Original Article

Effects of sub-chronic exposure to deltamethrin on shuttle-box avoidance and contents of amino acid neurotransmitters in hippocampus of mice

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ABSTRACT — The purpose of this study was to evaluate the effects of sub-chronic exposure to deltamethrin in lower doses on the acquisition of a two-way avoidance task and the levels of amino acid neurotransmitters in hippocampus of mice measured using shuttle-box and LC-MS/MS system. Deltamethrin was given to mice respectively at doses of 0.46, 0.92, 1.80 mg/kg BW daily for 60 days by gavage. Deltamethrin was found to decrease the number of avoidance responses, increase response latency, and increase glutamate levels in the 0.92 and 1.80 mg/kg BW-dose group. As revealed by electron microscopy, in 0.92 and 1.8 mg/kg-dose group mice, morphology of cells were changed and degeneration and necrosis morphological characteristics obviously were appeared. Collectively, results from this study suggest that deltamethrin may have cumulative effects in mice following repeated dosing of deltamethrin using moderately effective doses, beside Na^+ and Ca^{2+} channels as well as Na^+ and Ca^{2+}-dependent glutamate release, may be involved with neurotoxic action of deltamethrin.

Key words: Deltamethrin, Active avoidance, Neurotransmitter, Neurotoxicity

INTRODUCTION

Pyrethroids are insecticides that are extensively used in agriculture, public health and veterinary applications on both farm animals and pets for the prevention and control of ectoparasites (Casida *et al*., 1983; Llewellyn *et al*., 1996; Tayebati *et al*., 2009). They are commonly divided into two classes, namely type I (e.g. allethrin, pyrethrin, permethrin) and Type II derivatives (e.g. deltamethrin, fenvalerate, cyhalothrin) (Miyamoto *et al*.,1995; Gassner *et al*., 1997). Type I pyrethroids have no cyano-group at the carboxyl α position (α-carboxyl) whereas type II pyrethroids contain this cyano-group (Verschoyle and Aldridge, 1980). The type I compounds cause T syndrome that is characterized by hyperexcitation, incoordination, and whole body tremor. The type II, cyano compounds, cause CS syndrome that is characterized by profuse salivation, coarse tremor progressing to choreoathetosis and paralysis (Narahashi, 1985; Vijverberg and Van den Bercken, 1990). In spite of such differences in the symptoms of poisoning, the primary target site for type I and type II pyrethroids is sodium channel (Bloomquist, 1996; Narahashi, 1992, 1996). Electrophysiological studies found that neuroactive pyrethroids interacted with sodium channels in excitable tissue and prolonged sodium current evoked by membrane depolarization (Narahashi, 1992, 1996). Many target sites other than the sodium channel maybe relevant to pyrethroid action (Soderlund *et al*., 2002). For example, voltage-gated calcium channels and chloride channels are also involved with acute neurotoxic effects of some pyrethroids (Soderlund *et al*., 2002; Burr and Ray, 2004; Forshaw *et al*., 1993; Shafer and Meyer, 2004). A few studies found that deltamethrin enhanced Ca^{2+}-dependent neurotransmitter release in the isolated presynaptic nerve terminal (Symington *et al*., 2007) and prolonged Ca^{2+}-mediated action potential and depolarized the membrane of paramecium, which lack voltage-gated sodium channels (Symington *et al*., 1999).

Deltamethrin, α-cyano-group containing type II synthetic pyrethroid, is a potent neurotoxin for both insects and mammals. It is reported that deltamethrin has a high affinity for the central and peripheral nervous system (Anadon *et al*., 1996; Rickard and Brodie, 1985). Central nervous system is the major neurotoxicity target of

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deltamethrin. The acute effects of deltamethrin on learning and memory have been examined in a few of studies (Bloom *et al*., 1983; Husain *et al*., 1996; MacPhail *et al*., 1981; Stein *et al*., 1987). Rats orally exposured to a commercial formulation of deltamethrin at the concentration of 7 mg/kg/day (15 days) could cause a 30% decrease in a re-learning index for an avoidance response based on visual discrimination in a 24 hr Y-maze after the last treatment with deltamethrin (Husain *et al*., 1996). Other data indicated that 20 min after treatment with deltamethrin, operant behavior response rates were decreased (Stein *et al*., 1987). Few studies, however, have addressed the problem of whether sub-chronic exposure to deltamethrin at a lower dose can affect the function of learning and memory in mammals.

A few studies have been conducted on the action of deltamethrin on the central neurotransmitter system (Aldridge *et al*., 1978; Eells and Dubocovich, 1988; Hossain *et al*., 2004, 2006). For example, deltamethrin treatment resulted in a dose-dependently increase in the extracellular acetylcholine release in the hippocampus of freely moving rat brain (Hossain *et al*., 2004). In addition, rats were treated i.p. with deltamethrin (10, 20, 60 mg/kg) increased the extracellular dopamine levels in the striatum (Hossain *et al*., 2006).

Amino acid neurotransmitters are major neurotransmitters in the brain which primarily include glutamate, gamma amino butyric acid (GABA) and aspartic acid (Asp), etc. Hossain *et al.* (2008) reported that intraperitoneal injection of deltamethrin (10, 20, 60 mg/kg) could dose-dependently increased extracellular glutamate level to about 190-275% of baseline while decreasing the level of GABA in the hippocampus of rats. Niu *et al.* (1999) found that intraperitoneal injection of deltamethrin at the dose of 20 mg/kg increased the contents of glutamate and Asp in the cerebral cortex, hippocampus, cerebellum of rats. Neuropharmacological study of deltamethrin showed that deltamethrin administration (orally, 150 mg/kg, 1 day) decreased the GABA levels significantly in the cerebellum of rats (Manna *et al*., 2006). The literature describing the effects of sub-chronic deltamethrin administration on amino acid neurotransmitters events are spare.

Most of the toxicological and physiological investigations concerning the mechanical effects of deltamethrin had been performed on rat using injection. The main sources of general population exposure to deltamethrin are contaminated food and water, and deltamethrin is readily absorbed by the oral route (Barlow *et al*., 2001). So the present study is therefore designed to assess the orally sub-chronic effect of deltamethrin on the acquisition of a complex learning task and cellular contents of amino acid neurotransmitters in mice. In our study, deltamethrin is administered orally in order to simulate main way of human exposure to deltamethrin and the effects of sub-chronic deltamethrin administration on hippocampal microstructure in mice are investigated with electron microscopy.

MATERIALS AND METHODS

Chemicals and reagents

All reagents used were analytical grade. Deltamethrin was donated by Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA, Beijing, China). GABA (purity 99.0%) was from Acros Organics (Morris Plains, NJ, USA) and glutamate (purity 99.0%) was from National Institute for the Control of Pharmaceutical and Biological Products (NICPBP, Beijing, China). HPLC grade methanol and acetonitrile were supplied by Fisher Scientific (Waltham, MA, USA). Ultrapure water was filtered through a Milli-Q system Millipore (Bedford, MA, USA). Formic acid (FA) and ammonium acetate were purchased from Dikma (Lake Forest, CA, USA).

Animals

A total of 60 female Kunming mice, with body weight of 20-23 g at arrival, were obtained from the animal facilities of NICPBP. The animals were housed five per cage in a temperature (22 ± 2°C) and humidity (55-60%) controlled room with 12:12 hr dark-light cycle (lights on at 08:00 am). Free access to food and water was allowed throughout the experiment. The animals were kept in these conditions at least 7 days prior to initiation of experiment.

Experimental groups and treatments

At the beginning of the experiment, the 60 mice were randomly divided into four experimental groups: control, low-dose, middle-dose and high-dose (n = 15 each group). The mice were selected in such a way that the average weights of these groups were similar. Deltamethrin was dissolved in corn oil and administered to mice by gavage once daily for 60 days. Based on the LD_{50} of 14.7 mg/kg bw calculated using the Horn's method. According to the document about the sub-chronic effect of deltamethrin in rats, deltamethrin caused impaired mobility, gait, postural changes and mortality at 800 ppm dietary level (Nemec, 1998). In order to avoid death and obvious poisoning syndromes in mice in the experiment, the 1/8, 1/16, 1/32 of the oral LD_{50} were selected as the doses based on the results of preliminary experiment. Treated mice were given deltamethrin at doses of 0.46, 0.92, 1.80 mg/kg BW daily for a period of 60 days by gavage. Deltamethrin was

dissolved in corn oil and gavage volume was 5 mL/kg bw. The corn oil only was administered as control.

Active avoidance responses

Starting from one day after the 60-day DM treatment, the animals were given one session of two-way active avoidance conditioning daily for four consecutive days. Immediately prior to the first session, the mice were allowed to freely ambulate in the shuttle-box for 3 min in order to become familiar with the training box. A shuttle-box consists of two chambers of equal size divided by a stainless steel partition with a gate providing access to the adjacent compartment (28 × 15.5 × 16 cm) (SLY-SRC, Sun Instruments Co. Ltd., Beijing, China) and was placed in a sound-attenuating room to avoid noise disturbance to the animals during tests. The gate in the partition was kept open. The warning stimulus, which functioned as a conditioning stimulus (CS), consisted of the cue light and sound presented simultaneously for 10 sec. After the CS, the aversive stimulus unconditioning stimulus (US) was applied. The US was an electric shock (0.5 mA for 10 sec) applied to the grid floor. If the animal avoided the US by running into the other chamber within 10 sec after the onset of the CS, the microprocessor recorder unit of the shuttle box recorded an avoidance response, the CS and US were terminated, and the inter-trial interval (ITI) was initiated. The ITI averaged 20 sec and ranged from 15 to 25 sec. If the mouse did not cross into the non-illuminated chamber during the CS and US, the US was stopped and the ITI began. During the ITI, the mouse could move freely between the two chambers. Each animal was given 30 trials daily for 4 days. The parameters recorded were: number of avoidance (the mouse crossing the barrier during CS) and response latency (latency to avoid or escape).

The measures recorded were: number of avoidance (the mouse crossing the barrier during CS) and response latency (latency to avoid or escape) (Matthew *et al*., 2003).

Chemical analysis

Sample preparation

A certain amount of cortex, hippocampus and cerebellum samples of mice were accurately weighed and transferred into a 2.5 mL centrifuge tube. Tissues were homogenized in 10 times volume (v/w) of 0.1 mol/L hydrochloric acid. The homogenates (0.05 mL) were removed and diluted 800 times in two steps with ultrapure water, and then centrifuged at 10,000 rpm for 10 min. Supernatants (10 μL) were used for analysis.

MS/MS optimization

Glutamate, Asp and GABA gave prorogated parent ion $[M+H]^+$ at *m/z* 148.4, *m/z* 134.3 and *m/z* 104.1, respectively. The fragment ions of the most significant intensity were observed at *m/z* 84.2 for glutamate, *m/z* 88.1 for Asp and *m/z* 84.1 for GABA, respectively. The mass transitions thus chosen accordingly for quantitation were *m/z* 148.4→84.2 for glutamate, *m/z* 134.3→88.1 for Asp and *m/z* 104.1→84.1 for GABA. The high-flow gas flow parameters were optimized by making successive flow injections while introducing mobile phase into the ionization source, and the instrument setting was adjusted to maximize the response for the analytes.

LC-MS/MS analysis

Samples were applied on the LC-MS/MS system consisting of an HPLC system (Perkin Elmer Series 200), a micro-pump (Perkin Elmer, Waltham, MA, USA), an API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster, CA, USA) equipped with electrospray ionization (ESI) source. Chromatographic separations were carried out on an Agilent Zorbax SB-C18, and column was 75 mm × 4.6 mm, 3.5 μm (Agilent Technologies, Little Falls, DE, USA). Column temperature was maintained at 25°C. The injected sample volume was 10 μL. The flow rate was 300 μL/min. Mobile phases A and B were water with 0.1% formic acid in water, 10 mM/L ammonium acetate solution and acetonitrile respectively. A gradient elution was made using gradient of LC as follows: the percentage of solvent B was linearly increased from 10% at time 0 to 100% at 12 min, remained at 100% for 1.5 min, and linearly decreased to 10% solvent B at 13.5 min. The column was then equilibrated at 10% for 4.5 min prior to the next injection. Mass spectrometric analysis was performed in positive ion mode and set-up in multiple reaction-monitoring (MRM) mode. Nitrogen serves as nebulizer (GS1), auxiliary (GS2), curtain (CRU) and collision gas (CAD) in the API 4000. Date processing was performed on ANALYST 1.4.2 version software.

Quality control

The precision and accuracy of the method was evaluated by analyzing Quality control (QC) samples, prepared separately from calibration standards at a glutamate concentration of 30, 175, 375 ng/mL, and 75, 437.5, 937.5 ng/mL for GABA, respectively. Six replicates of QC samples at each concentration were measured within one day or for consecutive five days. Recovery rates were calculated by comparing the peak areas of glutamate and GABA standards with those obtained in mobile phase solution with QC samples containing equal amounts of

amino acids. The intra-and inter day precision and accuracy for glutamate and GABA were determined by analyzing the concentrations of QC samples with six replicates on the same day and on separate days. Results indicated that the values were within the acceptable range and the method developed was sufficiently accurate and precise. The limit of detection (LOD) was 10 ng/mL for glutamate and 25 ng/mL for GABA, respectively.

Transmission electron microscopy analysis

At the end of the active avoidance test, six mice were selected randomly. The hippocampus was removed rapidly on the ice when the mice were anesthetized. Hippocampal tissues were cut into pieces (1 mm × 1 mm × 1 mm) and fixed in 2% paraformaldehyde containing 2.5% glutaraldehyde at 4°C for 2 hr. Hippocampal tissues were washed three times with dimethyl sodium arsenate buffer and fixed inosmium tetroxide at 4°C for 2 hr. Hippocampal tissues were washed three times with double distilled water. The samples were embedded in Epson812 and stained with azure-methylene blue, cut on an ELB ultramicrotome and finally examined on the HITACHIH-7650 transmission electron microscope.

Statistical analysis

Data were presented as mean ± S.E.M. A mixed repeated measurement ANOVA was used for the active avoidance task analysis with day of training considered as a within-factor, and dose (deltamethrin) as major factor. Contents of glutamate, ASP and GABA from the hippocampus were analyzed using one-way ANOVA with Tukey HSD tests for Post-hoc test. In constructing ANOVA model, interaction between factors was considered when interaction was significant, else interaction was not considered. Statistical significance was defined as $P < 0.05$.

RESULTS

General appearance and body weight

All mice were visually inspected daily and weighted twice a week. Mice in each experimental group were generally in good condition. No animals died during the experiment. No signs of deltamethrin poisoning or gross behavioral abnormalities were observed throughout the experimental period except for the 0.92 and 1.80 mg/kg-dose group mice showing some excitability. The body weight of mice in each experimental group (including the control group) had no statistically significant differences [$F = 1.249$, $P > 0.05$]. (Fig.1)

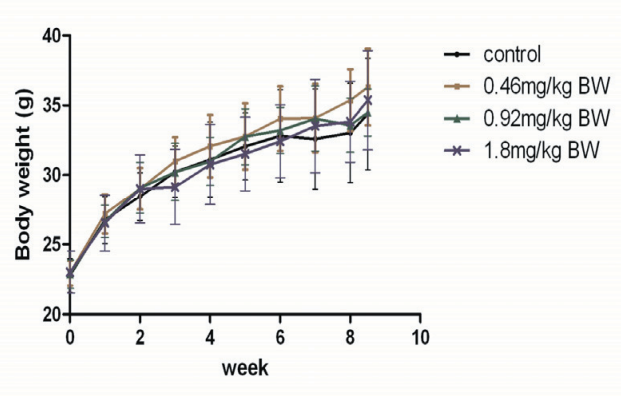

Fig. 1. The mean weekly body weights of mice during 60-day experiment. (mean ± S.E.M., n = 15).

Effect of deltamethrin exposure on the acquisition of an active avoidance task

Number of avoidance response

The avoidance data were analyzed with a 4 × 4 (Dose × Day) repeated measurement ANOVA. The 4 levels of concentration were 0, 0.46, 0.92, 1.80 mg/kg BW, and the days 1-4 served as the repeated measure. For number of avoidance, there was a significant main effect of day [$F = 33.087$, $P < 0.001$], and a significant main effect of dose [$F = 13.257$, $P < 0.001$]. However, these two main effects were of little interest on the basis of the significant two-way interaction (Dose × Day) obtained for the number of avoidance response [$F = 6.948$, $P < 0.001$]. The interaction term showed a significant difference in the slopes of the avoidance curves across groups, with 0.92 and 1.8 mg/kg-dose group achieving lower levels of avoidance response than solvent group and 0.46 mg/kg-dose group (Fig. 2A). Post-hoc tests indicated that both 0.92 and 1.8 mg/kg-dose group had less avoidance responses than the solvent group ($P < 0.01$), and 1.8 mg/kg-dose group had less avoidance responses than 0.46 mg/kg-dose group ($P < 0.01$).

Response latency

A 4 × 4 (Dose × Day) repeated measurement ANOVA revealed that the main effect of day was significant [$F = 79.273$, $P < 0.001$], and a significant main effect of dose [$F = 50.523$, $P < 0.001$]. A two-way interaction (Dose × Day) for response latency was significant [$F = 8.516$, $P < 0.001$]. Post-hoc tests showed that 1.80 mg/kg -dose group mice displayed longer response latency than control, 0.46 mg/kg-dose and 0.92 mg/kg-dose group mice ($P < 0.05$). 0.46 and 0.92 mg/kg-dose group mice had extended response latency than the control ($P < 0.05$). (Fig. 2B)

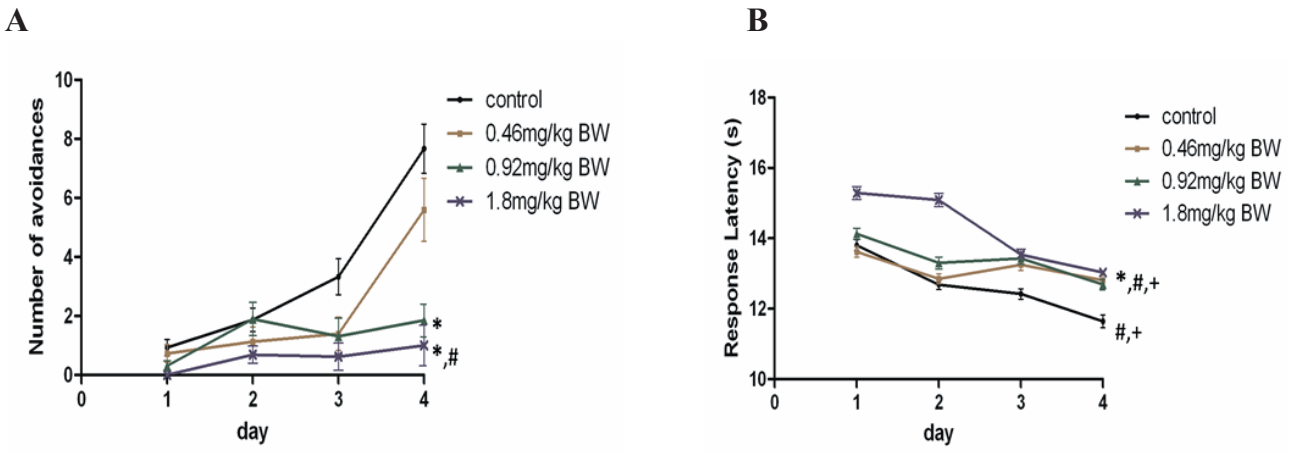

Fig. 2. Effect of 60-day deltamethrin administration on shuttle-box avoidance in mice. (A) The average active avoidance response of mice after the 60-day deltamethrin administration. (B) The average response latency of mice after the 60-day deltamethrin administration. Each value presented is the mean ± S.E.M. (n = 15). *significantly different from control values ($P < 0.05$). #, + significantly different from 0.46 mg/kg BW or 0.92 mg/kg BW respectively ($P < 0.05$) .

Contents of amino acid neurotransmitters in hippocampus of mice

The contents of glutamate, Asp and GABA was respectively analyzed with the one-way ANOVA [$F = 5.7925$, $P < 0.01$; $F = 1.274$, $P > 0.05$; $F = 2.081$, $P > 0.05$]. Post-hoc tests showed that compared to corn oil-treated (control) mice, the content of Glutamate was increased in the 0.92 mg/kg BW-dose and 1.8 mg/kg BW-dose group mice ($P < 0.01$). Meanwhile, compared to the 0.46 mg/kg BW-dose group mice, the content of glutamate was also increased in the 1.8 mg/kg BW-dose group mice ($P < 0.05$). (Fig. 3)

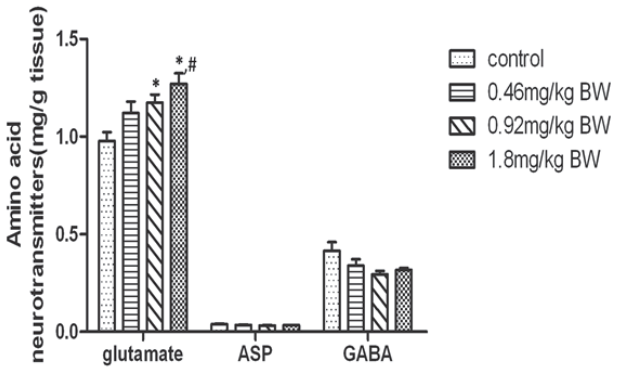

Fig. 3. The average contents of amino acid neurotransmitters in hippocampus of mice. Each value presented is the mean ± S.E.M. (n = 8). *significantly different from control values ($P < 0.05$). # significantly different from 0.46 mg/kg BW ($P < 0.05$) .

Histopathology

As revealed by electron microscopy, a large number of hippocampal neurons and astrocytes showed intact cell structure in the control and 0.46 mg/kg-dose group mice. The morphology of hippocampal neuron was round and oval, morphology of astrocyte was irregular. The intact cells had nucleus, homogeneous cytoplasmic matrix, and complete cell membrane. In 0.92 and 1.8 mg/kg-dose group mice, morphology of cells were changed; degeneration and necrosis morphological characteristics obviously were appeared, such as pyknosis, karyorrhexis, breakup and lysis of nuclear membrane. (Fig. 4)

DISCUSSION

Shuttle-box avoidance test can quantitatively measure the ability of animal to avoid an aversive event, and this method is the usual behavior measurement used in assessment of rodent's associative learning and memory (Grauer *et al*., 2009; Samardzic *et al*., 2012). In the two-way active avoidance program, animals are required to run from one compartment of the apparatus to another in order to avoid a foot-shock (Podhorna *et al*., 2002). We measured not only the number of avoidance response but also the escape latency. The results of present study shown that sub-chronic exposure to deltamethrin at the dose of 0.92, 1.8 mg/kg/day decreased the number of avoidance responses and increased response latency, which supported that sub-chronic exposure to deltamethrin at the dose of 0.92 mg/kg/day caused acquisition deficits in active

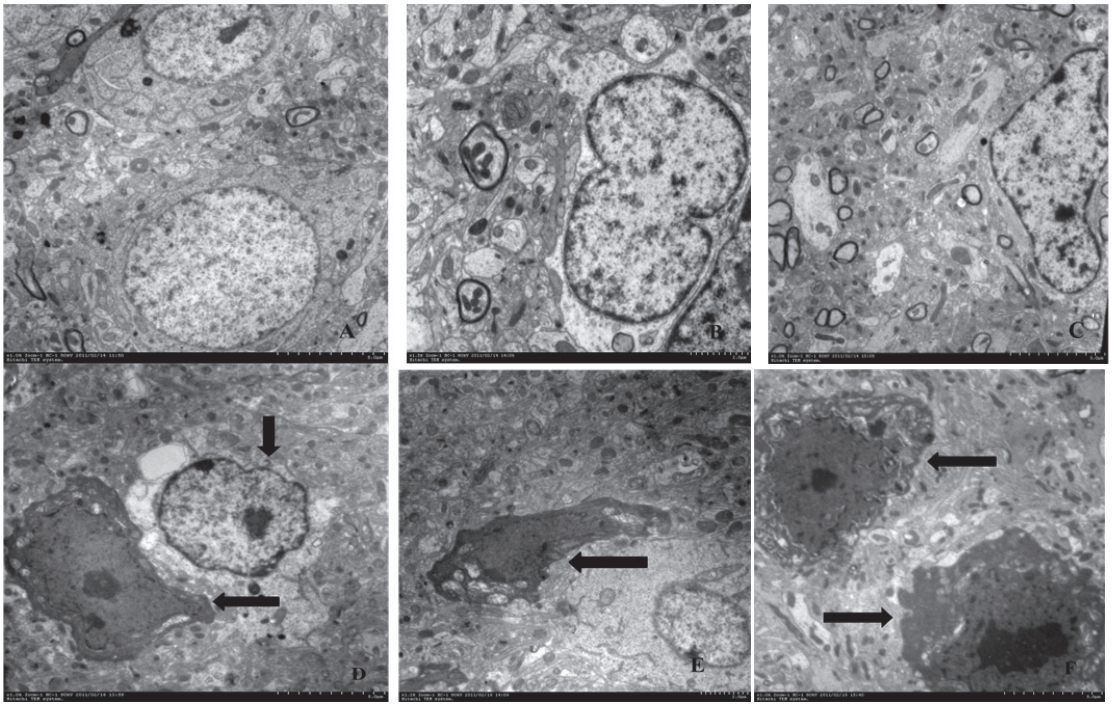

Fig. 4. Photomicrographs of the hippocampal neuron and astrocyte in hippocampaus of mice. (A) Normal hippocampal neurons. (B)-(C) Normal astrocyte. (D)-(F) Degeneration and necrosis morphological characteristics in hippocampal neuron and astrocyte in 0.92, 1.8mg/kg-dose group of mice. The samples were stained with uranyl acetate/lead citrate × 12,000.

avoidance task. Noteworthy, there was a significant interaction effect between dose and day, indicating the acquisitions of the treatment mice were slowed. This suggests a learning mechanism.

Analogous results were obtained in a positive reinforcement paradigm by Bloom *et al*. (1983), who utilized the schedule-controlled operant response approach with adult rats. Intraperitoneal injection of deltamethrin at the dose of 2 mg/kg could produce significant decreases in response rate. Y-maze by Husain *et al*. (1996), who reported that rats repeated oral exposed to a commercial formulation of deltamethrin at the dose of 7 mg/kg for 15 days could cause a 30% decrease in a re-learning index for an avoidance response based on visual discrimination. On the contrary in the study of Glowa (1986), no evident change in the deltamethrin (3 mg/kg)-induced decrease in responding rates was observed after repeated (once daily) administration for 10 days in mice. This is consistent with Crofton and Reiter (1984)'s the study, which found that there was no cumulative effects after repeated exposure to deltamethrin in rat using motor activity as an endpoint. However, toxicokinetics of deltamethrin and its metabolite after single doses of 26 mg/kg (oral) or 1.2 mg/kg (intravenous) of deltamethrin in rat found that deltamethrin was rapidly but partially absorbed after oral administration, relatively high concentrations were obtained in various brain regions, and its metabolism and disposition may be cause the deltamethrin-induced neurotoxicity (Anadon *et al*., 1996). In our experiment, deltamethrin administration at the dose of 0.92 mg/kg/day for 60 days in mice caused acquisition deficits in active avoidance task. Our result suggested that deltamethrin may have cumulative effects in mice following repeated oral dosing of deltamethrin using moderately effective doses once a day for 60 days. In addition, differences in experimental conditions, route of exposure, animal species and end of point maybe another reason to produce the disparity of these effective dose ranges.

The strong evidence implicated that the voltage-sensitive sodium channel was the principal site of action of deltamethrin. Voltage-sensitive sodium chancels are important to the function of most excitable cells, which is responsible for the generation of the Na^+ influx and action potential in most cells (Marban *et al*., 1998; Conley and

Brammar, 1999; Soderlund *et al*., 2002). It is reported that deltamethrin slow the activation and inactivation gates of Na^+ channels, resulting in prolonged opening of channels. Neurons depolarization can cause the nervous system excited, and the excited state cause the damage in the neurons (Soderlund *et al*., 2002). In addition to voltage-sensitive sodium channel, voltage-sensitive calcium channels have been proposed as a target of deltamethrin (Symington and Clark, 2005; Symington *et al*., 2007; Hossain *et al*., 2008). Deltamethrin administration at the low dose on extracellular glutamate in the hippocampus in rats was blocked by tetrodotoxin, and at dose of 60 mg/kg deltamethrin on extracellular glutamate was prevented by L-type calcium channel blocker (Hossain *et al*., 2008). This result suggested that deltamethrin may act on both Na^+ and Ca^{2+} channels to increase extracellular glutamate in the hippocampus. In addition, biochemical researches findings have validated the evidence that deltamethrin, with the typical CS-syndrome pyrethroid, acts at isolated presynaptic nerve terminals synaptosomes by increasing Ca^{2+} influx and subsequently enhancing endogenous glutamate release under depolarizing conditions (Symington and Clark, 2005; Symington *et al*., 1999; Lei *et al*., 1992). The results of our present experiments found that the levels of glutamate were increased in hippocampus of 0.92 and 1.8 mg/kg BW-dose groups after treating with deltamethrin for 60 days in mice. Taken together, the results of our experiment and other finds mentioned above suggested that orally 60-day exposure of deltamethrin may act on Na^+ and Ca^{2+} channels in mice producing Na^+ and Ca^{2+}-dependent glutamate release.

It is reported that a large proportion of the septohippocampal projection is GABAergic and the septal GABAergic afferents terminate exclusively on hippocampal interneurous (Amaral and Kurz, 1985; Baisden *et al*., 1984; Freund, 1989; Freund and Buzsaki, 1996). The findings in Hossain *et al*. (2008)'s study suggested that the effects of deltamethrin on hippocampal release of glutamate may involve direct effects on glutamatergic neurons and indirect effects on GABAergic and other neurons. Now, in our experiment we found that, compared to corn oil-treated mice, the levels of glutamate were increased in hippocampus of 0.92 and 1.8 mg/kg BW-dose groups. This result suggested that deltamethrin may preferential affect sodium channels on glutamatergic neurons within glutamatergic to GABAergic projections, thus increasing release of glutamate. However, in our study, deltamethrin had no effects on the levels of GABA in the hippocampus of mice. In the hippocampal network of glutamatergic terminals and inhibitory neurons (Freund, 1989; Freund and Buzsaki, 1996), deltamethrin may have affected the glutamatergic terminals preferentially at low doses (0.92 and 1.8 mg/kg BW), whereas the doses of 0.92 and 1.8 mg/kg BW deltamethrin were not large enough to act on the inhibitory neurons.

Several published studies have indicated that exposure to deltamethrin has the potential to produce neuronal death (Ray and Fry, 2006). Intravenous injection of deltamethrin at the dose of 12.5 mg/kg produced severe neuronal loss in the hippocampus and in cortical areas of rats, and this neuronal death involved both necrosis and activation of markers of apoptotic death (Wu and Liu, 2000). It was also reported that orally treatment with deltamethrin at a dose of 7.0 mg/kg/BW produced neuropathological damage in brains in rats (Husain *et al*., 1994). In the morphology results, degeneration and necrosis morphological characteristics obviously were appeared in hippocampal neurons and astrocytes in 0.92 and 1.8 mg/kg-dose group mice, such as pyknosis, karyorrhexis, breakup and lysis of nuclear membrane. Astrocytes are the most numerous cell types in the central nervous system. They provide structural, trophic, and metabolic support to neurons and modulate synaptic activity (Allen and Barres, 2009; Caito *et al*., 2014). As the functions of astrocyte, which include glutamate uptake, glutamate release, water transport and nitric oxide (Chen and Swanson, 2003). The degeneration and necrosis of astrocytes maybe related to the neurotoxicity of the excessive glutamate neurotransmitter.

In conclusion, the results from this study indicated that sub-chronic exposure to deltamethrin at the dose of 0.92 and 1.80 mg/kg/day could affect the acquisition in the active avoidance task and increase the glutamate level in the hippocampus of mice. These findings may provide some insights into mechanisms of deltamethrin neurotoxicity and might implicate deltamethrin as a risk factor leading to the cumulative effects. However, we still do not know the complete mechanism of deltamethrin neurotoxicity. Therefore, the potential mechanisms of deltamethrin neurotoxicity in mammals are considered to further research about other neurochemistry indicators and channels that maybe involve in neurotoxicity.

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Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES

Anadon, A., Martinez-Larranaga, M.R., Fernandiz-Cruz, M.L., Diaz, M.J., Fernandez, M.C. and Martinez, M.A. (1996): Toxicokinetics of deltamethrin and its 4'-HO-metabolite in the rat. Toxicol. App. Pharmacol., **141**, 8-16.

Aldridge, W.N., Clothier, B., Froshaw, P., Johnson, M.K., Parker, V.H., Price, R.J., Skilleter, D.N., Verscholyle, R.D. and Stevens, C. (1978): The effect of DDT and the pyrethroids cismethrin and decamethrin on the acetyl choline and cyclic nucleotide content of rat brain. Biochem. Pharmacol., **27**, 1703-1706.

Amaral, D.G.and Kurz, J. (1985): An analysis of the origins of the cholinergic and non cholinergic septalprojecti ons to the hippocampalformation of the rat. J. Comp. Neurol., **240**, 37-59.

Allen, N.J and Barres, B.A. (2009): Neuroscience: Glia-more than just brain glue. Nature, **457**, 675-677.

Baisden, R.H., Woodruff, M.L. and Hoober, D.B. (1984): Cholinergic and noncholinergic septo-hippocampal projections: a double-label horseradish peroxidase– acetylcholinesterase study in the rabbit. Brain Res., **290**, 146-151.

Bloomquist, J.R. (1996): Ion channels as targets for insecticides. Annu. Rev. Entomol., **41**, 163-190.

Burr, S.A. and Ray, D.E. (2004): Structure-activity and interaction effects of 14 different pyrethroids on voltage-gated chloride ion channels. Toxicol. Sci., **77**, 341-346.

Bloom, A.S., Staatz, C.G. and Dieringer, T. (1983): Pyrethroid effects on operant responding and feeding. Neurobehav. Toxicol. Teratol., **5**, 321-324.

Barlow, S.M., Sullivan, F.M. and Lines, L. (2001): Risk assessment of the use of deltamethrin on bednets for the prevention of malaria. Food Chem. Toxicol., **39**, 407-422.

Casida, J.E., Gammon, D.W., Glickman, A.H. and Lawrence, L.J. (1983): Mechanisms of selective action of pyrethroid insecticides. Annu. Rev. Pharmacol. Toxicol., **23**, 413-438.

Crofton, K.M. and Reiter, L.W. (1984): Effects of two pyrethroids on motor activity and the acoustic startle response in the rat. Toxicol. Appl. Pharmacol., **75**, 318-328.

Conley, E.C. and Brammar, W.J. (1999): The ion channel facts book. pp.768-838, SanDiego, Academic Press.

Caito, S.W., Yu, Y.C. and Aschner, M. (2014): Differential inflammatory response to acrylonitrile in rat primary astrocytes and microglia. Neurotoxicology, **42**, 1-7.

Chen, Y. and Swanson, R.A. (2003): Astrocytes and brain injury. J. Cereb. Blood. Flow. Metab., **23**, 137-149.

Eells, J.T. and Dubocovich, M.L. (1988): Pyrethroid insecticides evoke neurotransmitter release from rabbit striatal slices. J. Pharmacol. Exp. Ther., **246**, 514-521.

Forshaw, P.J., Lister, T. and Ray, D.E. (1993): Inhibition of a neuronal voltage-dependent chloride channel by the type II pyrethroid, deltamethrin. Neuropharmacology, **32**, 105-111.

Freund, T.F. (1989): GABAergic septohippocampal neurons contain parvalbumin. Brain Res., **478**, 375-381.

Freund, T.F. and Buzsaki, G. (1996): Interneurons of the hippocampus. Hippocampus., **6**, 347-470.

Gassner, B., Wuthrich, A., Scholtysik, G. and Solioz, M. (1997): The pyrethroids permethrin and cyhalothrin are potent inhibitors of the mitochondrial complex I. J. Pharmacol. Exp. Ther., **281**, 855-860.

Grauer, S.M., Pulito, V.L., Navarra, R.L., Kelly, M.P., Kelly, C., Graf, R., Langen, B., Logue, S., Brennan, J., Jiang, L., Charych, E., Egerland, U., Liu, F., Marquis, K.L., Malamas, M., Hage, T., Comery, T.A. and Brandon, N.J. (2009): Phosphodiesterase 10A inhibitor activity in preclinical models of the positive, cognitive, and negative symptoms of schizophrenia. J. Pharmacol. Exp. Ther., **331**, 574-590.

Glowa, J.R. (1986): Acute and sub-acute effects of deltamethrin and chlordimeform on schedule-controlled responding in the mouse. Neurobehav. Toxicol. Teratol., **8**, 97-102.

Hossain, M.M., Suzuki, T., Sato, I., Takewaki, T., Suzuki, K. and Kobayashi, H. (2004): The modulatory effect of pyrethroids on acetylcholine release in the hippocampus of freely moving rats. Neurotoxicology, **25**, 825-833.

Hossain, M.M., Suzuki, T., Sato, N., Sato, I., Takewaki, T., Suzuki, K., Tachikawa, E. and Kobayashi, (2006): Differential effects of pyrethroid insecticides on extracellular dopamine in the striatum of freelymoving rats. Toxicol. Appl. Pharmacol., **217**, 25-34.

Hossain, M.M., Suzuki, T., Unno, T., Komori, S. and Kobayashi, H. (2008): Differential presynaptic actions of pyrethroid insecticides on glutamatergic and GABAergic neurons in the hippocampus. Toxicology, **243**, 155-163.

Husain, R., Husain, R., Adhami, V.M. and Seth, P.K. (1996): Behavioral, neurochemical, and neuromorphological effects of deltamethrin in adult rats. J. Toxicol. Environ. Health, **48**, 515-526.

Husain, R., Malaviya, M., Seth, P.K. and Husain, R. (1994): Effect of deltamethrinon regional brain polyamines and behaviour in young rats. Pharmacol. Toxicol., **74**, 211-215.

Llewellyn, D.M., Brazier, A., Brown, R., Evans, M.L., Hampton, J., Nutley, B.P. and White, J. (1996): Occupational exposure to permethrin during its use as a public hygiene insecticide. Ann. Occup. Hyg., **40**, 499-509.

Lei, F.G., Marion, J.R. and Clark, J.M. (1992): Suppression of pyrethroid-dependent neurotransmitter release from synaptosomes of knock down-resistant house flies under pulsed depolarization conditions during continuous perfusion. Pestic. Biochem. Physiol., **42**, 64.

Miyamoto, J., Kaneko, H., Tsuji, R. and Okuno, Y. (1995): Pyrethroids, nerve poisons: how their risks to human health should be assessed. Toxicol. Lett., **82-83**, 933-940.

Macphail, R.C., Gordon, W.A. and Johnston, M.A. (1981): Behavioral effects of deltamethrin. Fed. Proc., **40**, 678.

Manna, S., Bhattacharyya, D., Mandal, T.K. and Dey, S. (2006): Neuropharmacological effects of deltamethrin in rats. J. Vet. Sci., **7**, 133-136.

Marban, E., Yamagishi, T. and Tomaselli, G.F. (1998): Structure and function of voltage-gated sodium channels. J. Physiol., **508**, 647-657.

Matthew, G.C., Sam, V. and Todd, M.M. (2003): Air and shock two-way shuttlebox avoidance in C57BL /6J and129X1/SvJ mice. Physiol. Behav., **78**, 117-123.

Narahashi, T. (1985): Nerve membrane ionic channels as the primary target of pyrethroids. Neuro. Toxicology, **6**, 3-22.

Narahashi, T. (1992): Nerve membrane Na+ channels as targets of insecticides. Trends. Pharmacol. Sci., **13**, 236-241.

Narahashi, T. (1996): Neuronal ion channels as the target sites of insecticides. Pharmacol. Toxicol., **79**, 1-14.

Niu, Y.J., Shi, N., Li, L. and Liu, Y.G. (1999): Effect of pyrethroids on the content of Glutamate and Aspartate in rat brain. Journal of Hebei medical university, **1**, 1-4.

Nemec, M. (1998): A subchronic (13-week) neurotoxicity study of deltamethrin in rats. Agr. Evo. USA Company study No.53014.

Podhorna, J., McCabe, S. and Brown, R.E. (2002): Male and female C57BL/6 mice respond differently to diazepam challenge in avoidance learning tasks. Pharmacol. Biochem. Behav., **72**, 13-21.

Rickard, J. and Brodie, M.E. (1985): Correlation of blood and brain levels of the neurotoxic pyrethroid deltamethrin with the onset of symptoms in rats. Pestic. Biochem. Physiol., **23**, 143-156.

Ray, D.E. and Fry, J.R. (2006): A reassessment of the neurotoxicity of pyrethroid insecticides. Pharmacol. Ther., **111**, 174-193.

Samardzic, J., Strac, D.S., Obradovic, M., Opric, D. and Obradovic, D.I. (2012): DMCM, a benzodiazepine site inverse agonist, improves active avoidance and motivation in the rat. Behav. Brain Res., **235**, 195-199.

Soderlund, D.M., Clark, J.M., Sheets, L.P., Mullin, L.S., Piccirillo, V.J., Sargent, D., Stevens, J.T. and Weiner, M.L. (2002): Mechanisms of pyrethroid neurotoxicity : implications for cumulative risk assessment. Toxicology, **171**, 3-59.

Shafer, T.J. and Meyer, D.A. (2004): Effects of pyrethroids on voltage-sensitive calcium channels: a critical evaluation of strengths, weaknesses, data needs, and relationship to assessment of cumulative neurotoxicity. Toxicol. Appl. Pharmacol., **196**, 303-318.

Symington, S.B., Frisbie, R.K., Lu, K.D. and Clark, J.M. (2007): Action of cysmethrin and deltamethrin on functional attributes of isolated presynaptic nerve terminals from rat brain. Pestic. Biochem. Physiol., **87**, 172-181.

Symington, S.B., Zhang, A., Karstens, W., Van Houten, J. and Clark, J.M. (1999): Characterization of pyrethroid action on ciliary calcium channels in *paramecium tetraurelia*. Pestic. Biochem. Physiol., **65**, 181-193.

Stein, E.A., Washburn, M., Walczak, C. and Bloom, A.S. (1987): Effects of pyrethroid insecticides on operant responding maintained by food. Neurotoxicol. Teratol., **9**, 27-31.

Symington, S.B. and Clark, M. (2005): Action of deltamethrin on N-type(Cav2.2)voltage-sensitive calcium channels in rat brain. Pestic. Biochem. Physiol., **82**, 1-15.

Tayebati, S.K., Di Tullio, M.A., Ricci, A. and Amenta, F. (2009): Influence of dermal exposure to the pyrethroid insecticide deltamethrin on rat brain microanatomy and cholinergic/dopaminergic neurochemistry. Brain Res., **1301**, 181-188.

Verschoyle, R.D. and Aldridge, W.N. (1980): Structure–activity relationships of some pyrethroids in rats. Arch. Toxicol., **45**, 325-329.

Vijverberg, H.P. and Van den Bercken, J. (1990): Neurotoxicological effects and the mode of action of pyrethroid insecticides. Crit. Rev. Toxicol., **21**, 105-126.

Wu, A. and Liu, Y. (2000): Apoptotic cell death in rat brain following deltamethrin treatment. Neurosci. Lett., **279**, 85-88.