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Acetamiprid Residues in Male Mice and its Effect on Liver Function

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Abstract: This study was performed to identify whether acetamiprid could residue in liver and kidney and its effect on liver function. Fifty adult Kunming male mice weighting 25-30 g were randomly grouped and ten mice in each group (five groups: control, blank, acetamiprid alone, acetamiprid+vitamin E, vitamin E alone); all groups were administered for 35 days by intragastric gavage. The residue in the tissues had been detected by the Liquid Chromatography coupled with tandem Mass Spectrometry (LC-MS/MS) and the serum biochemistry parameters were analyzed by an automatic chemistry analyzer. Acetamiprid induced a wide range of nervous signs in mice; the concentration of acetamiprid in liver was higher than that of kidney $p<0.05$. Furthermore, acetamiprid increased the activity of Alanine Transaminase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP) ($p<0.05$ for all) but reduced the activity of Total Protein (TP) and Albumin (ALB) ($p<0.05$, for both). However, Vitamin E ameliorated the effects of acetamiprid on these parameters, compared to the acetamiprid only group ($p<0.05$). The results indicated that the liver was the main organ of acetamiprid residues and acetamiprid could affect liver function but VE could reduce acetamiprid-induced impairment.

Key words: Acetamiprid, mice, liver, residues, liver function, impairment

INTRODUCTION

Acetamiprid, also called mospilan is synthetic chlorinated nicotimine insecticide with strong contact and stomach toxicity (Brunner *et al*., 2005). Due to the advantages of long-term retention, high-efficiency and low-poisoning of acetamiprid (El-Hassani *et al*., 2008), acetamiprid is a replacement for other insecticides and has been widely used in agricultural pest control including killing all kinds of aphid in crop vegetables and fruit protections (Brunner *et al*., 2005; Carletto *et al*., 2010; Elbert *et al*., 2008; He *et al*., 2007; Sanyal *et al*., 2008) and also used to protect the domestic areas against a wide range of animal parasites, thereby its yearly output is about 1,000 ton. Initially, It is widely accepted that acetamiprid is previously a safe pesticide in the vicinity of animals and human beings as it has no role in neurotoxicity, mutagenesis and interference endocrine. However, recently, inhaled acetamiprid resulted in people suffering from headaches, dizziness, nausea, vomiting and other symptoms (Todani *et al*., 2008) and genetic toxicity of mutagenesis in human peripheral blood lymphocytes (Kocaman and Topaktas, 2007, 2010) which inferred that acetamiprid possibly has the acute or chronic toxic effects. In order to study the deleterious effect of acetamiprid further, the study was conducted in male mice (25-30 g) to detect the concentration of acetamiprid in tissues and to detect the effect of acetamiprid residues on liver function.

MATERIALS AND METHODS

Acetamiprid (E)-N1-[(6-chloro-3-pyridyl)methyl]-N2-cyano-N1-methylacetamidine, C10H11ClN4) (>97% pure) was purchased from Shanghai Yongyuan Chemical and biochemical Co., Ltd. (Shanghai, China). Vitamin E naturally was made by Yang Shengtang (Hainan, China). Peanut oil was used to dissolve acetamiprid and vitamin E into 0.1 mL peanut oil. Kunming male mice weighing 25-30 g were provided by the Chongqing Institute of Traditional Chinese Medicine.

Experimental design: According to the preliminary test (Zhang *et al*., 2011), the concentration of acetamiprid and vitamin E was 30 and 20 mg kg^{-1} wt.day, respectively. Fifty adult Kunming male mice (25-30 g) were divided into five groups (n = 10 per group) including control, blank (0.1 mL peanut oil), acetamiprid alone (30 mg per kg of mouse per day), acetamiprid (30 mg per kg of mouse per day)+ vitamin E (20 mg per kg of mouse per day) and vitamin E alone (20 mg per kg of mouse per day). All mice were housed in rooms with a controlled temperature

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22±2°C, with a 12 h L/12 h D photoperiod and relative humidity (50-60%) with *ad libitum* access to water and food. All groups were treated for 35 days by gavage with observing their clinical symptoms. Then on day 36th, all mice were put to death by severing the neck vessels after anesthesia with halothane. All animal experiments were performed in compliance with institutional guidelines and approved by Committee on Research Animal Care at Southwest University, China.

Hematological biochemical analysis: Blood samples were taken from the eye sockets of mice prior to being sacrificed then after centrifugation at 5000 rpm min^{-1} for 4 min and the serum samples were stored at 4°C for hematological biochemical analysis. The serum biochemistry parameters were analyzed by an automatic chemistry analyzer (the Olympus Auto400, Japan) including Alanine Transaminase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP), Total Protein (TP) and Albumin (ALB).

Acetamiprid total residue in liver and kidney: Liver and kidney tissues (0.2 g), respectively were ultrasonically extracted for 1 h with anhydrous sodium carbonate (1 g) and diamine methane (5 mL) using a homogenizer and centrifuged at the speed (10,000 rpm) for 5 min. Then, the samples were dried after pouring out the diamine methane and added the acetonitrile (20%). The control sample was treated by the above means. While different concentrations of standard acetamiprid (2, 5, 10, 50 and 100 ng mL^{-1}) were used and detected in duplicate. Each sample was carried out in a total volume of 50 μL at one time. High Performance Liquid Chromatography (HPLC) was equipped with a C18 column, methanol for the mobile phase A, 1% acetic acid water for the mobile phase B and flow rate (0.3 mL min^{-1}). Mass Spectrometry (MS) were performed as follows: the method of acquisition was selected reaction monitoring (SEM), the parent ion was 223.054, the qualitative ion was 73.054 m z^{-1} and the quantitative ion was 126.047 m z^{-1}. The standard curve was drew with the mass concentration (ng mL^{-1}) for horizontal coordinates and the peak area for the vertical coordinates then the regression equation was to be calculated. Tissue residues were calculated by the following equation:

$$\text{Residues (ng g}^{-1}) = [(A\times C\times V)/(As\times m)] \times n$$

Where:

A = Sample size
C = Concentration of standard preparation
V = Volume size
As = Standard peak area
m = Sample quality
n = Dilution factor

Statistical analysis: Each group had 10 mice in the number and the experiments were performed for 2-3 times. All data are reported as mean±SE. Statistical analysis was performed by using SPSS (Ver. 16.0, SPSS Inc., Chicago, IL, USA). Data was analyzed by one-way ANOVA and treatment difference was determined by the Fisher's Least Significant Difference (LSD) Method. A probability of $p<0.05$ suggested that the difference was statistically significant.

RESULTS AND DISCUSSION

Effect of acetamiprid on clinical signs: During the course of intragastric gavage, the mice were normal in control, blank (peanut oil) and vitamin E. However, all animals in administrated acetamiprid firstly showed excitement then tended to be a depression, sleepiness and tiredness as well as anorexia, polydipsia and hypopnea syndrome. In some animals, there were some neurologic symptoms arisen such as motor incoordination, impaired mental state, coma, occasional convulsions, tremors. However, vitamin E could reduce acetamiprid-induced nervous signs.

Preparation of standard curve of acetamiprid: Different concentrations of standard acetamiprid (2, 5, 10, 50 and 100 ng mL^{-1}) were used and detected by LC-MS/MS. The results were the following equation:

$$Y = 6330.77+51964.6*X$$

with linear correlation coefficients higher than 0.9998 (R^2). It represented that the result coincided well with the experimental data of the samples, therefore it totally met the requirements of detection of acetamiprid (Fig. 1).

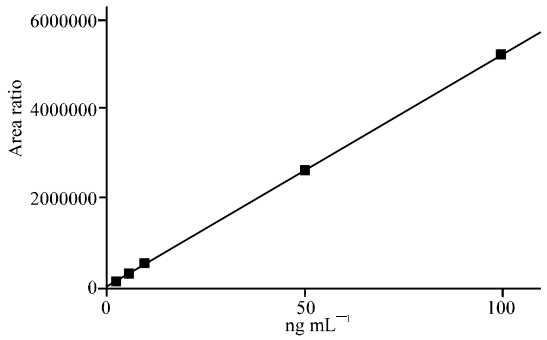

Fig. 1: Standard curve during the course of detecting acetamiprid

Acetamiprid residue in the livers and kidneys: The acetamiprid residues in the livers and kidneys were quite different. The amount of acetamiprid residue was 453.93±38.907 ng g^{-1} in the livers of acetamiprid group while it was 183.30±23.617 ng g^{-1} in the livers of acetamiprid and vitamin E group. The acetamiprid residue significantly decreased 59.61% ($p<0.05$) by adding vitamin E. Compared to acetamiprid alone and acetamiprid and vitamin E groups, the acetamiprid residue was not detected in the liver of the control, peanut oil and vitamin E groups.

Additionally, the amount of acetamiprid residue in the kidney of acetamiprid group and acetamiprid and vitamin E was 136.80±30.829 and 121.40±43.840 ng g^{-1}, respectively. Adding vitamin E, the acetamiprid residue also dropped by 11.26% ($p<0.05$) (Fig. 2).

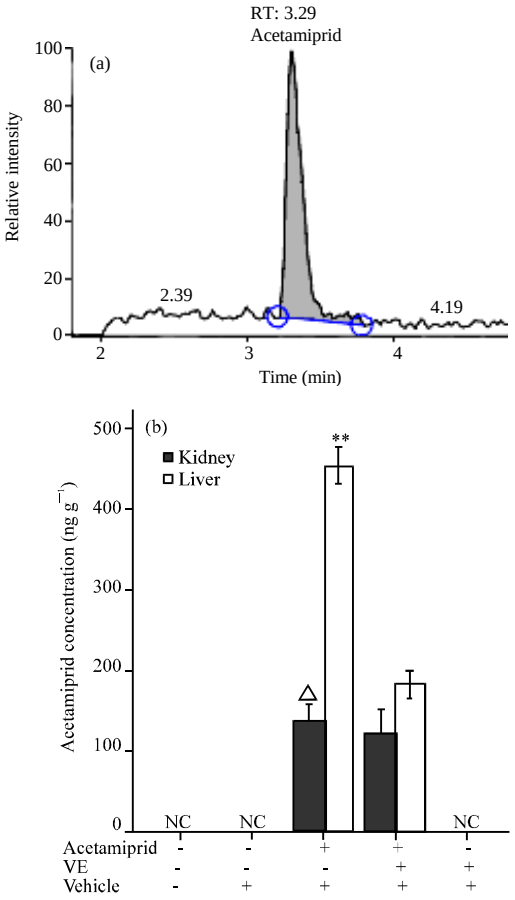

Fig. 2: Concentrations of acetamiprid in tissues; a) Representative LC-MS/MS profiles of livers from mice given a single oral dose of acetamiprid; b) Concentrations of acetamiprid in tissues. *ΔRepresent differences among different treatments of liver or kidney, respectively

Effect of acetamiprid on serum biochemistry parameters of liver: Compared to the control, the acetamiprid increased the activity of Alanine Transaminase (ALT), Aspartate aminotransferase (AST) and Alkaline Phosphatase (ALP) by 22.1, 20.2 and 64.51%, respectively ($p<0.05$, for all). Whilst compared to blank group, the activity of ALT, AST and ALP increased by 19.3, 23.1 and 39.13% in the acetamiprid only group ($p<0.05$, for all). In contrast, the activity of ALT, AST and ALP was declined by 5.7, 6.2 and 31.25% ($p<0.05$, for all) in the acetamiprid and vitamin E group in comparison to the acetamiprid only group. The only vitamin E had no effect on the activity of ALT, AST and ALP ($p>0.05$, for all) (Table 1).

Effect of acetamiprid on hepatic synthetic function: Compared to the control group, acetamiprid declined the activity of Total Protein (TP) and Albumin (ALB) by 7.1 and 7.73%, respectively ($p>0.05$, for all). Vitamin E prevented the decrease of Protein (TP) and Albumin (ALB) with a growth by 1.7 and 4.65% ($p>0.05$) (Table 2). In the study, researchers found that all mice administrated by acetamiprid had mainly neurological symptoms, consistent with insect nicotinic Acetylcholine Receptors (AChR) acting on central nervous system (Honda *et al*., 2006; Tan *et al*., 2007). Therefore, the mouse model was well-build to detect the acetamiprid as well.

Acetamiprid has a character of fast-metabolism so that acetamiprid could be detected in tea, soil, water and serum by the combination of High Performance Liquid Chromatography (HPLC) and Solid Phase Extraction (SPE, carbon black) Methods with the minimum detection limit (mg kg^{-1}) (Di Muccio *et al*., 2006; Chen *et al*., 2007). In leek, samples were treated with microwave treatment and convenient reverse solid phase dispersion clean-up and

Table 1: Effect of acetamiprid on liver's serum biochemistry parameters in mice (n = 10/group)

Groups	ALT (U L^{-1})	AST (U L^{-1})	ALP (U L^{-1})
Control	4.30±1.05^{b}	48.50±2.69^{bc}	14.88±2.40^{b}
Blank	4.40±1.13^{b}	47.35±2.47^{c}	14.90±2.10^{b}
Acetamiprid	5.25±1.10^{a}	58.30±2.97^{a}	24.48±5.58^{a}
Acetamiprid+vitamin E	4.95±1.18^{ab}	54.67±2.04^{ab}	16.83±3.64^{b}
Vitamin E	4.35±1.20^{b}	49.35±2.76^{bc}	14.80±2.27^{b}

Within the same column, different letter represents significant difference ($p<0.05$); Alanine Transaminase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP)

Table 2: Effect of acetamiprid on hepatic synthetic function in mice (n = 10/group)

Groups	TP (g L^{-1})	ALB (g L^{-1})
Control	13.840±0.8019^{a}	7.7667±0.45092^{a}
Blank	12.960±0.5595^{a}	7.7333±0.23094^{a}
Acetamiprid	12.860±0.8933^{a}	7.1667±0.15275^{a}
Acetamiprid+vitamin E	13.080±0.4970^{a}	7.5000±0.18708^{a}
Vitamin E	13.333±1.4980^{a}	7.8333±0.19304^{a}

Within the same column, different letter represents significant difference ($p<0.05$); Total Protein (TP), Albumin (ALB)

were in samples of surface waters by Reverse Phase Ultra Performance Liquid Chromatography analysis with quadrupole mass detection (RP-UPLC-MS/MS). Then acetamiprid could be determinated by reversed phase high performance liquid chromatography. Acetamiprid in drinking water, bee honey, fruits and vegetables could be detected by a high performance liquid chromatography tandem mass spectrometric method in which the weight of samples was >2 g for tea, 10 g for leek. However, the weight of tissues for mice was less about 0.2 g which was not suitable to be detected by above methods. In this study, the method of the Liquid Chromatography coupled with tandem Mass Spectrometry (LC-MS/MS) was established to detect acetamiprid in fewer amounts of samples and organic solvent. Besides, the results showed that this method was reproducible, high recovery and has the Lower Detection limit (LODs) (ng g^{-1}) than other methods.

Acetamiprid was metabolized into eight metabolites in which the major ones were 6-choronicotinic acid (Drozdzynski, 2008). Acetamiprid was absorbed in the human or mammals intestines *in vivo* which was combined with glycine to get the mixture called guanidine then to enter blood circulation (Brunet *et al*., 2005, 2008; Tomizawa and Casida, 2005). Even though, the low toxicity of acetamiprid reflected its rapid metabolism, the results inferred that acetamiprid tended to persist in tissues and the acetamiprid residue mainly in the liver was higher than that in the kidney. However, vitamin E lowered the acetamiprid residue in the liver and kidney as vitamin E causes the inhibition of phospholipase A2 activity and enhance the activity of enzymes resulting in the metabolism in tissues such as the liver and kidney (Ford and Casida, 2006a, b).

To study whether acetamiprid affected liver function, researchers detected serum biochemistry parameters. The concentrations of AST and ALT were significantly increased which was similar to the effect of the dystrophic damage (Patnaik *et al*., 2011; Vorobeva and Spiridonov, 2010). This gave further evidence that the detrimental effects of acetamiprid were mediated by the free radical generated in metabolic pathway (Zhang *et al*., 2011) because the free radical caused directly the toxic action to attack cell membranes and damage cell structure and function. On the other hand, this was possibly due to the fact that the accumulating of neurotransmitter acetylcholine by acetamiprid caused the increase of serum ALT and AST. Also, the increase of ALP was significant which show acetamiprid could induce intrahepatic cholestasis. However, ALB and TP showed a downward trend slightly. The main reason was that liver had sufficient compensatory capacity.

Moreover, researchers found that vitamin E could lower the acetamiprid residues and decrease the activity of ALT, AST and ALP while vitamin E could increase the activity of TP and ALB. The results suggest vitamin E ameliorate the effects of acetamiprid on liver function which is consistent with that previous research in which vitamin E known as antioxidants had a pivotal role in maintaining the bio-membrane and protecting the permeability of cell membrane ,delayed oxidation saturated fatty acid and lowered the proliferation of the free radicals generated (Zhang *et al*., 2011).

CONCLUSION

The study shows that acetamiprid was absorbed into the body of mice by gastrointestinal tract to mainly reside higher in liver than in kidney resulting in damage of liver function. However, the amelioration of vitamin E could lower the damage of liver function.

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