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ACETAMIPRIDE INDUCED HISTOPATHOLOGICAL ALTERATION IN LUNG AND LIVER OF MALE MICE

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Abstract

Description of the subject: Oral toxicity of low doses of acetamiprid remains poorly explored at the tissue and molecular level.

Objective : Evaluation of the two low doses acetamiprid effects on lung and liver structure and on liver function.

Methods : Two batches of treated mice were fed daily with acetamiprid at rate of 1/20 LD50 and 1/10 LD50 respectively for 15 days. The witnesses were force-fed with distilled water.

Results : Liver enzymes assay showed non-significant changes in plasma concentration of AST and ALT. In addition, the histological examination showed haemorrhages, leukocyte infiltrates and thickening of the endothelial walls of capillaries and alveolar septa.

Conclusion : According to the results, we can conclude that even with low doses, acetamiprid is toxic at tissue level in male mice.

Keywords: Acetamiprid; Lung; Liver; Oral toxicity; Male mice.

ALTÉRATIONS HISTOPATHOLOGIQUES INDUITES PAR L'ACÉTAMIPRIDE AU NIVEAU DU POUMON ET DU FOIE CHEZ LES SOURIS MALES

Résumé

Description du sujet : La toxicité par voie orale de l'acétamipride à faibles doses reste mal explorée à l'échelle tissulaire et moléculaire.

Objectifs : Evaluation des effets à faibles doses de l'acétamipride sur la structure du poumon et du foie et sur la fonction hépatique.

Méthodes : Deux lots de souris traités ont été gavés quotidiennement avec l'acétamipride à raison de 1/20 DL50 et 1/10 DL50 respectivement pendant 15 jours. Les témoins ont été gavés avec de l'eau distillée.

Résultats : Les teneurs plasmatiques des enzymes hépatiques, ASAT et ALAT, restent stables. Cependant, l'examen des coupes histologiques a montré des hémorragies, d'infiltrat leucocytaire et l'épaississement des parois endothéliales des capillaires et des cloisons alvéolaires.

Conclusion : D'après les résultats obtenus, nous pouvons conclure que même à faible dose, l'acétamipride est toxique à l'échelle tissulaire chez les souris mâles.

Mots clés: Acétamipride, Poumons, Foie, Toxicité orale, Souris mâles.

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INTRODUCTION

Neonicotinoid are a group of insecticides that has quickly became one of the most insecticides used around the world [1]. They dominate the market with total global sale's part of 25% [2] and a turnover exceeding one billion dollars [3]. These insecticides act selectively on nicotinic acetylcholine receptors (nAChRs) [4][5]. The nAChRs activation results of intracellular Ca^{2+} increase while their over-activation generates a blockage causing a fatal paralysis [6][7]. Acetamiprid (ACE) is a first-generation neonicotinoid insecticide mainly used in fruit and vegetable farming [3] to control fungal infections of crops [8], and sucking insects [9][10]. Neonicotinoids can negatively affect non-target fauna by causing neurological dysfunctions in insects [11][12], affecting memory and learning ability in rats [13], inducing hepatotoxicity and pneumotoxicity in fish [14], decreasing haemoglobin level and leukocyte count [15] and unbalancing plasma concentration of liver enzymes in mice [16] and electrolytes in rats [17]. They can also affect reproductive activity by decreasing testosterone level and inducing abnormalities in mitochondria and endoplasmic reticulum of Leydig cells in mice [18] [19]. However, low dose toxicity's is poorly explored at tissue level. In this context, we are interested by searching the oral toxicity of ACE at rate of two low doses in adult male mice. The study targets biochemical parameters, changes in body weight and histopathological study of pulmonary and hepatic parenchyma. Results can help proving the toxic effects of ACE and guide research in order to understand the molecular effectors in toxicity at tissue level.

MATERIAL AND METHODS

1. Animals

Our study was conducted on 17 male mice of the *Naval Medical Research Institute* strain (N.M.R.I.). Male mice were chosen to avoid the influence of hormonal changes that exist in females. Animals were allowed to acclimatize for a period of one week prior to experiment, at room temperature and an alternating photoperiod of 12 hours of darkness followed by 12 hours of light. The mice were housed in polyethylene cages, which were lined with litter made of woodchips. The cages were cleaned and the litter was changed every two days until the end of the experiment. All animals received water and food ad-libitum. The food was composed of a balanced mixture of proteins, carbohydrates, lipids, vitamins and minerals.

2. Chemical

Acétamiprid, insecticide marketed by MOPISTOP, is presented in aluminium bag containing 50g of powder with 20% of purity. It was used as solutions prepared at a rate of 1/10 (19.8 mg/kg/d) and 1/20 (9.9 mg/kg/d) of the LD50. ACE suspension was administered by oral gavage with a volume of 10 ml/kg. The daily oral administration was continued for 15 days.

3. Experimental design

After adaptation to laboratory conditions, mice were marked and divided into three groups: the first group contained 6 control mice that were fed daily with distilled water at a rate of 10 ml / kg. The second group contained 5 mice treated daily by 1/20 of ACE LD50 dissolved in distilled water at a rate of 10ml / kg. The third group contained 6 mice treated daily by 1/10 of ACE LD50 dissolved in 10 ml / kg of distilled water. Period and doses were chosen to deepen previous studies [20]. Body weight gain was measured three times: before treatment, after one week and the day of sacrifices. The doses were adjusted weekly. At the end of the experiment on day 15^{th}, sacrifices were made in the morning, by rapid decapitation and without anaesthesia. Plasma samples were collected in heparinized tubes for the estimation of some biochemical parameters. The organs: lungs and liver were rapidly removed, fixed and then intended for histologic study.

4. Histopathology

Histologic study was conducted by using standard method [21] in which, tissue samples were collected in 10% neutral buffered formalin. After dehydration and inclusion, the blocks were cut using a microtome to have a paraffin tape of 3µm of thickness. The cuts were stained with haematoxylin and eosin and Masson Trichrom staining.

5. Plasma analysis

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) plasma level were measured by using kit on automate. The unit for the measurement of lever enzymes was expressed as IU/L.

6. Statistical analysis

The results are presented as mean ± standard deviation. The normality of samples distribution was verified by using a *Shapiro-Wilk* test via the Statistic Package for Social Science (S.P.S.S). Intra and intergroup comparisons were carried out by using a *student test* after applying a *Ficher test*. The differences were considered as statistically insignificant when $p < 0.05$.

RÉSULTATS

1. Animals behaviour

Under our experimental conditions, we noticed that treated mice showed a decrease in activity with a tendency to stay in the corners of the cages and diarrhoea. also, we noticed that mice treated with 1/10 LD50 of ACE had a probable difficulty of breathing characterized by an acceleration or a slowing down of respiratory rhythm. This became remarkable and make gavage difficult during the 2nd week.

2. Biochemical assay

According to results, the two groups of treated mice show a regression in plasma level of AST. In mice treated with 1/20 DL50, regression is of the order of 258.00 IU/L ± 4.24 vs. 338.33 IU/L ± 52.73 in controls (P = 0.13). Mice treated with 1/10 LD50 have an average rate of 261.00 IU/L ± 50.91 (P = 0.20). Compared to controls, mice treated with 1/20 LD50 show a sharp increase in plasma level of ALT (142.00 ± 73.54 vs. 75.00 ± 20.95) with P=0.21. However, mice treated with 1/10 LD 50 show a slight increase of 93.50 ± 27.58 with P=0.45. All deference between control and treated mice are statistically insignificant (Tableau 1).

Tableau 1: Effect of acetamiprid on plasma level of liver enzymes after exposure.

Parameters	Treated groups		Control
	1/20 LD50	1/10 LD50	
AST (UI/L)	258.00 ± 4.24	261.00 ± 50.91	338.33 ± 52.73
ALT (UI/L)	142.00 ± 73.54	93.50 ± 27.58	75.00 ± 20.95

3. Weight change

Our results show an increase in body weight of control and treated mice. In control mice, the increase is of the order of 2.07% during the 1st week (24.17g ± 4.71 vs. 24.67g ± 4.32) with a P=0.85 and 12.41% during the 2nd week (24.17g ± 4.71 vs. 27.17g ± 3.92) with a P=0.26. In mice treated with 1/20 LD50, the increase is of the order of 10.61% during the 1st week (26.40g ± 3.44 vs. 29.20g ± 3.70 with a P = 0.25) and 15.15% during the 2nd week (26.40g ± 3.44 vs. 30.40g ± 3.44 with a P = 0.10). Mice treated with 1/10 LD50 show an increase in weight of the order of 1.32% during the first week (25,17g ± 4.88 vs. 2.88 ± 25,50g with a P = 0.89) and 10.60% during the 2nd week (25.17g ± 4.88 vs. 27.83g ± 4.62 with a P = 0.35). Statistical analysis of weight differences during the 1st and 2nd weeks show a statistically insignificant changes ($p < 0.05$) in all mice groups (Fig. 1).

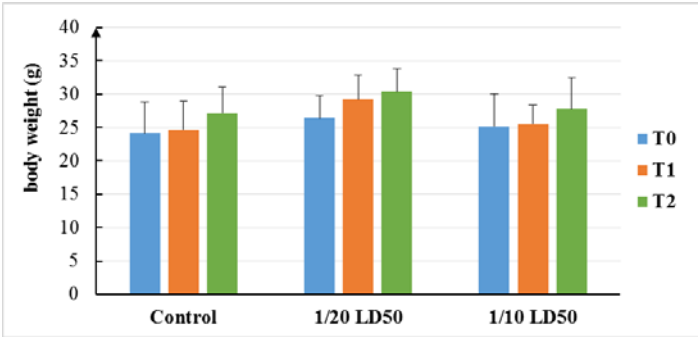

Figure 1 : Body weight evolution of control and mice treated with 1/10 and 1/20 LD50 of acetamiprid.

4. Histopathology

4.1. Lung parenchyma

Histological examination of lungs tissue sections in mice treated with 1/20 LD50 showed an haemorrhagic appearance with intra-alveolar congestions (fig. 2 part (c) and (d) and fig. 3 part (c)) and an invasion of the terminal and respiratory bronchial lumen by blood (fig. 2 part (c) and figure 3 part (c)). In addition, the intra-alveolar space contains red blood cells and cell debris probably caused by cell lyses (fig. 2 part (d)) , with thickness of the alveolar walls, causing a reduction of alveolar sacs and alveolus diameters (fig. 2 par (d) and fig. 3 part (d)).

The results show a similar appearance in both mice treated. However, the alterations are more important in mice treated with the highest dose (fig. 2 part (e) and figure 3 part (e)). We notice more bleeding with very frequent congestions (fig. 2 part (e) and (f), fig. 3 part (e) and (f)) and thickness of blood vessels endothelial wall (fig. 3 part (f)).

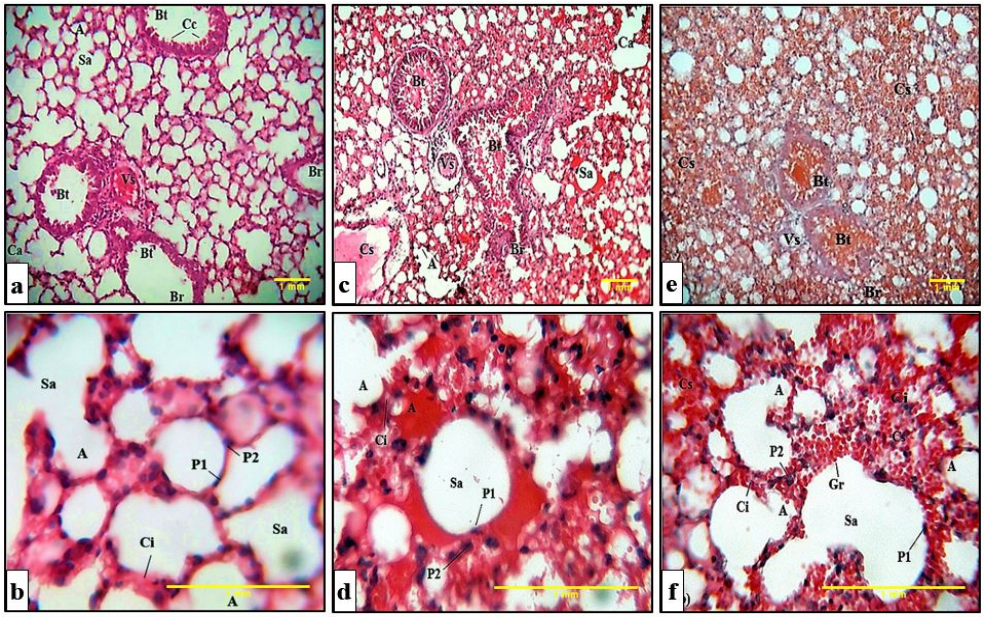

Figure 2 : Lung parenchyma structure in control (left), mice treated with 1/20 LD50 (middle) and mice treated with 1/10 LD50 of acetamiprid (right). H.E staining; magnification: x100 and x400

A : alveolus; Br : respiratory bronchiole; Bt : terminal bronchiole; C : blood capillary; Ca : alveolar duct; Cc : Clara cell; Ci : alveolar wall; Cs : blood congestion; Gr :red blood cells; Nl : lymph node; P1 : pneumocyte type I ; P2 : pneumocyte type II ; Sa : alveolar sac; Vs : blood vessel.

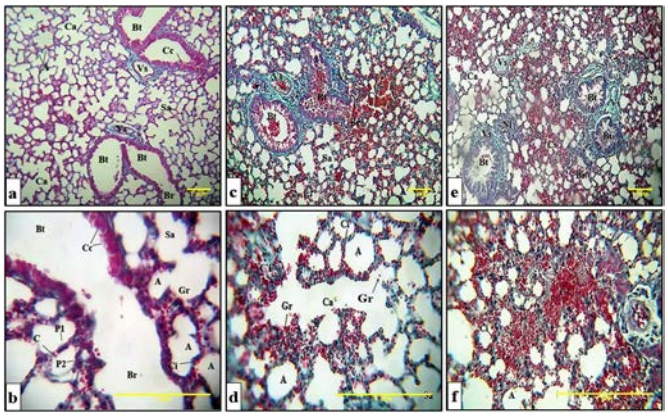

Figure 3 : Lung parenchyma structure in control (left), mice treated with 1/20 LD50 (middle) and mice treated with 1/10 LD50 of acetamiprid (right). Masson Trichrome staining; magnification: x100 and x400

A : alveolus; Br : respiratory bronchiole; Bt : terminal bronchiole; C : blood capillary; Ca : alveolar duct; Cc : Clara cell; Ci : alveolar wall; Cs : blood congestion; Gr :red blood cells; Nl : lymph node; P1 : pneumocyte type I ; P2 : pneumocyte type II ; Sa : alveolar sac; Vs : blood vessel.

4.2. *Liver parenchyma*

The observation of liver sections in treated mice shows structural alterations in liver parenchyma. The central veins present blood congestion (fig. 4 part (c) and (e)) and portal triads are dilated (fig. 4 part (e)). An abundant presence of leukocytes (fig. 4 part (d) and (f)) with cases of margination, leukocytes infiltration (fig. 4 part (d)) and endothelial membrane thickening are shown (fig. 4 part (c) and (d)).

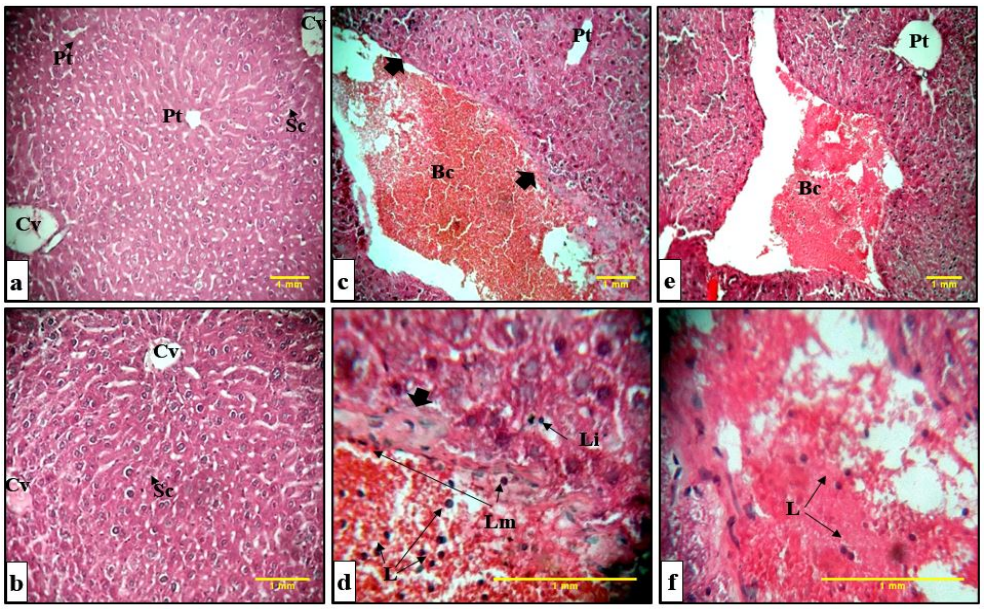

Figure 4: liver parenchyma structure in control (left), mice treated with 1/20 LD50 (middle) and mice treated with 1/10 LD50 of acetamiprid (right). H.E staining; magnification: x100, x100 with zoom and x400

Bc : blood congestion; Cv : central vein; L : leukocytes; Li : leukocytes infiltration; Lm : leukocytes margination; Pt : portal triad; Sc : sinusoidal capillary.

DISCUSSION

In our study witch investigate the short-term toxicity of ACE administered orally at rate of 1/20 LD50 (9.9 mg / kg / day) and 1/10 LD50 (18.9 mg / kg / day) in adult male mice of *N.M.R.I.* strain, several toxic effects were noted especially at structural level. The clinical signs like respiratory depression and diarrhoea were also observed by Mondal in female rats given orally acetamiprid [22].

All control and treated mice show a non-significant increase in body weight. These results corroborate with those found by Singh & *al.* who reported that male mice treated with ACE for 28 days showed an increase in body weight until the day 15th and then a decreased until the day 28th [20]. Zhang & *al.* found that the decrease in body weight was related to an increase in markers of oxidative stress, including activation of P38 mitogen-activated protein kinases, and decrease of the activity of antioxidant enzymes [18]. However, Jain & *al* reported no effect on body weight in male Wister rats treated with imidacloprid [23].

AST and ALT are liver enzymes, useful in measuring liver toxicity and particularly cell injury and necrosis [24]. Though enzymes are not known to have any function or role in the plasma, but their increase level in the blood indicate cellular damage and increased membrane permeability [25]. However, ALT is considered as more sensitive indicator of hepatocellular damage than AST [26]. Our results show a non-significant decrease in plasma AST concentration and also a non-significant increase in plasma ALT concentration in both treated groups. Our findings are correlated with findings of Zhang & *al.* which reported a significant increase in ALT plasma concentration of male mice in acetamiprid sub-acute toxicity [27], Wang & *al.* confirmed the significant increase of liver enzymes in a sub-acute exposer of male mice to ACE [28].

Structurally, microscopic observation of lung parenchyma in treated mice reveals frequent haemorrhages with intra-alveolar congestions,

cell lyses and thickening of alveolar and endothelial walls. Alteration degree seems to be dose dependent. The invasion of bronchial lumen and alveolar sacs by blood may probably explain the observed respiratory discomfort in mice treated with the highest dose. Similar results were reported in albino rats intoxicated with nitrosodiethylamine, in which severe congestion and moderate interstitial inflammation in lungs were observed [29]. Haemorrhages and thickened intra-alveolar septa with infiltration of mononuclear cells in lungs were reported after benzalkonium administration by Swiercz & *al*. [30]. According to Mondal & *al*., the lung lesions may be the result of cyclic reduction oxidation of ACE, which were brought via circulation with subsequent release of superoxide radicals, leading to lipid peroxidation in the cell of the alveolar wall [31]. In our study, congestion was found in blood vessels, bronchi and bronchioles which were filled with red blood cells. Because of infiltration, the intra-alveolar walls were thickened. According to Rivolta & *al*. The poisonous substances cause damage in vascular endothelium as well as to the alveolar epithelial cells. As a result of damage in vascular endothelium and increased vascular permeability excessive fluid and plasma proteins leaks out initially into the interstitium and subsequently into the alveoli [32].

In liver, structural alterations seem to be non-dose dependant. Optical microscopy shows the presence of congestion at the central veins and dilated portal triads. Similar results were reported in intoxication studies in which, an enlargement of sinusoids, degeneration of hepatic cords and hepatocytes and vacuole formation in hepatocyte in rat liver were observed [33]. According to Mondal & *al*. congestion and reduction in blood circulation may cause anoxia, which resulted in vacuolar degeneration in the hepatocytes. ACE could possibly be a hepatotoxin that alters mitochondrial and microsomal functions, increase free fatty acid synthesis, diminished triglyceride utilization and decrease fatty acid oxidation [31].

CONCLUSION

According to the results, we can conclude that low-dose ACE causes toxicity at tissue level in male mice. These results remain preliminary. It would be interesting to deepen the research in other organs and molecular scale.

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