CrossMark

RESEARCH ARTICLE

Neuroprotective effects of curcumin against acetamiprid-induced neurotoxicity and oxidative stress in the developing male rat cerebellum: biochemical, histological, and behavioral changes

Ines Bini Dhouib[1,2] · Alya Annabi[2] · Raoudha Doghri[3] · Ines Rejeb[2] · Yosra Dallagi[2] ·
Yassin Bdiri[2] · Mohamed Montassar Lasram[2] · Amel Elgaaied[4] · Raja Marrakchi[4] ·
Saloua Fazaa[2] · Asma Gati[4]

Abstract Curcumin is a molecule found in turmeric root that has anti-inflammatory, antioxidant, and anti-tumor properties and has been widely used as both an herbal drug and a food additive to treat or prevent neurodegenerative diseases. This study aimed to investigate the effect of curcumin on neurobehavioral and neuropathological alterations induced by acetamiprid on male rats. Three groups of ten male Wistar rats each were used for the study: the first was a control group (CTR) that did not consume acetamiprid (ACE); the second was an experimental group (ACE) that consumed 40 mg/kg body weight/day of acetamiprid; and the third group (CUR) received curcumin (100 mg/kg) and acetamiprid (40 mg/kg) in combination. Neurobehavioral evaluations including inclined plane performance and forepaw grip time were studied. Treatment with CUR significantly prevented ACE-treated rats from impairments in the performance of neurobehavioral tests, indicating the presence of deficits on sensorimotor and neuromuscular responses. In addition, Curcumin administration protects rats against acetamiprid-induced cerebellum toxicity such as increase in AChE and BChE activities, decrease on cells viability, oxidative stress, and an increase of intracellular calcium. Taken together, these results demonstrate for the first time that ACE treatment substantially impairs the survival of primary neuronal cells through the induction of necrosis concomitantly with the generation of an oxidative stress. Additionally, curcumin reduced histopathological changes caused by ACE.

Keywords Acetamiprid · Curcumin · Cerebellum · Necrosis

✉ Ines Bini Dhouib
dhouib_ines@yahoo.fr

[1] Laboratory of Venoms and Therapeutic Biomolecules, LR11IPT08, Pasteur Institute of Tunis, University of Tunis El Manar, Tunis, Tunisia

[2] Department of Biology, Faculty of Sciences of Tunis, Laboratory of Aggression Physiology and Endocrine Metabolic Studies, University of Tunis El Manar, Tunis, Tunisia

[3] Laboratory of Anatomo-Pathology, Institut Salah Azaiez, Tunis, Tunisia

[4] Department of Biology, Faculty of Sciences of Tunis, Laboratory of Genetics, Immunology and Human Pathology, University of Tunis El Manar, Tunis, Tunisia

Introduction

The neonicotinoids (NCs) belong to a new class of insecticides that are commonly used to protect crops from pest insects and domestic animals from fleas (Tomizawa et al. 2000). The neonicotinoids have been reported to act as agonist of nicotinic acetylcholine receptors (nAChRs), and their high toxicities to insects have been attributed to its selective binding affinity to insect nAChRs (Tomizawa et al. 2000). A few reports have been interested on the study of neonicotinoid-induced toxicity in the nervous systems of vertebrates, and the majority of them were conducted principally with neonicotinoids, such as imidacloprid, thiamethoxam, clothianidin, and acetamiprid.

NCs act as agonists of nAChRs in rat cells and induce changes on the membrane properties of neurons in the mouse cochlear nucleus (Casida 2010). Therefore, NC exposure is a consistent producer of neuronal toxicity, particularly when the exposure occurs during periods of enhanced neuronal vulnerability. In addition, according to some authors, the cerebellum is one of the brain parts that is particularly sensitive to environmental insult, including pesticide exposure (Thomas et al. 1998; Tran et al. 2005).

In the developing cerebellum, NC exposure leads to the degeneration of cognitive and behavioral functions and causes impairment in Purkinje and granule cells and in glia (Baltazar et al. 2014). Purkinje cells are the dominant elements involved in the processing of cerebellar information, so, Purkinje cell dysfunction can damage motor coordination, the main feature of Parkinson's disease (PD) (Baltazar et al. 2014). In fact, several studies reported that exposition to NC pesticides can cause PD (Cicchetti et al. 2009). Parkinson's disease is a neurodegenerative pathology characterized by a damage of the dopaminergic neurons present in the substantia nigra associated with a dopamine loss in the basal ganglia. Among the early events that are highly involved on the generation of neurodegeneration are oxidative stress and mitochondrial dysfunction (Cicchetti et al. 2009; Jeschke et al. 2011).

The central nervous system and particularly cerebellum are susceptible to oxidative stress due to its high oxygen consumption rate, elevated levels of polyunsaturated fatty acids, and relatively low content of antioxidative enzymes (Fahn and Cohen 1992). Tremendous research efforts have been made to identify potential neuroprotective agents that can ameliorate pesticide-induced developmental central nervous system damage, so targeting NC pesticide-induced ROS and oxidative stress would be a logical preventative approach.

Synthetic drugs or natural products have been more and more examined for potential use in attenuation of toxicities induced by environmental toxicants, such as curcumin. Curcumin, a non-toxic component, is a polyphenol with antioxidant, antimitogen, and anticancer properties and therapeutic potential in neurological disorders (Lonare et al. 2014; Sahu et al. 2016; Marzouki et al. 2017). Curcumin have the capacity to cross the blood-brain barrier in many neurological alterations such as Alzheimer's and Parkinson's disease (Chattopadhyay et al. 2004). Recently, we showed that curcumin protects mitochondria against nitrosative and oxidative stress both in vitro and in vivo (Bayomi et al. 2015). Although, these studies represent a large range of actions for curcumin, the neuroprotective effects of curcumin against damage in the developing cerebellum have not fully been explored. Therefore, the present study investigates the recovery effect of curcumin against acetamiprid-induced toxicity in cerebellum using behavioral studies, as well as biochemical and histological analysis to arrive at a conclusion.

Material and methods

Animals and chemicals

Adult male Wistar rats weighting 150 ± 20 g (10 weeks old) were procured from Tunisian Society of Pharmaceutical Industries and we housed two per clean plastic cage and

allowed them to acclimatize in the laboratory environment. Animals were maintained in a mass air displacement room with a 12-h light: 12-h dark cycle at 24 ± 2 °C with a relative humidity of $50 \pm 10\%$. Balanced food and drinking water were given to the animals ad libitum. Acetamiprid technical 98% pure was obtained from the Ministry of Agriculture, Tunisia.

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Animal experiments were carried out under strict compliance with the Guidelines for Ethical Control and Supervision in the Care and Use of Animals. All efforts were taken to minimize suffering.

Experimental design

After a pilot study and determination of optimum ACE dose (40 mg/kg), the rats were randomly divided into three groups of ten animals each. One group was orally given equal amount of vehicle (corn oil) and used as the control; the second group received a dose of 40 mg/kg of acetamiprid during 21 consecutive days. The last group received a dose of 40 mg/kg of acetamiprid and 100 mg/kg of curcumin. No overt sign of toxicity or mortality were observed in any group of rats during the experimental period.

Behavioral analysis

The analysis of the behavior was employed in these studies included of sensorimotor reflexes and motor strength evaluation. The test was performed by an observer who was blind to the animal's treatment status and was carried out in a soundproof room with subdued lighting.

Inclined plane (Fig. 1)

Rats were placed on a flat plane in a horizontal position, with the head facing the side of the board to be raised (Abou-Donia et al. 2008). Inclined plane performance was measured with a standard protractor to the nearest 5°. A trial ended when the rat began to slip backward; therefore, there was no specific trial duration. The angle at which the rat began to slip downward was recorded. The results of the two trials were averaged. Trials were separated by 1 h.

Forepaw grip time (Fig. 2)

The rat's forepaw strength was assessed by having it grip on a 5-mm-diameter wood dowel that was held horizontally and raised so that the animal supported its body weight, as described by Andersen et al. (1991) and Abou-Donia et al. (2008). Time to release grip was recorded in seconds. All rats attempted to grip the dowel during this grip strength testing.

Fig. 1 Schematic representation and experimental protocol of the inclined plane. The inclined plane, which assesses an animal's ability to maintain its position on a board which is raised in 5° increments and thus can be used as an index of hind limb strength. The maximum angle at which a rat is able to maintain its position for at least 5 s constitutes the inclined plane score
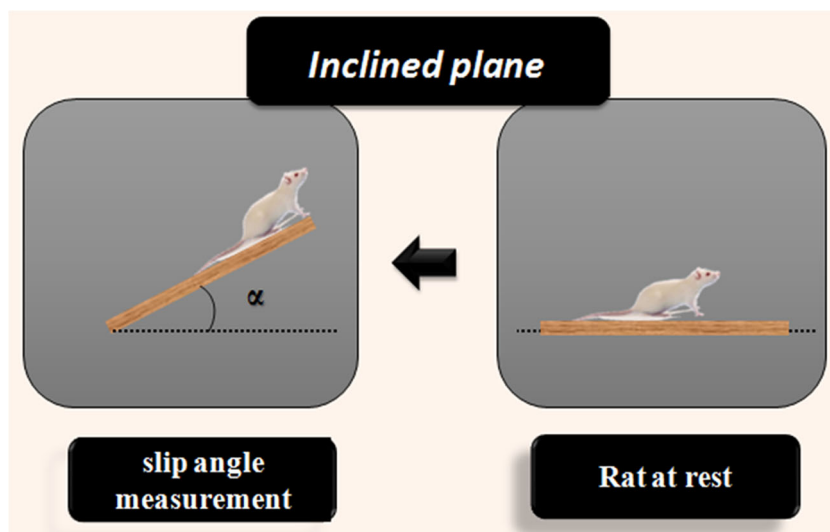
The results of the two trials were averaged. Trials were separated by 1 h.

Biochemical studies

After 21 days of treatment, fasted animals were decapitated without preliminary anesthesia and the cerebellum was removed for the determination of cell viability and biochemical analysis.

Cholinesterase determination

Plasma acetylcholinesterase (E.C.3.1.1.7) activity was determined at 25 °C in phosphate buffer Tris (0.1 M; pH7.4) with 0.3 mM DTNB using 1.0 mM ATCh by the Ellman spectrophotometric method (Ellman et al. 1961). Also, cerebellum homogenate was used for the estimation of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities (Ellman et al. 1961).

Oxidative stress determination

Lipid peroxidation is detected by the determination of malondialdehyde (MDA) production with the method published by Begue and Aust et al. (1978). Superoxide dismutase (SOD) was assayed according to Misra and Fridovich (1972). Catalase (CAT) activity was assayed by the method previously described by Aebi (1984). Thiol group (SH) level was assayed according to the method of Hu and Dillard (1994).

Cellular viability determination

Tissue calcium release was measured by spectrophotometer according to the methods of Stern and Lewis (1957).

MTT assay (3[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) was used to evaluate cellular viability. The formazan formation was spectrophotometrically analyzed at 570 nm (Denizot and Lang 1986). Results are indicated as percentage of reduction viability taken as reference control rats for which 100% viability was attributed.

Evaluation of cell apoptosis/necrosis rate by flow cytometry To quantify the apoptotic/necrotic cells, the staining of cerebellum cells was performed using the Annexin V-FITC/PI Apoptosis Detection Kit (BD Pharmingen, Heidelberg, Germany) according to the manufacturer's instruction. The cells were analyzed by flow cytometry (Accuri C6, BD, USA).

Histopathological studies

Small pieces of cerebellum of each animal of control and treatment groups were fixed in 10% formal saline solution. After washing and dehydration in alcohol, paraffin-embedded sections were cut (5-6 μm thickness) and stained with hematoxylin (H) and eosin (E) for microscopic examination at × 400 magnification.

Statistical analysis

All data were normally distributed and presented as the mean ± standard deviation (mean ± SD). In cases of multiple comparisons, data were analyzed using a one-way analysis of variance (one-way ANOVA). A p value of less than 0.05 was considered statistically significant.

Fig. 2 Schematic representation and experimental protocol of the forepaw grip test. The forepaw grip time was used to evaluate the motor strength of the rats. This was conducted by having rats hung down from a 5-mm diameter wood dowel gripped with both forepaws. The time spent by each rat before releasing their grips was recorded in seconds
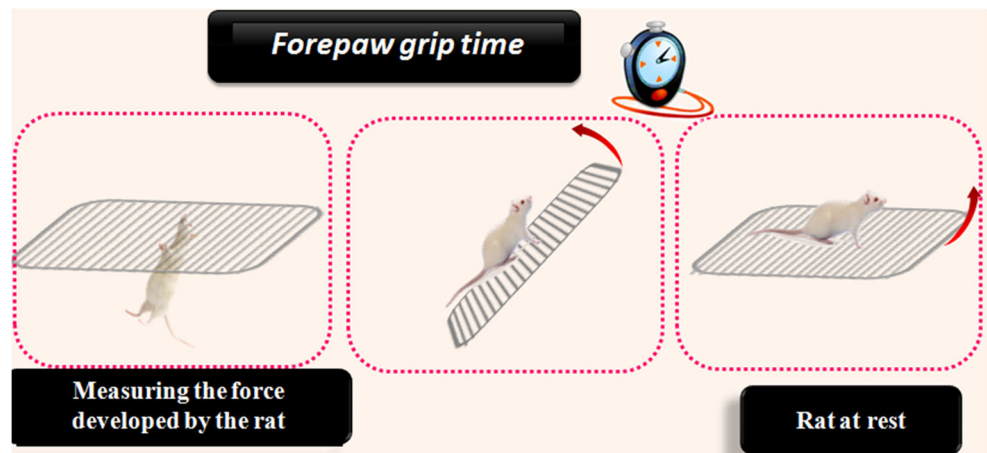
Results

Effects on locomotor activity

The data for inclined plane and grip time of rats exposed to ACE and CUR are presented in Fig. 3. The inclined plane test showed a significant increase of angle degree following ACE treatment (Fig. 3a). Grip time also showed a significant deficit (Fig. 3b). The simultaneous treatment of CUR with ACE showed significant improvement in inclined plane test.

Effects on plasmatic AChE activity

Changes in acetylcholinesterase activity following acetamiprid dosing are shown in Fig. 4. Acetylcholinesterase activity in the plasma was significantly increased in ACE rats compared with the control ones.

Curcumin supplementation reversed significantly the ACHE activity by 124.08% in CUR + ACE-treated group when compared to ACE-treated group.

Effects on cerebellum BChE and AChE activities

Exposure to acetamiprid induced a significant increase in cerebellum AChE (124.23%) and BChE (143.72%) activities. The results are presented in Table 1.

CUR rats showed significant decrease in cerebellum BChE and AChE activities compared to ACE rats (Table 1).

Effects on cerebellum Ca^{2+} level

Results show a significant increase in cerebellum calcium level in acetamiprid-treated rats compared with the corresponding control values. CUR seems to have gender-related differences when calcium level increases significantly tending to normal values (Table 1).

Effects on cerebellum weight and on oxidative stress

Mean cerebellum weights are shown in Table 2. There was no significant difference in cerebellum weight between the control and treated group (1.401 ± 0.04 vs 1.35 ± 0.03 mg).

Table 2 depicts the effect of 40 mg/kg of acetamiprid on lipid peroxidation (LPO) in the cerebellum of rat. LPO was measured in terms of malondialdehyde (MDA) produced in the cerebellum of rat. MDA formed was significantly ($p < 0.05$) higher in ACE-treated rats when compared with control. Concurrent administration of curcumin in the ACE-treated rats resulted in significant decrease ($p < 0.05$) in the MDA level.

Results of total thiol in the cerebellum are presented in Table 2. Total thiol was significantly decreased ($p < 0.05$) at this dose of ACE as compared to control. Co-administration of curcumin in ACE group caused significant increase ($p < 0.01$) in the total thiol content compared to ACE-alone-treated group.

Acetamiprid treatment significantly increased SOD and CAT activities in the cerebellum of rat. SOD activity was significantly restored by co-administration of curcumin in the ACE rat group, while there was no change in the activity of catalase in this tissue of rats administered with curcumin when compared with ACE rats (Table 2).

Effects on cell viability

As an initial approach, neuronal viability following ACE exposure was determined by measuring the reduction of MTT (Fig. 5). ACE resulted in reduction of viability on 29.05% compared to control rats (100% viability). On the other hand, supplementation of curcumin nullified the loss of mitochondrial function produced by ACE. Indeed, viability was increased by 130% compared with ACE rats (Fig. 5).

To determine the type of cell death (apoptosis or/and necrosis), we evaluated the apoptotic/necrotic effect of ACE on rat cerebellum using annexin V-FITC/PI staining (Fig. 6).
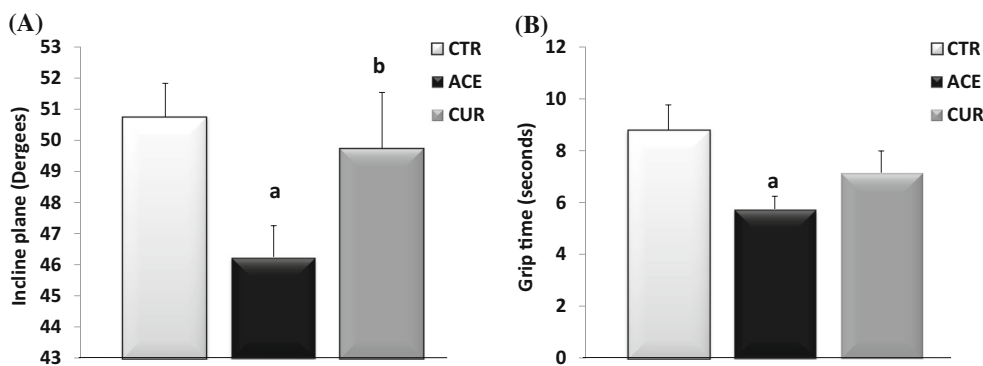
Fig. 3 Effects of curcumin and/or acetamiprid exposure on inclined plane and grip time on male rats. The inclined plane test showed a significant effect following acetamiprid treatment (Fig. 3a). Grip time also showed a significant deficit (Fig. 3b). These results indicate that male rats treated with acetamiprid have sensorimotor deficits. On the other hand, curcumin present a recovery effect for behavioral study.

CTR, control group; ACE, treated group with acetamiprid; CUR, treated group with ACE and supplemented with curcumin. Values are expressed as mean ± SD (n=10). a Significantly different from the control group ($p < 0.05$). b Significantly different from the ACE group and CUR group($p < 0.05$)

These results indicate that the increased significant proportion of AV +/PI + cells observed under acetamiprid treatment results from necrotic membrane disruption. Overall, these data demonstrate that ACE induces primary necrosis in rat cerebellum (Fig. 6a). Also, results show that necrotic cells in CUR treatment group were decreased compared to ACE group (Fig. 6a). These results indicate that CUR can reduce ACE-induced necrosis in rat cerebellum.

In addition, after treatment with 40 mg/kg of ACE for 21 days, percentage of annexin V-positive cells was 41.11%. Apoptotic cells increased slightly on ACE groups compared with the control group (Fig. 6b).To evaluate the role of curcumin in ACE-induced damage to rat cerebellum,

apoptotic cells exposed to ACE with curcumin (100 mg/kg) were detected by annexin V-FITC staining. Results showed that apoptotic cells in CUR treatment group were decreased but not significantly compared to ACE group (Fig. 6b).

Histopathology

Figure 7 and Table 3 depict the effect of acetamiprid exposure on the cerebellum of male rat. Control group showed a distinct Purkinje layer with well-defined cells; acetamiprid exposure (40 mg/kg) produced disturbances in the Purkinje cells and a significant damage to the Purkinje layer. Consequently, ACE altered the integrity of the Purkinje cells. However, curcumin supplementation preserved partially the histopathological changes.
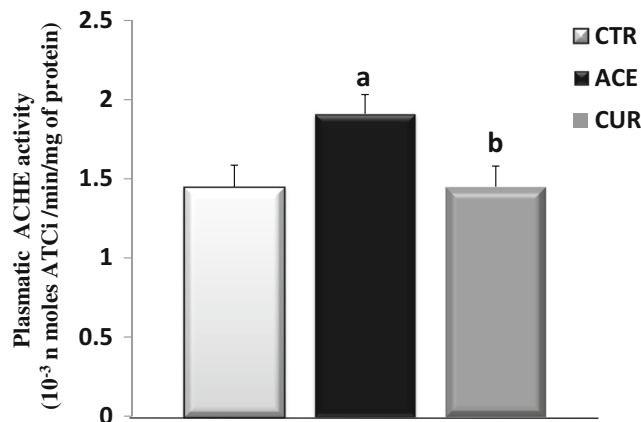
Discussion

In the present investigation, we employed advanced methods to uncover the mechanisms of neurotoxicity and apoptotic

Fig. 4 Effects of curcumin and/or acetamiprid exposure on plasmatic AChE activity. Plasmatic AChE activity in the male rats treated with acetamiprid showed a significant increase compared with control group. Result indicated that co-administration of curcumin (100 mg/kg) and acetamiprid (40 mg/kg) has a significant effect and decreased AChE activity in comparison to acetamiprid-treated group. CTR, control group; ACE, treated group with acetamiprid; CUR, treated group with ACE and supplemented with curcumin. Values are expressed as mean ± SD (n=10). a Significantly different from the control group ($p < 0.05$). b Significantly different from the ACE group and CUR group($p < 0.05$)

Table 1 Effects of curcumin and/or acetamiprid exposure on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities, and calcium levels in the cerebellum of adult rats

	CTR	ACE	CUR
AChE (u/mg protein)	1.91 ± 0.122	2.521 ± 0.122[a]	1.9 ± 0.107[b]
BChE (u/mg protein)	0.157 ± 0.004	0.279 ± 0.043[a]	0.284 ± 0.016[b]
Ca²⁺ (mg/dl)	1.35 ± 0.03	1.74 ± 0.03[a]	1.33 ± 0.06[b]

Values are expressed as mean ± SD ($n = 10$)

CTR control group, *ACE* treated group with acetamiprid, *CUR* treated group with ACE and supplemented with curcumin

[a] Significantly different from the control group ($p < 0.05$).

[b] Significantly different from the ACE group ($p < 0.05$).

Table 2 Effects of curcumin and/or acetamiprid exposure on tissue weight and oxidative status in the cerebellum of adult rats

	CTR	ACE	CUR
Cerebellum weight (g)	1.401 ± 0.04	1.35 ± 0.03	1.37 ± 0.02
MDA (nmoles MDA/mg protein)	0.891 ± 0.03	1111 ± 0.06^a	0.898 ± 0.066^b
TSH (mM)	0.065 ± 0.004	0.039 ± 0.01^a	0.065 ± 0.006^b
SOD (nmoles SOD/mg protein)	0.021 ± 0.001	0.033 ± 0.003^a	0.02 ± 0.004^b
CAT (nmoles CAT/mg protein)	$0.47 \times 10^{-3} \pm 0.06 \times 10^{-3}$	$0.93 \times 10^3 \pm 0.14 \times 10^{-3a}$	$0.98 \times 10^{-3} \pm 0.12 \times 10^{-3}$

Values are expressed as mean $\pm$ SD ($n = 10$)

CTR control group, *ACE* treated group with acetamiprid, *CUR* treated group with ACE and supplemented with curcumin

[a] Significantly different from the control group ($p < 0.05$)

[b] Significantly different from the ACE group ($p < 0.05$)

effect of acetamiprid in male rat cerebellum. We assessed the effect of ACE on oxidative status, cell viability, and histopathology and also evaluated the ameliorative role of curcumin; hence, the neurodegenerative studies of ACE are limited.

Our results showed that subacute exposure to a dose of 40 mg/kg of ACE produced significant sensorimotor and neuromuscular impairments that were reflected in the inclined plane performance and forepaw grip in rats. Similar to previous reports by Abou-Donia et al. (2008), they found significant impairment in hippocampus and cerebellum after imidacloprid, a neonicotinoid similar to ACE, exposure in rat pups. This change was resulting on decrease in hanging wire grip strength observed in imidacloprid-exposed pups indicating that it affected motor coordination by altering neurogenesis and brain development. These neurobehavioral deficits may reflect dysfunction at multiple anatomical areas in the cerebellum. These effects are mediated by a complex

Fig. 5 Effects of curcumin and/or acetamiprid exposure on the percentage of cell viability. Cell viability was evaluated by MTT test. In fact, acetamiprid decreased the percentage of cell viability in the cerebellum of male rats. Curcumin, at the dose of 100 mg/kg, had a neuro-cytological effect. CTR, control group; AC, treated group with acetamiprid; CUR, treated group with ACE and supplemented with curcumin. Values are expressed as mean $\pm$ SD (n=10). a Significantly different from the control group ($p < 0.05$). b Significantly different from the ACE group and CUR group($p < 0.05$).

array of multiple pathways. Indeed, inclined plane performance and forepaw grip are an integrated form of behavior necessitating pertinent levels of consciousness, memory and sensorimotor, peripheral nervous system, neuromuscular junction, and muscular functions (Abou-Donia et al. 2008).

Acetamiprid, like other neonicotinoid insecticides, acts as an agonist at the postsynaptic nicotinic acetylcholine receptor (nAChR) of insects (Tomizawa and Casida 2005). Herein, plasmatic and cerebellum AChE activities were significantly increased in male rats. AChE, an enzyme involved in the metabolism of acetylcholine (ACh) and a neuromodulator at the cholinergic synapses also plays a major role in synaptic plasticity, specifically in the control of locomotor activity (Lane et al. 2004). Increased AChE activity in this region may contribute to the neurotoxic effects resulting from decreased ACh and less than optimal function of ACh receptors. Furthermore, studies showed that increased expression of AChE produces neurodegeneration in vivo and in vitro (Yang et al. 2002; Day and Greenfield 2003). Like AChE, butyrylcholinesterase (BChE) inactivates the acetylcholine (ACh) neurotransmitter and is hence a viable therapeutic target in Parkinson's and Alzheimer's disease characterized by a cholinergic deficit (Greig et al. 2005; Baltazar et al. 2014).

Ours results showed that ACE exposure increases the level of Ca^{2+}. This raise may be due to inhibition in the activities of Na^+/K^+, Ca^{2+}, and Mg^{2+} ATPases (Mani and Sadiq 2014). Inhibition of these enzymes could be due to the interaction of pesticide with Mg^{2+} and Na^+/K^+ ATPases thereby: (i) disturbs the Na^+/K^+ pump, (ii) inhibition of Mg^{2+}ATPase activity might be due to the damage of the mitochondrial membranes, (iii) inhibition of Ca^{2+}ATPase activity might be due to lipophilic nature of acetamiprid and (iiii) degradation products of lipid peroxidation and target voltage-sensitive sodium channels.

In fact, in the present study, we showed an increase in lipid peroxidation (LPO) and in the enzymatic antioxidants activities in ACE-induced group. Acetamiprid-induced oxidative stress, leading to the formation of free radicals and causing
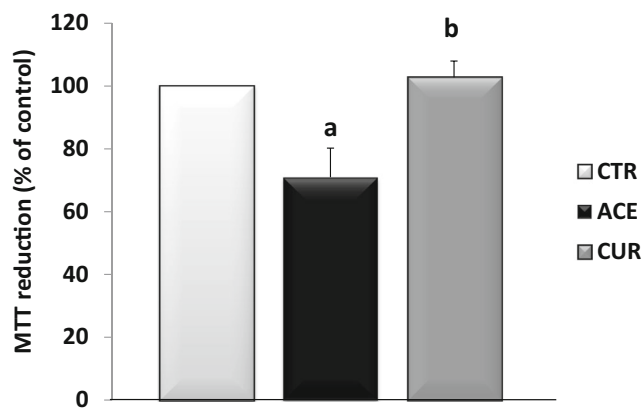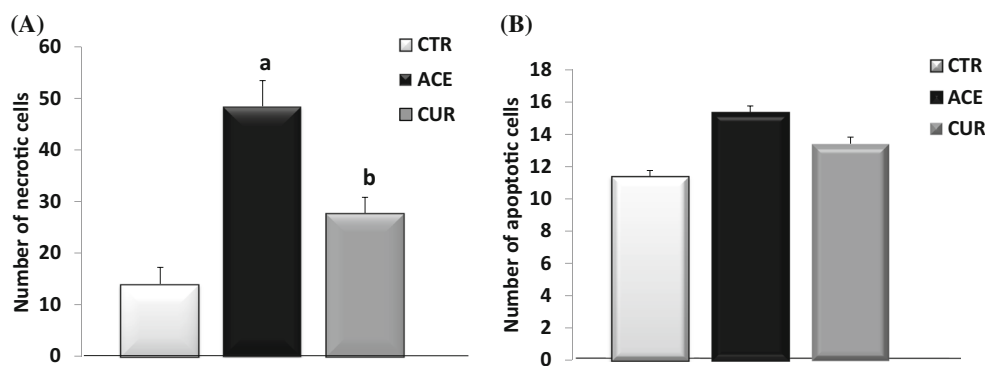
Fig. 6 Effects of curcumin and/or acetamiprid exposure on the number of cell death. Figure 6 presents the quantification of acetamiprid-induced cell death and the effect of curcumin at the dose of 100 mg/kg on the number of necrotic (Fig. 6a) and apoptotic cells (Fig. 6b). Annexin V-positive cells were counted in the same area (600 × 900 μm) and then analyzed statistically. In Fig. 6a, we showed that ACE induces primary necrosis in rat cerebellum. Also, results show that necrotic cells in CUR treatment group were decreased compared to ACE group. CTR, control group; ACE, treated group with acetamiprid; CUR, treated group with ACE and supplemented with curcumin. Values are expressed as mean ± SD (n=10). a Significantly different from the control group ($p < 0.05$). b Significantly different from the ACE group and CUR group($p < 0.05$)

lipid peroxidation, leads to molecular mechanism of neonicotinoid pesticide-induced neurotoxicity (Lonare et al. 2014).

Herein, we investigated the possible role of oxidative stress in ACE-induced neurotoxicity in order to evaluate its participation in the cerebellum damage mechanisms responsible for the neurological impairment. Membrane lipids in the cerebellum contain high levels of polyunsaturated fatty acids and are therefore particularly sensitive to oxidation. LPO is plausibly the most extensively investigated process induced by free radical and hence regarded as excellent indexes of oxidative stress.

This prompted us to investigate possible ACE-provoked protein oxidation. Subsequently, we examined the level of thiol profile in which we have observed that there was a significant decrease in the TSH contents in the cerebellum. TSH includes free amino thiols ($-$SH) which are natural reservoirs of the reductive capacity of the cell. It has been widely recognized that $-$SH plays an integral part in homoeostasis and has an extensive role in oxidative physiology (Gultekin et al. 2001). TSH buffers free radicals in tissue and provides protection to the cells from oxidative damage by reducing disulphide groups of cellular molecules or by scavenging free radicals (Khan et al. 2010). Thus, TSH inhibition could increase the susceptibility of cell membrane towards peroxide attacks (El-Gendy et al. 2010).

In addition, SOD and CAT are the most important defense mechanisms against toxic effects of oxygen metabolism. These antioxidant enzymes can, therefore, alleviate the toxic effects of ROS (Duzguner and Erdogan 2012). In the present investigation, the activity of SOD was increased in cerebellum. The superoxide radical is the most well-known oxygen-derived free radical and can lead to the formation of additional reactive species. H_2O_2, because of its non-ionized state, is able to diffuse through hydrophobic membranes and can form hydroxyl radicals that react with organic lipids to act like highly reactive free radicals (Duzguner and Erdogan 2012). These can cause cellular damage and cell death. The increased activity of SOD can also demonstrate the high enzymatic activity to lead to the formation of superoxide radicals and H_2O_2, which in turn can form hydroxyl radical. The high CAT activity induced by ACE may be due to the flux of superoxide radicals.

On the other hand, many studies have shown that ROS production, responsible for oxidative stress, induces the death of nervous cells. In this data, cell viability and death were measured by both MTT assay and annexin V test, as index of apoptosis and necrosis. In fact, we found that MTT assay showed higher degree of loss in cell viability on treated rats compared with CTR group. MTT assay revealed that at 40 mg/kg of ACE, the cell mortality was 31%. Now, the question is: does ACE cause death of Purkinje and granule cells in the cerebellum of adult rats? To answer this query, we analyzed the cerebellum cell suspension using annexin V test. Flow cytometry revealed that ACE induced less apoptosis and more necrosis on rat cerebellum cells. Similarly, Singh et al. (2012) noted that exposure to ACE produced necrosis of Purkinje cells with loss of dendrites and granules in the granular layer of the cerebellum in female rats. The cell death induced by ACE in cerebellum cells may be mediated through intrinsic pathway of cell death (Kumi-Diaka et al. 1999).

Further investigations are required to study how cell lesions induced by neonicotinoid pesticides interfere with cerebellar connections to other cortical regions during the performance of motor, behavioral, and cognitive functions. To answer this question, Curtin et al. (2002) indicated that increased oxidative stress has been shown to have a correlation with increased cell death. In fact, ROS generation can cause cell death either by apoptosis or necrosis, two distinct cell death pathways. In
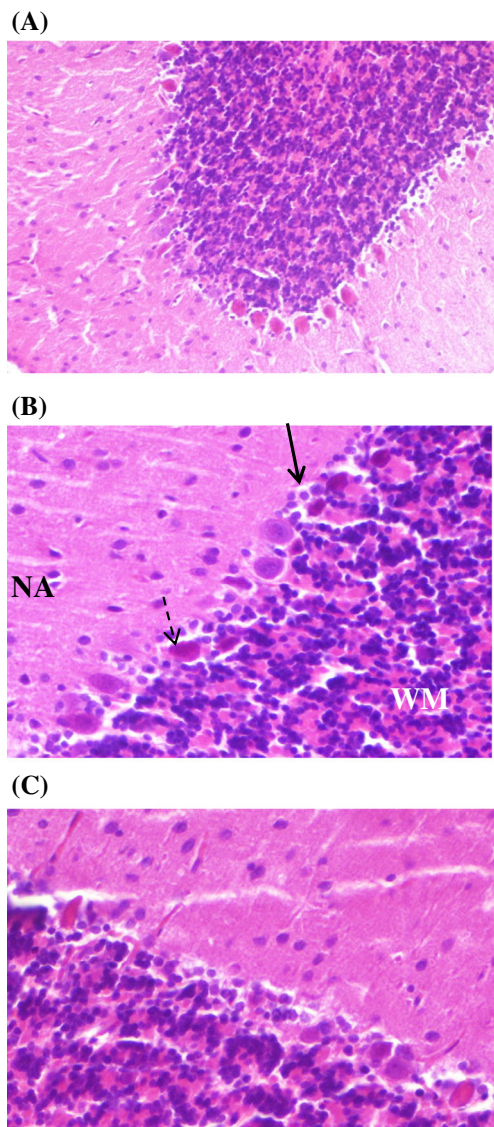
(A)

(B)

NA

WM

(C)

Fig. 7 Histological architecture of the cerebellum of male rats. The cerebellum were collected after 21 days of exposure. Microscopic examination at × 400 magnification was performed after staining with hematoxylin and eosin. Representative images are shown for the three following groups of rats control (CTR), acetamiprid (ACE), and acetamiprid-curcumin (CUR) groups. **a** Histological architecture of the cerebellum in CTR group with normal structure of molecular and granule cell layer and with single intervening Purkinje cells. **b** Cerebellum of ACE-treated group rat showing meningeal congestion (arrow) and degeneration changes in Purkinje cells (broken arrow). **c** Cerebellum of ACE plus CUR-treated male rat showing almost normal structure and perivascular space. NA, necrotic area; WM, white matter

Table 3 Grading of the histopathological changes in the cerebellum of experimental groups of adult rats

	Molecular layer	Purkinje cells	Granular layer	White matter
CTR	Two types of cells are present	Typically arranged in a single row at junction of molecular and granular layers	Contains numerous small granular cells with dispersed irregularly glomeruli	Consists of myelinated nerve fibers also contains dendrites and numerous neuralgia
ACE	Vacuolated spaces	Degenerative changes in Purkinje cells	Congestion	
	Highly vacuolated cytoplasm in stellate and basket cells Some cells showing degenerative changes	Shrink Reduced in size Showing cytoplasmic vacuolation and faintly stained nuclei	Revealed sever sings of damage Lost their normal organization Both cell types glomeruli and Golgi II were deeply stained and representing clear coagulative necrosis	Showing degenerated nerve fibers Contained large vacuoles
CUR	Two types of cells are present	Typically arranged in a single row	Contains numerous small granular	Myelinated nerve fibers

CTR control group, *ACE* treated group with acetamiprid, *CUR* treated group with ACE and supplemented with curcumin

this data, ACE induced cell death by necrosis. In physiological conditions, necrosis occurs usually in response to severe trauma/injury to the cell and is characterized morphologically by cytoplasmic and mitochondrial swelling, plasma membrane rupturing, and release of the cellular contents into the extracellular space.

Our findings concerning the neurotoxicity of ACE were correlated with histopathological changes observed in cerebellum's rats. Under microscopic examination, severe distortions in cellular architecture were observed in the cerebellum of rats exposed to 40 mg/kg of ACE (Fig. 7), which was consistent also with the results of flow cytometry. Necrosed Purkinje cells and loss of granules in the granular layer of the cerebellum in this data have also provided support to the neurobehavioral effects indicating accumulation of ACE and its metabolites in the cerebellum. Our results corroborate with findings previously published by Singh et al. (2015). This can be due to reactive oxygen species which may contribute to histopathological changes in cerebral cortex of adult rats submitted to ACE treatment.

As the cerebellum has crucial role to control motor coordination, balance, muscle tone, motor learning, and cognition (Oliveira et al. 2014), damage at the cholinergic system in the cerebellum in ACE-treated rats may be associated with neurobehavioral alteration as observed in the present study. Thus,

our results demonstrate that these alterations may be caused by both cell death and oxidative stress. In addition, we evaluated the action of the curcumin, a natural product. Indeed, curcumin co-treatment in ACE-treated rats improved spontaneous inclined plane values and prevented cerebellum damage. Ours results corroborate with those of Lonare et al. (2014); they noticed that curcumin at a dose of 100 mg/kg restored locomotor activity after subchronic exposure to imidacloprid in rat. Besides, our results showed amelioration in AChE and BChE activities after curcumin supplies to the diet of ACE group. This product probably protected AChE and BChE activities via their antioxidant properties. Our hypothesis agrees with the previous findings of Tsakiris et al. (2000) showing that AChE and BChE activities are decreased by free radicals and prevented by antioxidants. Also, CUR treatment modulates the level of Ca^{2+} near to control level by its antioxidant, free radical scavenging, and anti-lipid peroxidation activities. In fact, curcumin co-exposure with ACE significantly alters the levels of lipid peroxidation by reducing MDA formation and decreases the enzymatic antioxidants. Similarly, Lonare et al. (2014) show that CUR administration resulted in decrease lipid peroxidation; improvement in enzymatic and non-enzymatic antioxidants status in brain with exposure of imidacloprid in rats was observed. The protective effect of curcumin against ACE-induced oxidative stress could be both: (i) direct by inhibiting lipid peroxidation and scavenging free radicals and (ii) indirectly through the enhancement defensive antioxidant systems to scavenge free radicals in the cerebellum.

In addition, curcumin provides protection against neurodegenerative diseases through scavenging free radicals, interaction with oxidative cascade and preventing its outcome, oxygen quenching and making it less available for oxidation reactions, and inhibiting oxidative enzymes like cytochrome P450 (Yang et al. 2005; Zhang et al. 2011; Lonare et al. 2014). Also, the presence of phenolic, methoxyl, and diketonic groups in curcumin structure gives it the possibility to cross the blood-brain barrier and to report a neuroprotective potential (Yang et al. 2005). Indeed, experimental and clinical studies reported that curcumin has been found to be effective in the treatment of Alzheimer's and Parkinson's diseases (Zhang et al. 2011; Sahu 2016). In other study, the neuroprotective effects promoted by curcumin thought the regulation of important enzymes and molecules involved in inflammation, such as cyclooxygenase-2 (COX-2), lipoxygenase, nuclear factor-kappa B (NF-κB), and cytokines (Goel et al. 2008). Furthermore, some studies have demonstrated that curcumin is able to modulate intracellular signaling pathways through the activity of the calcium-dependent protein kinase enzymes A, B, and C, as well as the inositol 1,4,5-triphosphate receptor, both of which are important to the neurotransmission (Jaques et al. 2011).

Conclusion

In summary, the present study findings suggest that exposure to 40 mg/kg of acetamiprid substantially impairs the survival of primary neuronal cells. In addition, curcumin at the dose of 100 mg/kg reduced histopathological changes caused by ACE and may be involved in cholinergic system modulation and exert an effect on motricity in part through its antioxidant action and inhibiting the peroxidation of lipids and improving the activity of antioxidant enzymes. However, we know that the protective effects of curcumin against primary neuronal cell death observed during acetamiprid toxicity may be mediated by multiple factors in addition to oxidative stress. Therefore, further investigations are required to investigate the potential therapeutic use of curcumin in preventing the cerebellum from acetamiprid-induced oxidative damage.

References

Abou-Donia MB, Goldstein LB, Sarah B, Tu T, Khan WA, Dechkovskaia AM, Abdel-Rahman AA (2008) Imidacloprid induces neurobehavioral deficits and increases expression of glial fibrillary acidic protein in the motor cortex and hippocampus in offspring rats following in utero exposure. J Toxic Environ Health A 71:119–130

Aebi HE (1984) Catalase in vitro. Methods Enzymol 105:121–126

Andersen CS, Andersen AB, Finger S (1991) Neurological correlates of unilateral and bilateral "strokes" of the middle cerebral artery in the rat. Physiol Behav 50:263–269

Baltazar MT, Dinis-Oliveira RJ, de Lourdes Bastos M, Tsatsakis AM, Duarte JA, Carvalho F (2014) Pesticides exposure as etiological factors of Parkinson's disease and other neurodegenerative diseases—a mechanistic approach. Toxicol.Lett 230(2):85–103

Bayomi SM, El-Kashef HA, El-Ashmawy MB, Nasr MNA, El-Sherbeny MA, Abdel-Aziz NI, El-Sayed MA-A, Suddek GM, El-Messery SM, Ghaly MA (2015) Synthesis and biological evaluation of new curcumin analogues as antioxidant and antitumor agents: molecular modeling study. Eur J Med Chem 101:584–594

Begue JA, Aust SD (1978) Microsomal lipid peroxidation. Methods Enzymol 52:302–310

Casida JE (2010) Neonicotinoid metabolism: compounds, substituents, pathways, enzymes, organisms, and relevance. J Agric Food Chem 59:2923–2931

Chattopadhyay I, Biswas K, Bandyopdhyay U, Banerjee RK (2004) Turmeric and curcumin: biological actions and medicinal applications. Curr Sci 87:44–53

Cicchetti F, Drouin-Ouellet J, Gross RE (2009) Environmental toxins and Parkinson's disease: what have we learned from pesticide-induced animal models? Trends PharmacolSci 30:475–483

Curtin JF, Donovan M, Cotter TG (2002) Regulation and measurement of oxidative stress in apoptosis. J Immunol Methods 265(1–2):49–72

Day T, Greenfield SA (2003) A peptide derived from acetylcholinesterase induces neuronal cell death: characterization of possible mechanisms. Exp Brain Res 153:334–342

Denizot F, Lang R (1986) Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. J Immunol Methods 89:271–277

Duzguner V, Erdogan S (2012) Chronic exposure to imidacloprid induces inflammation and oxidative stress in the liver and central nervous system of rats. Pestic Biochem Physiol 104:7

El-Gendy KS, Aly NM, Mahmoud FH, Kenawy A, El-Sebae AK (2010) The role of vitamin C as antioxidant in protection of oxidative stress induced by imidacloprid. Food ChemToxicol 48:215–221

Ellman GL, Courtney KD, Andres VJ, Featherstone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. BiochemPhamacol 7:88–95

Fahn S, Cohen G (1992) The oxidant stress hypothesis in Parkinson's disease: evidence supporting it. Ann Neurol 32:804–812

Goel A, Kunnumakkara AB, Aggarwal BB (2008) Curcumin as "Curecumin": from kitchen to clinic. BiochemPharmacol 75:787–809

Greig NH, Utsuki T, Ingram DK, Wang Y, Pepeu G, Scali C, Yu QS, Mamczarz J, Holloway HW, Giordano T, Chen D, Furukawa K, Sambamurti K, Brossi A, Lahiri DK (2005) Selective butyrylcholinesterase inhibition elevates brain acetylcholine, augments learning and lowers Alzheimer b-amyloid peptide in rodent. Proc Natl Acad Sci U S A 102:17213–17218

Gultekin F, Delibas N, Yasar S, Kilinc I (2001) In vivo changes in antioxidant systems and protective role of melatonin and a combination of vitamin C and vitamin E on oxidative damage in erythrocytes induced by chlorpyrifos-ethyl in rats. Arch of Toxicol 75:88–96

Hu ML, Dillard CJ (1994) Plasma SH and GSH measurement. MethodsEnzymol 233:385–387

Jaques JAS, Ruchel JB, Schlemmer KB, Pimentel VC, Bagatini M, SouzaVdo C et al (2011) Effects of curcumin on the activities of the enzymes that hydrolyse adenine nucleotides in platelets from cigarette smoke-exposed rats. Cell Biochem Funct 29:630–635

Jeschke P, Nauen R, Schindler M, Elbert A (2011) Overview of the status and global strategy for neonicotinoids. J Agric Food Chem 59:2897–2908

Khan RN, Matharoo-Ball B, Shaw RW (2010) Antioxidant enzyme expression, lipid peroxidation, and protein oxidation in human myometrium with parturition. Reprod Sci 17:78–84

Kumi-Diaka J, Nguyen V, Butler A (1999) Cytotoxic potential of the phytochemical genisteinisoflavone (4′, 5′, 7-trihydroxyisoflavone) and certain environmental chemical compounds on testicular cells. Biol Cell 91:515–523

Lane RM, Kivipelto M, Greig NH (2004) Acetylcholinesterase and its inhibition in Alzheimer disease. ClinNeuropharmacol 27:141–149

Lonare M, Kumar M, Raut S, Badgujar P, Doltade S, Telang A (2014) Evaluation of imidacloprid-induced neurotoxicity in male rats: a protective effect of curcumin. NeurochemInt 78:122–129

Mani VM, Sadiq AMM (2014) Naringin modulates the impairment of memory, anxiety, locomotor, and emotionality behaviors in rats exposed to deltamethrin; a possible mechanism association with oxidative stress, acetylcholinesterase and ATPase. Biomed Prev Nutr 4(4):527

Marzouki S, Bini Dhouib I, Benabdessalem C, Rekik R, Doghri R, Maroueni A, Bellasfar Z, Fazaa S, Bettaieb J, Barbouche MR, Ben Ahmed M (2017) Specific immune responses in mice following subchronic exposure to acetamiprid. Life Sci 188:10–16

Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and simple assay for superoxide dismutase. J BiolChem 247:3170–3175

Oliveira SA, Chuffa LGA, Fioruci-Fontanelli BA, Neto FSL, Novais PC, Tirapelli LF, Oishi JC, Takase LF, Stefanini MA, Martinez M, Martinez FE (2014) Apoptosis of Purkinje and granular cells of the cerebellum following chronic ethanol intake. Cerebellum 13:728–738

Sahu PK (2016) Design, structure activity relationship, cytotoxicity and evaluation of antioxidant activity of curcumin derivatives/analogues. Eur J Med Chem 121:510–516

Singh TB, Mukhopadhayay SK, Sar TK, Ganguly S (2012) Acetamiprid induces toxicity in mice under experimental conditions with prominent effect on the hematobiochemical parameters. J Drug MetabToxicol 3:6

Singh V, Hussein M, Singh AK, Hassan MA, Gupta P (2015) Histological and immunohistochemical changes in cerebellum of chick embryos after exposure to neonicotinoid insecticide imidacloprid. J Anat Soc India 64(2):122–127

Stern J, Lewis WHP (1957) The colorimetric estimation of calcium in serum with O-cresolphtaleincomplexone. Clin Chim Acta 2:576–580

Thomas JD, Goodlett CR, West JR (1998) Alcohol-induced Purkinje cell loss depends on developmental timing of alcohol exposure and correlates with motor performance. Brain Res Dev Brain Res 105(2):159–166

Tomizawa M, Lee DL, Casida JE (2000) Neonicotinoid insecticides: molecular features conferring selectivity for insect versus mammalian nicotinic receptors. J Agric Food Chem 48:6016–6024

Tomizawa M, Lee DL, Casida JE (2005) Neonicotinoid insecticide toxicology: mechanisms of selective action. Annu Rev Pharmacol Toxicol 45:247–268

Tran TD, Jackson HD, Horn KH, Goodlett CR (2005) Vitamin E does not protect against neonatal ethanolinduced cerebellar damage or deficits in eyeblink classical conditioning in rats. Alcohol Clin Exp Res 29:117–129

Tsakiris S, Angelogianni P, Schulpis KH, Stavridis JC (2000) Protective effect of phenylalanine on rat brain acetylcholinesterase inhibiton induced by free radicals, Clin. Biochemist 33:103–106

Yang L, Heng-Yi H, Zhang XJ (2002) Increased expression of intranuclearAChE involved in apoptosis of SK-N-SH cells. Neurosci Res 42:261–268

Yang L, Calingasan NY, Chen J, Ley JJ, Becker DA, Beal MF (2005) A novel azulenyl nitrone antioxidant protects against MPTP and 3-nitropropionic acid neurotoxicities. Exp Neurol 191:86–93

Zhang J, Wang Y, Xiang H, Li M, Li W, Ma K, Wang X, Zhang J (2011) Oxidative stress: role in acetamiprid-induced impairment of the male mice reproductive System. Agric Sci China 10(5):786–796