



Effect of Acetamiprid on spatial memory and hippocampal glutamatergic system

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Abstract

Acetamiprid (ACE) is one of the widely used neonicotinoid insecticides. In mammals, in spite of the low-affinity nAChRs, neurotoxic effects following the Acetamiprid exposure have recently been reported, which suggests some concerns regarding the impacts on the nervous system of mammals. This study aims to investigate the effect of Acetamiprid on spatial memory and possible vulnerability of hippocampal glutamatergic system following the Acetamiprid exposure. 10, 20, and 40 mg/kg doses of Acetamiprid were administered to male rats by gavage once per day for 28 days. The spatial memory was examined with the Morris water maze apparatus. The amount of Acetamiprid in the serum and hippocampus was measured. In addition, glutamate level and changes in the expression of NR1, NR2, and NR2B genes were measured in the hippocampus; also, the hippocampus tissue was histologically evaluated. A significant increase in training parameters which consist of escape latency and traveled distance was observed on the first and second day of training in Acetamiprid-treated groups (20 and 40 mg/kg) compared to the control group ($p < 0.001$). In the probe test, rats in all Acetamiprid-treated groups significantly spent less time in the target quadrant compared to the control group ($p < 0.001$). Acetamiprid concentration dose dependently increased in the serum and in the hippocampus followed by Acetamiprid exposure. In all Acetamiprid-treated groups, a significant reduction of glutamate level in the hippocampus was observed ($p < 0.05$). The reduction of NR1, NR2A, and NR2B gene expression in the hippocampus was observed at a dose of 20 mg/kg. The histological evaluation showed neural degeneration in the dentate gyrus area of the hippocampus at a dose of 40 mg/kg in the Acetamiprid-treated group. The results of the present study indicate that Acetamiprid impairs memory consolidation through the reduction of glutamate and the expression of NMDA receptor subunits in the hippocampus at low doses, along with the loss of neural cells in dentate gyrus at high dose.

Keywords Acetamiprid · Memory · Hippocampus · NMDA · Glutamate

Introduction

Acetamiprid belongs to neonicotinoid insecticides—a new class of systemic insecticides. All neonicotinoids are structurally related to nicotine; subsequently, they stimulate nicotinic

acetylcholine receptors (nAChRs); however, in vitro binding assay has indicated that neonicotinoids have more binding affinity to insects' nAChRs than mammals', due to the particular chemical structure they have, and therefore neonicotinoids are supposed to be safe in mammal groups (Tomizawa and Casida 2005). Accordingly, in addition to the resistance of insect pest to other insecticides, neonicotinoids have become an increasingly used replacement for other insecticides during the recent decades, to remarkably control insects, including aphids, bees, and mosquitoes, which cause damage to a large number of crops such as cotton, cabbage, and carrot (Jeschke et al. 2011). Although neonicotinoid insecticides are supposed to be safe, recent studies have presented evidence which indicates their toxicity to mammal organs, particularly the nervous system (Han et al. 2018). In human cases, people who had been exposed to neonicotinoid insecticides showed neurotoxic symptoms such as seizure (Imamura et al. 2010), and in animal

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studies, neurobehavioral alterations such as learning and memory impairment were reported (Özdemir et al. 2014). Therefore, the safety of these insecticides is questionable and needs further study to be confirmed.

The neurotoxic effects of Acetamiprid on brain tissue have been detected. After oral exposure to Acetamiprid, rapid absorption and penetration through blood-brain barrier were observed. Moreover, it tends to accumulate in different regions of the brain such as the hippocampus and disturbs the expression of nAChR subunits (Terayama et al. 2016); also, Acetamiprid has the highest affinities for mammalian nAChRs among neonicotinoid insecticides (Tomizawa and Casida 1999).

In the hippocampus, cholinergic system, along with glutamatergic system, plays an essential role in learning and memory formation processes (Aigner 1995). The nAChRs modulate glutamatergic neurotransmission (Yakel 2014). Several studies have been conducted to identify the role of glutamatergic neurotransmission in the hippocampal memory formation. As an excitatory neurotransmitter, glutamate is involved in the modulation of synaptic plasticity and long-term potentiation (LTP) associated with learning and memory (Marmioli and Cavaletti 2012). Glutamate exerts its function through its various receptors which include ionotropic and metabotropic receptors. Among them, N-methyl-D-aspartate (NMDA) receptors—ionotropic receptors permeable to calcium ion (Ca^{2+})—have a great importance in synaptic plasticity. Ca^{2+} influx through these receptors initiate signaling cascades involved in LTP and memory formation (Riedel et al. 2003). NMDA receptors consist of different subunits including NR1, NR2 (A–D), and NR3. The classical composition of the NMDA receptor comprises two NR1 subunits and two NR2 subunits; moreover, NR2A and NR2B are common subunits in the hippocampus (Morris 2013). There is a well-known association between the deficit of hippocampal NMDA receptors and memory impairment. NMDA receptor antagonists impair spatial memory in laboratory animals (Morris et al. 2013). Disturbance in subunit expression and assembly impairs its function which results in memory deficit (Bannerman 2009). Additionally, there is a close relation between the nACh and the NMDA receptor functions in the hippocampus. They are co-localized in the synapses and nAChRs modulate NMDA receptor functions (Marchi et al. 2015).

Since there is no study thus far revealing precisely the effects of Acetamiprid on hippocampus and spatial memory, prolonged Acetamiprid consumption in agriculture to control pest insects causes a matter of human safety. In the present study, we investigated the role of glutamatergic system and possible adverse effects of Acetamiprid on mammal organ systems. Regarding this matter that the safety of this insecticide is questionable, based on recent studies, further studies is needed to evaluate possible adverse effects of Acetamiprid on different organs of mammals. According our literature review,

so far, there is no study about the effect of Acetamiprid on hippocampus and its main function, spatial memory; therefore, in this study, our aim is to investigate the possible destructive effect of Acetamiprid on memory and the role of the glutamatergic system in this regard.

Materials and methods

Animals

Several male Wistar rats (180–200 g) from the animal house of Tarbiat Modares University were kept in standard conditions, including temperature of 25 °C, 12-h light/dark cycle, and free access to water and food ad libitum. All experimental procedures such as working with animals and tissue collection were approved by the ethical committee of the Tarbiat Modares University (ethical code: IR.MODARES.REC.1397.115).

Chemicals

RNX-Plus kit (SinaClonBioScience, Iran), cDNA Synthesis kit (Takara, Japan), Master Mix SYBR green kit (Ampliqon, Denmark), Methanol and Acetonitrile, HPLC Grade (AmertateShimico, Iran), and all other chemicals were purchased from Merck (Germany). Acetamiprid, technical grade (purity 99%) was provided by Gol Sam Co. (Iran).

Treatments

The male Wistar rats were randomly allocated into four groups (ten rats per group), including a control group (without treatment) and three Acetamiprid-treated groups (10, 20, and 40 mg/kg). In the first place, Acetamiprid was dissolved in a 10% DMSO solution in water and then it was administered orally via gavage needle for 28 consecutive days. This study aimed to evaluate the subacute effects of Acetamiprid, so according to the Organization for Economic Co-operation and Development (OECD) protocol for the subacute repeated dose study (OECD Test NO 407), it was administered for 28 days (OECD 2008). The dose levels were selected based on Acetamiprid LD50 which is 200 mg/kg according to the previous studies (Chakroun et al. 2016; Yamada et al. 1999). Therefore, the selected doses correspond to 1/20, 1/10, and 1/5 of LD50, according to a previous study (Chakroun et al. 2016). The body weight was also measured weekly.

Morris water maze

The spatial memory of animals was examined by the Morris water maze (MWM) test conducted during the 24th to 28th day of treatment. The MWM test which is widely used in the hippocampus-dependent spatial memory was performed on

rodents. The MWM apparatus is a circular black-painted tank with a diameter of 150 cm and a height of 60 cm filled with water (25 ± 2 °C) to a height of 25 cm. The tank is hypothetically divided into four quadrants, and a plexiglass platform (10-cm diameter) is located in the center of one of the quadrants (target quadrant). Then, it was submerged to 2 cm under the water surface in order to be hidden. The animals were trained for four consecutive days which consisted of four trials per day. In each trial, the rat was released into the tank from a randomly selected quadrant facing the wall and allowed to swim for 90 s to find the hidden platform; otherwise, it was manually guided to the platform by the researcher and allowed to stay on the platform for 30 s. Rats learn how to find the hidden platform during training sessions through external visual cues located near the tank. The animal swimming path was recorded via a video camera located above the tank with a computer connected to it. The learning measurements including escape latency (time to find the platform) and traveled distance (distance moved to find platform) were calculated through a video tracking software. The probe test was performed 1 day after the last training session on the fifth day in which the platform was removed from the tank and then the rat was released into the tank and allowed free swimming for 60 s. Swimming time spent in target quadrant and opposite quadrant was recorded and compared as the memory consolidation and recall was measured (Soodi et al. 2016).

Serum and tissue collection

Immediately after the behavioral test, blood samples were collected through cardiac puncture under ether anesthesia, then the rats were decapitated and their hippocampal tissue was dissected. After being rinsed in ice-cold phosphate buffer (pH = 7.4), they were kept in the temperature of -70 °C in freezer. Two brains from each group were collected and kept in 10% formalin solution for histological evaluation.

HPLC equipment

The Agilent HPLC system (Agilent1200series) was equipped with binary pump, autosampler, column oven, and UV and fluorescent detectors. The analytes were separated on Nucleodur C18 column with the size of 25-cm length, 4.6-mm diameter, and 5- μ m particle.

Measurement of Acetamiprid in serum and hippocampus

Acetamiprid was extracted from serum and hippocampus samples as previously described with slight modification (Terayama et al. 2016). Briefly, the serum sample was mixed with two volume of chilled acetone and then shaken for 5 min. Following that, it was centrifuged at 2000 g for 10 min. The

supernatant was collected and the resultant pellet was extracted with acetone again, then the supernatants were pooled and evaporated under nitrogen stream to dry, then dissolved in 1 ml of mobile phase, and filtered through a 0.45-mm filter. The same procedure was employed for hippocampus samples homogenized in acetone (10% w/v). The optimized HPLC conditions for measuring Acetamiprid include acetonitrile/water (40/60 v/v) as mobile phase, 1 ml/min flow rate, 40 °C column temperature, and 20- μ l injection volume. The Acetamiprid was detected at 244 nm with UV detector.

Measurement of glutamate in hippocampus

A pre-column derivatizing technique was employed to measure glutamate by HPLC with minor modification. The hippocampus tissue was homogenized in 50 volume of methanol and water (85, 15 v/v) solution and centrifuged at 12000g for 15 min, then the supernatant was utilized for derivatization after filtering through a 0.22 μ m filter. Derivatization solution was freshly prepared by dissolving 8.1 mg o-phthalaldehyde (OPA) in 150 μ l methanol, 6.3 μ l 2-mercaptoethanol, and 1.35 ml sodium borate buffer (0.1 M, pH = 10). One hundred microliters of this solution was mixed with 100 μ l of sample or of glutamate standard solution and allowed to stay at room temperature for 1 min and after that injected to HPLC system. The following isocratic HPLC condition was used: mobile phase consisting of methanol, tetrahydrofuran, and sodium acetate buffer (0.05 mM, pH = 4) (49:1:50 v/v), flow rate of 0.7 ml/min, and 25 °C column temperature. The eluent was monitored by a fluorescence detector (Ex: 330, Em: 440 nM) (de Freitas Silva et al. 2009; Reisi et al. 2009).

Quantitative real-time PCR

The relative change in NR1, NR2A, and NR2B gene expression was measured by quantitative real-time PCR (qRT-PCR) technique and it was compared with control and treated groups. Total RNA of hippocampus tissue was extracted with RNX-Plus kit according to the manufacturer instruction (SinaClonBioScience, Iran). The extracted RNA was reverse transcribed into cDNA using cDNA Synthesis kit (TakaRa, Japan). The cDNA was quantified utilizing Master Mix SYBR green kit (Ampliqon, Denmark) and Step One Plus Real-Time PCR System (Applied Biosystems). The primer sequences are shown in Table 1. Beta-microglobulin was used as a house-keeping gene to normalize mRNA expression of target genes. The fold change in target gene expression in relation to control group was calculated using the $2^{-\Delta\Delta Ct}$ method.

Histological evaluation

Fixed brain tissue was dehydrated in 10% formalin solution of the ethanol series, then it was embedded in paraffin, and then

Table 1 primers sequence used in Real-time PCR

Gene	Primer	Sequence
NR1	Forward 5'–3'	AAT GAC CCC AGG CTC AGA AAC
	Reverse 5'–3'	CCA CGG AGC TCT GCT TTA CAG
NR2A	Forward 5'–3'	GCC AGA TAA CAA TAA GAA CCA CAA G
	Reverse 5'–3'	TGC GGT CAA CAT CGC TAC AG
NR2B	Forward 5'–3'	GAC GAC TTC AAG CGA GAT TCG
	Reverse 5'–3'	GCC CGT TCC GTG TTT GAG
β 2M	Forward 5'–3'	CCG TGA TCT TTC TGG TGC TT
	Reverse 5'–3'	TTT TGG GCT CCT TCA GAG TG

sectioned at 5 μ M. Finally, the sections were stained with hematoxylin and eosin (H&E) for evaluation with light microscope.

Statistical analysis

Data are represented as mean \pm SEM. The data of the Morris water maze test was analyzed by repeated measures two-way ANOVA followed by the Tukey's multiple comparison test. Other data were analyzed with one-way ANOVA. All statistical analyses were performed through GraphPad prism 8 software. Additionally, real-time PCR data analysis was performed through Rest software.

Results

Changes in the body weight

The body weight was weekly measured during the treatment and the results are shown in Fig. 1. As shown in the graph, animals treated with Acetamiprid at 10 and 20 mg/kg doses had the same weight gain as the control group and not any significant difference was observed between the body weight of these groups and control group. Since animals received Acetamiprid at 40 mg/kg dose, their body weight significantly decreased on the 3rd and 4th weeks of treatment in comparison with the control group.

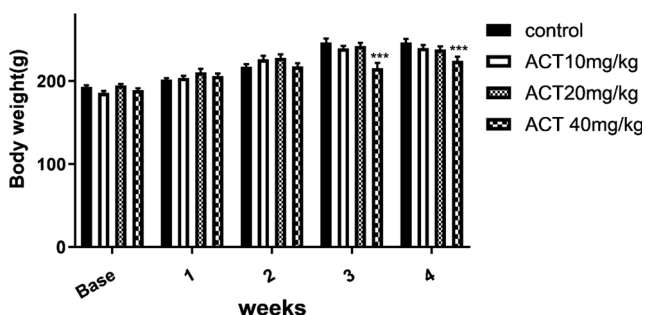


Fig. 1 Body weight changes in Acetamiprid treated and control groups. (mean \pm SEM, $n = 9$) *** $p < 0.001$ versus control

Concentration of Acetamiprid in the serum and hippocampus

Table 2 represents the result of Acetamiprid concentration measurement both in the serum and in the hippocampus tissue. The results showed that the level of Acetamiprid had dose-dependently increased in both the serum and hippocampus tissue after 28 days of treatment.

Effect of Acetamiprid exposure on learning and memory

The results of the animal performance during MWM test are summarized in Fig. 2. RM two-way ANOVA indicates significant treatment and day factor effect on escape latency and traveled distance parameters, and the interaction of these two factors (treatment \times day) was significant. Based on the Tukey's post-test analysis, there is a remarkable difference in escape latency and traveled distance parameters between the control and Acetamiprid-treated group at 20 and 40 mg/kg doses on the first and the second days of training. Acetamiprid exposure to 10 mg/kg dose did not affect learning parameters during training days. (Fig. 2A and B). There is no significant difference in swimming speed parameter between the control and Acetamiprid-treated groups (Fig. 2C). The result of the probe test shown in Fig. 3 represents that animals in all Acetamiprid-treated groups spent less time in target quadrants compared to the control group. Moreover, there was a notable difference between the time spent in target quadrant and opposite quadrant of the control group, but no significant difference was observed in Acetamiprid-treated groups.

The expression of NMDA receptor subunits

The expression of NR1, NR2A, and NR2B genes in the hippocampus was determined by real-time PCR. Figure 4 indicates changes of subunit gene expression in Acetamiprid-treated groups relative to the control group. Although the expression of NMDA receptor subunits relative to control group shows no significant change in 10 mg/kg Acetamiprid-treated

Table 2 Acetamiprid level in the serum and hippocampus

	Control	ACT 10mg/kg	ACT 20mg/kg	ACT 40mg/kg
Serum (µg/ml)	not detected	6.34±1.127	10.85±2.627	14.71±3.767
Hippocampus (µg/g)	not detected	4.606±1.044	5.914±0.73	7.276±0.968

Data is represented as mean ± SD

group, a remarkable gene expression decrease was observed in 20 mg/kg Acetamiprid-treated group.

Effect of Acetamiprid on glutamate level of the hippocampus tissue

The amount of glutamate in the hippocampus tissue was measured by a HPLC method. A significant reduction in the

hippocampus glutamate level was observed in all Acetamiprid-treated groups compared to control group (Fig. 5).

Histological evaluation of the hippocampus tissue

The hippocampus tissue sections were evaluated with a light microscope after H&E staining; as in Fig. 6, normal appearance of tissue was observed in the control group including intact neuron arrangement in a regular layer, whereas in 40 mg/kg Acetamiprid-treated group, morphological changes in neurons led to a neuronal shrinkage and pyknotic nuclei, along with an increase in glial cell numbers, where dentate gyrus area of hippocampus is located. Rats' hippocampal tissue shows slight morphological change in 20 mg/kg Acetamiprid-treated group, but normal structure in 10 mg/kg dose of Acetamiprid.

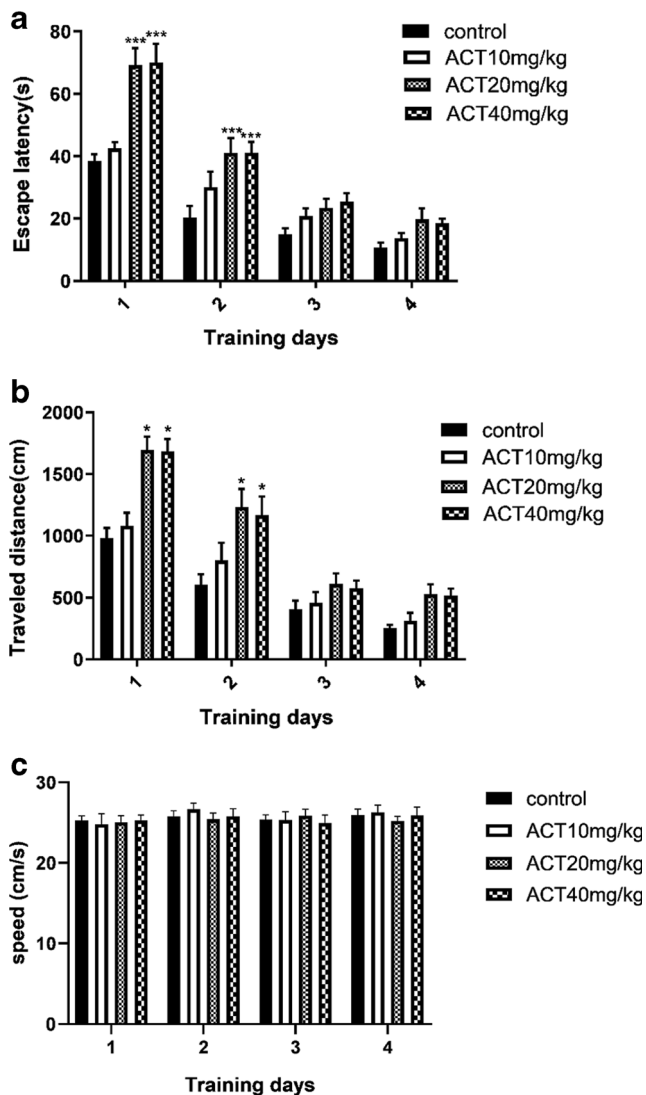


Fig. 2 Comparison of escape latency (A), traveled distance (B) and Speed (C) during training days between Acetamiprid-treated and control groups (mean ± SEM, n = 9), **p* < 0.01, ****p* < 0.001 versus control

Discussion

The present study investigated the effect of sub-acute oral administration of Acetamiprid at three different doses on rat spatial memory, and further analysis of Acetamiprid mechanism on the learning process in the hippocampus was conducted to delve deeply into its effect on the gene expression of NMDA receptor subunit and glutamate level in the hippocampus. Then, histological change of hippocampus was studied. The concentration of Acetamiprid in plasma and hippocampus was measured to determine at what concentration Acetamiprid has toxic effect on hippocampus.

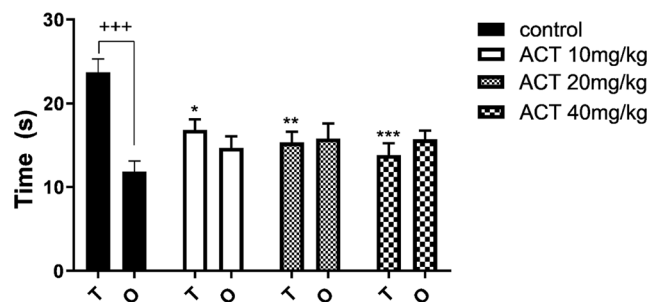


Fig. 3 Time spent in target quadrant (T) and opposite quadrant (O) in probe test. (mean ± SEM, n = 9) +++*p* < 0.001 represents significant difference between target quadrant and opposite quadrant. **p* < 0.05, ***p* < 0.01, ****p* < 0.001 represent significant difference between time spent in target quadrant of Acetamiprid-treated groups and control group

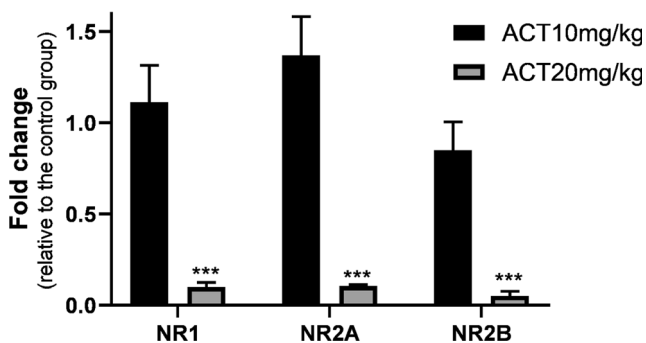


Fig. 4 Relative changes in NMDA receptor subunits gene expression in the hippocampus of Acetaminophen-treated (10 and 20 mg/kg) groups in compared to the control group. (mean \pm SEM, $n = 3$)

The result of our study demonstrated that Acetaminophen concentration dose-dependently increased in both serum and hippocampus, and therefore it confirms that Acetaminophen is absorbed from the gastrointestinal (GI) system, penetrates into BBB, and is subsequently distributed in the brain particularly in the hippocampus. This finding is in line with a previous study which illustrated the distribution of Acetaminophen in different areas of the brain after oral administration (Terayama et al. 2016). We found that even low-dose (10 mg/kg) Acetaminophen is distributed to the brain and detected in the hippocampus at concentration of $4.6 \pm 1 \mu\text{g/g}$ and at plasma concentration of $6.4 \pm 1.12 \mu\text{g/ml}$, but in that study, a high dose of Acetaminophen had been administered. Therefore, it seems that Acetaminophen has a tendency to accumulate in brain tissue and due to its neurotoxic potential, the brain could be a vulnerable target during the exposure to Acetaminophen.

The result of the MWM test indicated that Acetaminophen-treated groups at high doses (20 and 40 mg/kg) had higher escape latency and traveled distance compared to the control group on the first and second day of training which indicates that acquisition phase of learning is affected by the Acetaminophen exposure; However, Acetaminophen at 10 mg/kg dose had no effect on learning parameters during training days. The probe test indicated that rats in all Acetaminophen-treated groups spent less time in target quadrant in comparison to the control group, which indicates memory consolidation

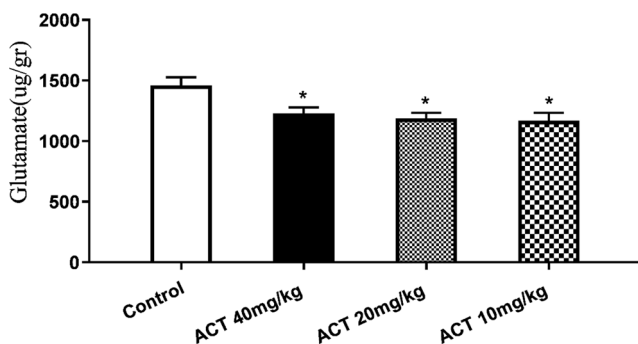


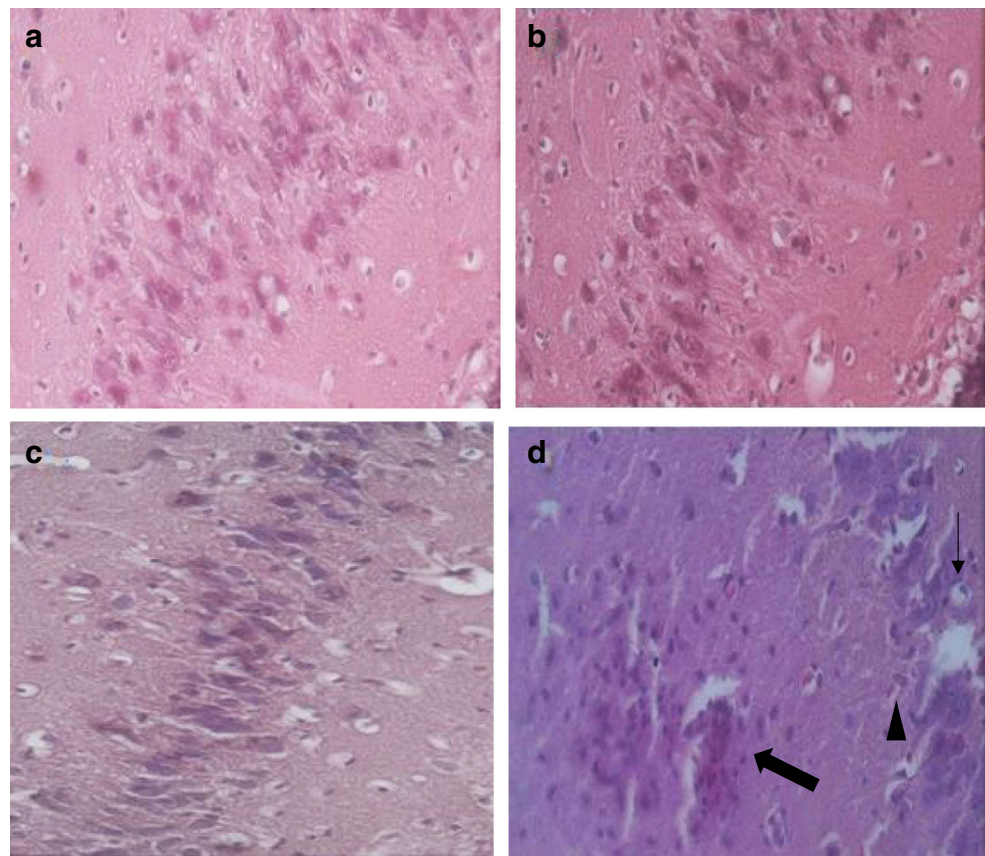
Fig. 5 Glutamate level of the hippocampus tissue (mean \pm SEM, $n = 3$), * $p < 0.05$ VS control

and retrieval impairments. It seems that memory consolidation and retrieval processes are more susceptible to Acetaminophen exposure due to the memory deficit at low dose. The memory impairment caused by some neonicotinoid insecticides has been shown in the previous study. For instance, it has been reported that a 90-day exposure to imidacloprid dose-dependently impairs learning and memory in neonatal rats (Kara et al. 2015), and high-dose clothianidin also impairs memory consolidation and retrieval (Özdemir et al. 2014). This study has been conducted to examine the effect of Acetaminophen on memory process for the first time.

The hippocampal formation is an important part of the brain involved in memory formation and hippocampal damage impairs spatial memory in MWM test (Jarrard 1993). The present study shows histological evaluation at high dose of Acetaminophen (40 mg/kg) causing neural cell shrinkage with pyknotic nuclei in dentate gyrus area of hippocampus with apoptotic neural cell death in this area. This finding is consistent with the previous study that indicated the vulnerability of dentate gyrus to Acetaminophen exposure (Nakayama et al. 2019). The hippocampal dentate gyrus area is associated with spatial memory formation (Bott et al. 2016) and previous study showed that loss of the dentate gyrus granule cell is associated with memory deficit and poor performance in MWM task particularly in acquisition phase (Xavier and Costa 2009; Xavier et al. 1999). Therefore, it is suggested that memory deficit in high dose of Acetaminophen-treated rats is due to the loss of dentate gyrus neural cell. The mechanism of how Acetaminophen can do damage to the dentate gyrus has not been investigated yet, but the role of oxidative stress may be involved. In a study, it has been shown that Acetaminophen induces mitochondrial dysfunction and increases reactive oxygen species (ROS) formation in the cerebrum which leads to apoptotic cell death (Dhouib et al. 2017).

The glutamatergic system in the hippocampal formation plays a pivotal role in memory formation processes, but inappropriate glutamate receptors function particularly NMDA receptors interferes with synaptic plasticity, and LTP leads to impaired learning and memory formation (Riedel et al. 2003). In the hippocampus, NMDA receptors are heterotetramers predominantly consisting of two NR1, NR2A, and NR2B subunits (Morris et al. 2013). It is well-known that alteration in the expression of NMDA receptor subunits dramatically affects its function by altering receptor channel kinetics and conductance which results in memory deficit. A reduction in the expression of NMDA receptor subunits in the hippocampus is observed at the advanced age which is associated with memory deficit in the elderly (Clayton et al. 2002; Kumar and Foster 2019). Also, decreased level of NR2A and NR2B gene expression in the hippocampus has been reported in Alzheimer's disease (Bi and Sze 2002). These studies indicate an important relationship between NMDA receptor subunit expression in the hippocampus and memory function. In the

Fig. 6 Photograph of the dentate gyrus area of the hippocampus (400 X). Control (A), Acetamiprid-treated 10 mg/kg (B), Acetamiprid-treated 20 mg/kg (C), Acetamiprid-treated 40 mg/kg (D). Tick arrow: irregularity in cells arrangement, arrow head: increase in glial cell numbers, thin arrow: pyknotic nuclei



present study, we investigated a significant decrease in the expression of NR1, NR2A, and NR2B genes of the hippocampus at Acetamiprid of 20 mg/kg, and therefore the observed memory deficit in this group might be the consequence of reduction in NMDA receptor subunit gene expression.

In the present study, amount of glutamate in the hippocampus decreased following the exposure to all Acetamiprid doses. It is suggested that Acetamiprid interferes with the metabolism of glutamate in the hippocampus. Recently, a study has reported that a 28-day exposure to imidacloprid—another member of neonicotinoids insecticides—reduces glutamate level in the hippocampus by disturbing glutamate metabolic cycle (Zheng et al. 2020). Glutamate as an excitatory neurotransmitter in glutamatergic system plays a vital role in the memory formation. Previous studies have indicated that decreased glutamate level in the hippocampus impairs memory consolidation (Szyndler et al. 2006). So, it is suggested that impaired memory consolidation in all Acetamiprid-treated groups is due to the decreased glutamate level in the hippocampus.

In the hippocampus, nAChRs modulate glutamatergic neurotransmission. Stimulation of $\alpha 7$ nAChRs triggers aspartate and glutamate excitatory amino acids in hippocampal synapses and increase the number of glutamatergic synapses (Lozada et al. 2012; Zappettini et al. 2010); it also potentiates NMDA receptor function (Cheng and Yakel 2015). On the

other hand, studies have indicated that the exposure to Acetamiprid reduces $\alpha 7$ nAChR expression in the hippocampus (Terayama et al. 2016). According to these studies, it is postulated that the effect of Acetamiprid on glutamatergic system is mediated through its effect on nAChRs.

Conclusion

Our study indicated that the subacute exposure to Acetamiprid impairs spatial learning and memory in MWM test. This behavioral deficit was due to the hippocampal glutamatergic system dysfunction at low doses and loss of neural cells in dentate gyrus area of hippocampus at high dose. In addition, Acetamiprid is largely absorbed from GI and crosses to the brain-blood barrier even at a low dose. Therefore, people who are repeatedly exposed to Acetamiprid such as agricultural workers and pesticide manufacturing factory workers are at neurotoxic risk of Acetamiprid.

Authors' contributions MS designed the study and interpreted the results and was a major contributor in writing the manuscript. MSh carried out the experiments and collect the data. SS was involved in the gene expression experiments. AO was involved in histological evaluation of the brain sections. All authors read and approved the final manuscript.

Data availability Not applicable

Compliance with ethical standards

Conflict of interest The author declares that they have no conflict of interest.

Ethics approval and consent to participate All experimental procedures such as working with animals and tissue collection were approved by the ethical committee of the Tarbiat Modares University (ethical code: IR.MODARES.REC.1397.115).

Consent for publication Not applicable

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