in liver of male mice were considered not to be related treatment this result of tumours in male mice supported by the liver tumours were age-related, occurring predominantly after 18 months of the study. (ii) The survival of the animals was not affected by administration of metalaxyl. (iii) Neither

general health nor body weight or food consumption were compromised by treatment. (iv) The presence of *Tyzzeria* did not obscure the presence of liver tumours, and the non-neoplastic lesions in the liver (hepatocytic fatty vacuolation and bile-duct proliferation) were no different in control and treated groups and would therefore not have affected the distribution of liver tumours. (v) The liver tumour incidence was not statistically significantly different between control and treated groups ($p = 0.12$). (vi) The liver tumour incidence did not show a dose-related trend (vii) the liver tumour incidence in the control group (23 %) was within the range of that in six other control groups in the same laboratory (12–37 %). Treatment with metalaxyl did not increase the incidence of beginning or malignant tumours in mice of either sex.

Groups of six male and six female beagle dogs were given diets containing technical-grade metalaxyl (purity, 92%) in acetone at a concentration of 0, 50, 250 and1000 p.p.m. for 6 months by **Beck and deWard (1981).** They found increase in plasma alkaline phosphatase activity in animals of each sex at the highest concentration from day 30 which became statistically significant during the second half of the treatment period. After 180 days of treatment, the activity was 170 % of the control value in males and 190 % in females. In the group allowed to recover, the alkaline phosphatase activity (ALP) was only minimally above the control values (120 % of control value in males and 110 % in females).

In a study of the toxicological effects of repeated inhalation of the pyrolysis products of cigarette tobacco treated with metalaxyl was carried out by **(Coatc 1982**). Groups of 10 male and 10 female Fischer 344 rats were exposed for 5 days per week, for 13 weeks, to the smoke from 18 cigarettes that had been spiked with 0, 130, 3900, and 13000 p.p.m. of metalaxyl (technical- grade, purity not specified).

The result showed that. No distinct clinical changes were noted in any of the treated animals during the study. No treatment–related effects or trends were observed in body-weight gain, food consumption, hematological, clinical chemical parameters, gross, histological appearance.

Metalaxyl (purity, 92.7–94.1%) was administered in gelatine capsules to groups of six male and six female beagle dogs at a dose of 0, 0.8, 8 and 80 mg/kg. per day by **Harada (1984).** The collected data showed that animals at 80 mg/kg. per day had significantly increased serum activities of alkaline phosphatase (ALP) and alanine amino transferase(ALT) as compared with controls. The values for alkaline phosphatase during the study were 1.5–2.5 times higher than those determined before initiation of treatment and 2–5.6 times those in the corresponding control groups. Also, Albumin, total protein and calcium concentration were increased in animals at the highest dose. While globulin, ALT activity and Creatinine concentration were decrease. The increase in alanine amino transferase activity in males at the intermediate dose was slight (maximum, 50%) and often not dose-related. The changes in alkaline phosphatase and alanine amino transferase activities observed in animals at the highest dose indicated hepatic effects of treatment Where, The liver was the target organ of metalaxyl, as indicated by the changes in blood chemistry and increased liver weight.

In a study of the effect of Metalaxyl-induced bradycardia in rats: mediated by alpha-adrenoreceptors. was carried out by **Naidu, and Radhakrishnamurty. (1988).** The effect of the fungicide metalaxyl [methyl N-(2-methoxyacetyl)-N-(2,6-xylyl)-DL-alaniate] on cardiac activity in albino rats anesthetized with phentobarbitone caused dose-dependent bradycardia, and at higher doses (250 and 300 mg/kg body weight) the sustained bradycardia led to cardiac arrest.

The acetylcholinesterase activities in brain and heart were not affected in metalaxyl-treated rats. However, the bradycardia induced by the fungicide was blocked by pretreatment of rats with the alpha-adrenoreceptor antagonist phentolamine (20 mg/kg, ip). The data suggest that the bradycardia-inducing effect of metalaxyl was not mediated by the cholinergic system but through alpha-adrenoreceptors.

The effect of metalaxyl on drug metabolizing enzymes were examined during a study of the kinetics and metabolism in rats by **Uesugi (1988).** Five male rats were given metalaxyl orally at dose of 40 mg/kg/day for 7day.The obtained results showed that, the activity of cytochrome P450 (CYP), aminopyrine-N-demethylase, para-nitroanisol- O-demethylase, para-nitrophenol -UDP-glucoronyl transferase and dinitrochlorobenzene glutathione transferase were significantly increased comparing with control in animals. Thus, administration of metalaxyl at 40 mg/kg. per day orally for 7 days significantly increased the activity of several hepatic enzymes.

The in vitro inhibition of monoamine oxidase (MAO) activity in rat heart by the fungicide metalaxyl was studied by **Naidu KA (1989)** .The results showed that Metalaxyl decreased MAO activity in a dose- dependant manner. The inhibitory concentration (IC^{50}) for metalaxyl was found to be 19 µmol Substrate-dependent kinetic studies demonstrated noncompetitive inhibition as evidenced by decreased velocity of enzyme activity (V_{max}) without significant change in enzyme –substrate affinity (K_m). The inhibition of MAO activity by metalaxyl suggests interference with amine metabolism.

In a study of metalaxyl effect on heart rate in rats: role of alpha1-adrenoreceptors carried out by **Naidu** and **Radhakrishnamurty (1989)**. The results showed that Intraperitoneal injection of metalaxyl (250 mg/kg) produced a decrease in heart rate

lasting for more than 60 min in anesthetized rats. Pretreatment of rats with phentolamine (a nonselective alpha- adrenoreceptor antagonist, ip at 20 mg/ kg) and prazosin (an alpha 1-adrenoreceptor antagonist, ip at 5 mg/kg) significantly reduced the bradycaridia induced by metalaxyl . yohimbine (an alpha 2- adrenoreceptor antagonist, ip at 10 mg/kg did not change the effect of metalaxyl on heart rate. The results suggest that alpha 2- adrenoreceptors mediate the bradycardic effect of metalaxyl.

Gerspach (1994). conducted a study to detect possible toxicological properties differences of metalaxyl-m and the racemate metalaxyl at doses 10, 50, 150 and 300 mg/kg/day by gavage administration for 28 days in Dawly rats. all animals were examined clinically and histopathologically. The results revealed that, Body weight gain and food consumption were similar in treated and untreated rats. Also, the data showed that, plasma sodium concentration was minimally lower. While, plasma chloride concentration was minimally higher in treated rats. Atendancy to decrease urea concentration was recorded among males at the highest dose of both Metalaxyl-M showed a decrease in albumin, globulin in females at the highest dose. The above mentioned results were significantly different untreated animals at dose 300 mg/kg/day for metalaxyl-m and metalaxyl. The same trend was noticed at dose 150 mg/kg/day in metalaxyl- treated rats.

The effect of Biomarkers in evaluating metalaxyl cocarcinogenesis was studied by **Paolini M et. Al., (1996).** The ability of metalaxyl, whose mutagenic/cocarcinogenic activity has as yet not been clarified, to affect specific biomarkers related to non-genotoxic cocarcinogenesis, was investigated. Several CYP-dependent reactions have been studied in liver, kidney and lung microsomes derived from male and female swiss albino mice treated

i.p. with single (200 and 400 mg/kg b.w.) or repeated (200 mg/kg b.w., 3 days) administrations of fungicide. No significant changes in both absolute and relative liver, kidney and lung weights were observed after metalaxyl treatment. Although a single dose did not significantly affect the considered monooxygenases, a clear example of selective CYP3A induction was recorded in different tissues after repeated treatment. A approximately-3 fold increase in CYP3A isozymes, probed by N-demethylation of aminopyrine, was observed in the liver (both sexes). Again, approximately-5 fold increase (averaged between male and female) in this oxidase activity was present in the kidney. No significant change of the selected biomarkers was observed in the lung. A weak, but significant reduction of CYP2B1 isoform in liver (male) was also recorded. Liver and kidney CYP3A overexpression was corroborated by means of Western immunoblotting analysis using rabbit polyclonal antibodies anti-CYP3A1/2. Northern blotting analysis with CYP3A cDNA biotinylated probe showed that, in the liver, the expression of this isozyme is regulated at the mRNA level. On the whole, these data seem to indicate the cotoxic and cocarcinogenic potential of this fungicide.

The toxicity of metalaxyl-M in dogs was investigated in a 13-week study of dietary administration by **Altman (1995).** Groups of four male and four female beagle dogs were given diets containing technical- grade metalaxyl-M (purity, 97 %) in acetone at a concentration of 0, 50, 125, 250 and 1250 p.p.m. for 13 weeks. The obtained data showed that, all dogs ate similar quantities of food, and the body-weight development was similar in treated and control groups and no treatment-related changes in haematological parameters. Also, increased alkaline phosphatase activity was seen in males and females at the highest concentration after 7 weeks (190 %

increase over controls for males and 220 % increase for females) and 13 weeks (210 % for males and 260 % for females) of treatment.

In a study where , groups of five male and five female fasted mice were given metalaxyl-M (purity, 97.1 %) orally at a dose of 500 or 1000 mg/kg b.w. for males and 200, 500 and 1000 mg/kg b.w. for females, and were observed for 14 days before sacrifice by **Winkler(1996c).** The results showed that at 1000 mg/kg b.w. male and female animals showed the following treatment-related symptoms: ventral or lateral recumbency, severe dyspnoea, reduced locomotor activity, conclusions, tremor and tonic spasms. Ataxia, hunched posture and piloerection were found in surviving males only, all of which recovered fully by day 7. At 500 mg/kg b.w. Ventral or lateral recumbency were seen in two males and two females, and dyspnoea, reduced locomotor activity, hunched posture and piloerection were seen in all animals, whereas tonic spasms, convulsions and ataxia were seen in individual animals. All males had recovered by day 4 and all surviving females by day 2. At 200 mg/kg bw, all females presented with hunched posture and piloerection but fully recovered within 1 day. At autopsy, no deviations from normal morphology were found. The body weights of surviving animals were not affected by the treatment

In a study conducted on chromosomal aberration, peripheral blood lymphocytes were obtained from two healthy donors, and an exogenous metabolic activation system was obtained from the livers of rats induced with phenobarbital and beta-naphthoflavone was carried out by **Hrelia et. Al., (1996).**The collected data showed that the frequency of aberrations reached statistical significance at doses of metalaxyl of 300 and 1000 µg/ml in the absence of metabolic activation; the maximum frequency observed at the highest dose was 3.4-fold greater than in the corresponding control. No cytotoxicity