The Involvement of Oxidative Stress, Neuronal Lesions, Neurotransmission Impairment, and Neuroinflammation in Acrylamide-Induced Neurotoxicity

Mengyao Zhao  
East China University of Technology

Linlin Deng  
East China University of Technology

Xiaoxuan Lu  
East China University of Technology

Liqiang Fan  
East China University of Technology

Yang Zhu  
Wageningen University

Liming Zhao (✉️ zhaoliming@ecust.edu.cn)  
ECUST: East China University of Science and Technology  https://orcid.org/0000-0002-8523-103X

Research Article

Keywords: Acrylamide, Oxidative stress, Neurotoxicity, Neurotransmission impairment, Neuroinflammation, NLRP3 inflammasome

DOI: https://doi.org/10.21203/rs.3.rs-691568/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Acrylamide (ACR) is a typically contaminate during environmental industry and poses potential health hazards that have been attracting increasing attention. Its neurotoxicity is known to cause significant damage to health. However, the mechanisms of ACR-induced neurotoxicity require further clarification. This study explores how ACR-induced oxidative stress, neuronal lesions, neurotransmission impairment, and neuroinflammation mutually contribute to neurotoxicity using a mouse model. According to the results, oxidative stress was indicated by the presence of a distinct increase in cellular reactive oxygen species levels, malondialdehyde, and 8-hydroxy-2-deoxyguanosine content, as well as a significant decrease in the glutathione content after ACR exposure. Moreover, ACR caused neurological defects associated with gait abnormality and neuronal loss while suppressing the levels of acetylcholine and dopamine and increasing the protein expression of α-syn, further inhibiting cholinergic and dopaminergic neuronal function. Additionally, ACR treatment caused an inflammation response via NF-κB activation and increased the protein expression of NLRP3. Consequently, ACR activated the NLRP3 inflammasome constituents, including Caspase-1, ASC, N-GSDMD, IL-1β, and IL-18. The results revealed the underlying molecular mechanism of ACR-induced neurotoxicity via oxidative stress, neurotransmission impairment, and neuroinflammation-related signal cascade. This information will further improve the development of an alternative outcome pathway strategy for investigating the risk posed by ACR.

Highlights

ACR inhibited cholinergic and dopaminergic neuronal functionality.

ACR caused NF-κB activation and increased NLRP3 priming.

ACR activated NLRP3 inflammasome constituents, including ASC, Caspase-1, N-GSDMD, and IL-1β, IL-18.

Introduction

Acrylamide (ACR) is a white crystal-chemical used to produce polyacrylamide (Friedman, 2003). It has been traditionally used as an industrial raw material in soil conditioning, wastewater treatment, the textile industry, and more (Erkekoglu and Baydar, 2014). Reports have indicated that workers can be heavily exposed to ACR, showing symptoms and signs of peripheral nervous dysfunction (Hagmar et al., 2001). In addition, ACR has been revealed as a typical contaminant originating from the heat processing of food (Mottram et al., 2002; Stadler et al., 2002; Tareke et al., 2002). Since the presence of ACR has been revealed in food, ways to reduce its content has been continually sought, but existing methods cannot completely inhibit its formation (Dias et al., 2017; Koszucka et al., 2019; Rannou et al., 2016; Xu et al., 2016). The abundance and widespread occurrence of ACR in the environment and food products mean that the risk of toxicity to human health can no longer be ignored (Pundir et al., 2019).

Studies have shown that ACR displays neurotoxicity, genotoxicity, reproductive toxicity, and potential carcinogenicity (Exon, 2006). Of the ACR-induced injuries, damage to the nervous system is the most
serious (Guan et al., 2018; LoPachin et al., 2003). During the early 1990s, researchers observed sub-chronic neurotoxic symptoms in workers exposed to ACR for an extended time (Abelli et al., 1991). Repeated exposure to ACR at levels between 0.5 mg/kg/day and 50 mg/kg/day induced similar neurotoxic phenotypes in several species of laboratory animals, such as rodents, rabbits, guinea pigs, cats, and dogs (Barber et al., 2007; Erkekoglu and Baydar, 2014; LoPachin, 2004). Chemical level analysis indicated that this might be due to the formation of a covalent adduct between the ACR and highly nucleophilic cysteine at the active presynaptic neuron site, leading to neuron inactivation and affecting the neurotransmitter transmission process, finally resulting in neurotoxicity (LoPachin and Gavin, 2012). From a physiological and biochemical perspective, oxidative stress, which acts as an activation signal, is associated with direct or indirect ACR-induced neurotoxicity (Zamani et al., 2017; Zhao et al., 2017b).

Oxidative stress is caused by an imbalance between the production and elimination of oxygen species (ROS) (Cook and Petrucelli, 2012), while the central nervous system actively participates in oxygen metabolism (Patel, 2016). However, it is highly susceptible to oxidative damage since the enzymatic antioxidant activity in this region is lower than in other tissues (Salim, 2017). Both in vivo and in vitro experiments have shown that ACR can induce oxidative stress in neurocytes (Pan et al., 2017; Yousef and El-Demerdash, 2006), further inducing mitochondrial-dependent apoptosis that leads to neurotoxicity (Chen et al., 2013; Lee et al., 2014). In addition, cholinergic and dopaminergic dysfunction reportedly play a role in various neurodegenerative diseases, as well as other pathologies of the brain, such as Parkinson's disease and Alzheimer's disease, and are associated with oxidative stress (Pepeu and Grazia Giovannini, 2017; Qamar et al., 2017). Furthermore, ACR reduces acetylcholinesterase (AChE) activity in the brain (Elblehi et al., 2020). However, the relationship between ACR-induced oxidative stress and the dysfunction of neurotransmission requires further investigation.

Additionally, inflammation can also activate an immune response and enhance ACR-induced neurotoxicity (Santhanasabapathy et al., 2015). A previous study found that ACR induces inflammation via NF-κB translocation, releasing the downstream cytokine, IL-1β, to enhance the neurotoxic effect (Zhao et al., 2017a). The NOD-like receptor family pyrin domain containing 3 (NLRP3) refers to a downstream site regulated by NF-κB that is involved in the IL-1β release pathway (Zhong et al., 2016b). NLRP3 inflammasome activation is essential in triggering the inflammatory responses in the central nervous system (Song et al., 2017; Zhou et al., 2016). Oxidative stress can activate and assemble NLRP3 inflammasome (Abderrazak et al., 2015), playing a crucial role in neurodegenerative disease development, such as cerebral arteriosclerosis and Parkinson's disease (Heneka et al., 2014; Libby and Everett, 2019; Pirzada et al., 2020; Sarkar et al., 2017). We hypothesized that the shared target during NLRP3 inflammasome activation, ACR-induced neurotoxicity, and neurodegenerative factors suggest that NLRP3 inflammasome activation may lead to ACR neurotoxicity and may ultimately contribute to pathological neurodegenerative processing.

Gait abnormality are considered classical morphological characteristics of the neurotoxicity resulting from ACR (Tan et al., 2019). Several studies have examined cerebellum damage and/or peripheral motor nerve injury to explain ACR-induced gait abnormality (Lebda et al., 2015; Yan et al., 2018). Locomotion is
a highly complex process involving afferent sensory input, central neuron system processing, efferent motor commands and skeletal muscle coordination, which can be compromised due to failure at multiple levels (LoPachin et al., 2002b). Thus, both the cerebellum and cerebrum are important targets of ACR-related neurotic injury and degeneration (He et al., 2017; Lehning et al., 2002). However, the systemic analysis of ACR-induced dysfunction in both the cerebellum and cerebrum remains lacking. Furthermore, the synergistic contribution of oxidative stress, neurotransmission impairment, and neuroinflammation to ACR-induced neuronal lesions in the cerebellum and cerebrum requires further clarification.

This study aims to determine the connection between ACR-induced neurotic toxicological significance and the molecular mechanism in the central neuron system. First, a mouse model exposed to low, medium, and high-ACR doses is established to present the different neurotoxic processes. Then, the ACR-mediated neurotoxic and neurodegenerative effects are assayed. Follow-up analyses revealed that ACR-induced neurotoxicity stems from oxidative stress, neurotransmission impairment, and neuroinflammation in the central nervous system. Moreover, the signal cascade of NLRP3 inflammasome prime and activation is analyzed. This study aims to establish a more complete theoretical basis to reveal the mechanism of ACR toxicity and improve the adverse outcome pathway, provide a novel approach to the final targeted search of its toxic intervention, and promote the coordinated development of health safety.

Materials And Methods

Chemicals

ACR (purity > 99%) was purchased from Titan (Shanghai, China) and diluted with physiological saline for use in mice.

Animal and experimental design

The Animal Care and Use Committee of Laboratory Animals (Chinese Academy of Sciences; license number: SYXK (HU) 2019-0013) provided research ethics approval for the use of animals. The SLAC Laboratory Animal Center (Shanghai, China, license number: SCXK (HU) 2017-0008) provided the wild-type C57BL/6 male mice.

The mice were accommodated separately in a humidity- and temperature-controlled room, with a 12-h light/dark cycle. The animals received tap water and a standard diet. Before the experiment, they were allowed one week to acclimatize to the environment. At six weeks of age, the mice were randomly divided into six groups according to their weight (three negative control groups and three ACR treatment groups at different doses; n = 10 in each group). ACR neurotoxicity is progressive, while the rate of progression is dose-dependent (LoPachin, 2004). Considering that the dose-rate determined the onset time and development of neurotoxicity, 5 mg/kg BW ACR was selected as the low dose, referring to the lowest observed adverse effect level (LOAEL) of ACR exposure (Liu et al., 2020; LoPachin, 2004). Furthermore, 25
mg/kg ACR was selected as the medium dose and 50 mg/kg ACR as the high dose, to observed obvious nerve terminal damage caused by ACR (LoPachin et al., 2006). Table 1 shows the experimental details.

| Groups            | Experimental dose                  | Experimental times |
|-------------------|------------------------------------|--------------------|
| Control group 1   | 15 mL/kg B.W./d (Normal saline)    | 60 d               |
| Low dose group    | 5 mg/kg B.W./d (ACR)               | 60 d               |
| Control group 2   | 15 mL/kg B.W./d (Normal saline)    | 30 d               |
| Medium dose       | 25 mg/kg B.W./d (ACR)              | 30 d               |
| Control group 3   | 15 mL/kg B.W./d (Normal saline)    | 15 d               |
| High dose         | 50 mg/kg B.W./d (ACR)              | 15 d               |

The negative control groups were fed a powdered standard diet only and given normal saline daily via oral administration. All ACR treatment groups had their negative control groups throughout the experimental progression. The low-dose group received ACR once daily via oral administration at a dose of 5 mg/kg BW for 60 successive days (sub-chronic toxicity). The medium-dose group received ACR once daily via oral administration at a dose of 25 mg/kg BW for 30 successive days (subacute toxicity). The high-dose group received ACR once daily via oral administration at a dose of 50 mg/kg BW for 15 successive days (acute toxicity). Then, 18 h after the last ACR administration, the mice were anesthetized, euthanized, and dissected immediately. The cerebrums, cerebellums, livers, and spleens were immediately removed and weighed. For the biochemical analysis, the brains of five mice from each group were removed and immersed in liquid nitrogen for later use. For the histopathological examination, the cerebrums and cerebellums of five mice from each group were fixed with a 4% paraformaldehyde (PFA) solution. This process was followed by the dehydration, embedding, and sectioning of the brains, after which they were stained with H&E and Nissl. The samples were photographed, followed by histopathological analysis.

**Gait score**

Ataxia and hind-limb muscle weakness are the primary neurological defects associated with the induction of distal axonopathy by chemicals such as ACR (Spencer and Schaumburg, 1974).

Gait score observations represent a relatively sensitive measure of the onset and progression of neurological changes during ACR exposure (LoPachin et al., 2002b). Here, gait scores were determined every 3 d as indices of developing neurotoxicity, which was assessed by an independent examiner not involved in the experiments using a previously described method (Yan et al., 2018). The abnormal gaits of the mice, such as hind-limb spread, foot splay, and balance disorder, were observed and recorded while the animals were permitted to move freely in an unobstructed environment for 3 min. The scores were divided into four grades ranging from 1 to 4 according to severity. Here, 1 indicated a normal gait, 2 indicated a slightly affected gait, 3 denoted a moderately affected gait, and 4 referred to a severely affected gait. Furthermore, to allow for the occurrence of random errors, three consecutive assessments were conducted.

**Landing foot splay**
Landing foot splay was used as a simple and sensitive assessment method to determine the degree of disability and peripheral neuropathy caused by ACR-induced neurotoxicity (Edwards and Parker, 1977). The landing foot splay was measured according to previously described methods to assess athletic muscle ability (Yan et al., 2018b.). Briefly, at the end of each exposure period, the rear limbs of each mouse were painted with ink. They were dropped from a height of 30 cm onto a soft surface, after which measurements were taken of the distance between the central points of the right and left rear limbs. Three measurements were performed for each mouse at 30 min intervals. The landing foot splay was represented by the average value between the three measurements.

**Histochemical analysis**

**H&E staining**

The fixed tissues were subjected to a routine follow-up process for 48 h. The samples were dehydrated and shaped into paraffin blocks, which were cut into sections of 4 µm thick for observation on slides. After H&E staining, the histological modifications were observed using light microscopy (Olympus BX51, Japan), while the pathological analysis of the brain was performed as described previously (Yan et al., 2018). Different colored arrows represented the histological results. The black arrow denoted the nucleus condensation and pycnosis, while the blue arrow indicated changes in the neuron morphology.

**Nissl staining**

The paraffin sections were subjected to Nissl staining to evaluate the number and morphological characteristics of the neurons using a light microscope (Olympus BX51, Japan). The evaluation criteria were established according to the toxicological pathology (Kaufmann et al., 2012). Moreover, three sections were assessed for each sample, while the number of Nissl body and the size of the neurons were quantified via Analyze Particles using Image J software (Wayne Rasband, NIH).

**Biochemical assays**

The oxidative stress-related indexes, including the ROS, MDA, 8-OHdG adduct levels, and GSH content of the complete brains of the mice, were measured after tissue homogenization using commercial kits according to standard protocols (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

The neurotransmission-related enzymes and metabolites, such as dopamine (DA), acetylcholine (Ach), acetylcholinesterase (AChE), and choline acetyltransferase (ChAT), were measured after homogenization of all the brain tissue, using commercial kits according to standard protocols (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Measurements of the TNF-α and IL-6 cytokines were obtained using an ELISA kit and the homogenized brains of the mice according to standard protocols (Thermo Fisher Scientific, USA).

**Immunohistochemical staining**
Sections of tissue were cut from the paraffin blocks and positioned on positively charged slides for immunohistochemical staining. These sections were placed in an oven for 1 h, after which they were dehydrated and deparaffinized by being submerged in a xylol-alcohol series. Then, phosphate buffer solution (PBS) was used to wash the tissue sections, followed by treatment for 10 min with a 3% hydrogen peroxide (H$_2$O$_2$) solution. Using an antigen retrieval solution, microwave treatment was performed twice at 500 W for 5 min to clarify the endogenous peroxidase-inactivated antigens in the tissues, which were then cooled at room temperature and washed with PBS employing a protein block to prevent the binding of non-specific antibodies. After the protein block was removed, the tissues were incubated with anti-NF-κB-p-p65 (1:200; Cell Signaling Technology, USA) and anti-TH (1:250; Abclonal, China) antibodies, followed by a PBS washing process and treatment with secondary antibodies. The tissues were subjected to 3–3′ diaminobenzidine (DAB) chromogen, after which they were washed with PBS for the last time and rinsed with tap water. Mayer’s hematoxylin was then used for counterstaining. The TH-positive neurons and NF-κB-p-p65-labeled cells in the cerebrums and cerebellums were observed and counted using Image J software (Wayne Rasband, NIH).

**Western blot analysis**

The expression of the related proteins in the tissues of the mice was measured via Western blotting. The proteins (10 µg-30 µg) were transferred to polyvinylidene fluoride (PVDF) membranes (from GE Healthcare, IL, USA) before they were separated using SDS-PAGE. Then, 5% fat-free milk was used to block (Sangon Biotech, Shanghai, China) the membranes at room temperature for 1 h, after which they were subjected to an overnight incubation process at 4°C with specific primary antibodies, namely anti-NLRP3 (diluted at 1:1000, Abclonal, A5652), anti-ASC (diluted at 1:1000, Abclonal, A1170), Caspase 1 (diluted at 1:1000, Abclonal, A16288), IL-18 (diluted at 1:1000, Abclonal, A16737), IL-1β (diluted at 1:1000, Abclonal, A16288), GSDMD (diluted at 1:1000, Abcam, ab20829408), TH (diluted at 1:1000, Abclonal, A0028), α-syn (diluted at 1:1000, Proteintech, 10842-1-AP), and β-actin (diluted at 1:1000, Abcam, ab8226). On the second day, secondary HRP-conjugated goat anti-rabbit IgG (diluted at 1:3000, CST, #7074) was to incubate the membrane for 1 h at room temperature after washing three times with Tris-buffered saline tween (TBST). Protein detection was accomplished using an enhanced chemiluminescence (ECL) assay (obtained from Bio-Rad, Shanghai, China), while scanning was performed using a Tannon 4200 imaging system (Tanon, Shanghai, China). The gray values were analyzed using Image J software (Wayne Rasband, NIH).

**Statistical analysis**

The Graph Pad Prism statistical software package (Version 7.0, Graph Pad Software, CA) was used for statistical analysis, curve fitting, and graph plotting. The data were expressed as the mean ± standard deviation (SD). Furthermore, the statistical comparisons between the experimental groups were evaluated via one-way ANOVA with Duncan’s multiple comparison test using the SPSS software program or two-way ANOVA with Sidak’s multiple comparison test using the Graph Pad Prism statistical software. Consequently, $P<0.05$ values were regarded as statistically significant and were denoted by different letters.
Results

ACR-induced abnormalities in gait in mice

Figure 1A illustrates the flow chart of the treatment administered to the animals. Due to the different experimental periods, ACR treatment at low, medium, and high doses had negative control groups throughout the experimental process. The behavior of the mice was monitored daily, while their body weights and gait scores were recorded every 3 d. The landing splay was measured on the last day of every exposure period, i.e., day 15 for the high-dose group and its control group (tan arrow); day 30 for the medium-dose group and its control group (blue arrow); and day 60 for the low-dose group and its control group (purple arrow).

No notable abnormal performances were observed in any of the negative control groups. At an ACR dosage of 5 mg/kg, splay foot evidence started to appear during the final 6 d of the 60 d period (Fig. 1E). All the mice in the 25 mg/kg/day ACR group started displaying symptoms of hypokinesia and lethargy after 21 d (Fig. 1E). The high-dose, 50 mg/kg ACR group exhibited tremors, walking difficulties, and weakness after 10 d (Fig. 1E). These results indicated that both the dosage and time influenced the gait score. In the 25 mg/kg/day and 50 mg/kg/day ACR groups, the distance between the two rear limbs was the widest of the three groups and increased significantly during the landing foot splay tests ($P < 0.05$, Fig. 1B and Fig. 1F). Therefore, the ACR exposure of the 25 mg/kg and 50 mg/kg dosage groups caused substantial gait abnormalities and weakness in the hind limbs ($P < 0.05$). Higher doses reduced the time required for ACR toxicity to induce gait abnormality. Figure 1C shows that the average body weight declined slightly in the 25 mg/kg/day and 50 mg/kg/day ACR groups, indicating that medium- and high-dose ACR treatment negatively affected the growth of the mice. However, no mortality or clinical signs were observed in the primary organs of the mice in any of the groups. Figure 1D shows that there were no statistical differences between the control group and the ACR treatment groups regarding organ coefficients, including the liver, spleen, cerebrum, and cerebellum ($P > 0.05$), indicating that ACR did not significantly affect the development of the organs of mice.

ACR-induced neuron loss and neuron lesions

The neurotoxic effect of ACR-induced brain injury was further confirmed by H&E staining and histological assessment. As shown in Fig. 2A and Fig. 2B, normal neurocytes in the hippocampus and dentate nucleus displayed a clear nucleus, a uniform cytoplasm, and a well-arranged structure. In contrast, obviously abnormal histo-architectures of hippocampus and dentate nucleus were observed after ACR exposure. All ACR treatments caused neurocytes degeneration, with vague cellular boundaries and neuron pyknosis, denoted by black arrows. Moreover, the neuron morphology was changed (blue arrow), and inflammatory cell infiltration was found after the 50 mg/kg high-dose ACR treatment (Fig. 2A and Fig. 2B).
ACR-induced neuronal lesions were observed after Nissl staining. In the hippocampus and cerebellum control groups, the granular cell layer displayed a uniform color and regular arrangement (Fig. 2C and Fig. 2D). As shown in Fig. 2E and Fig. 2G, the number of Nissl bodies in the hippocampal DG regions and the Purkinje cells in the dentate nucleus were decreased significantly after medium- and high-dose ACR treatment, respectively ($P < 0.05$). Moreover, a distinct decrease in staining intensity and vague cellular boundaries were evident after high-dose ACR treatment (Fig. 2D). It is worth noting that the size of the neurons and Purkinje cells significantly increased with obvious cell swelling in the hippocampal DG regions and dentate nucleus after all ACR treatment doses (Fig. 2F and Fig. 2H), exhibiting similar characteristics to ACR-induced cell pyroptosis. In summary, ACR exposure caused significant neuron loss and neuron lesions in the cerebrum hippocampus and cerebellum dentate nucleus of the mice.

**ACR-induced oxidative stress in mice**

Oxidative stress typically causes pathophysiological events in neurodegenerative diseases, as well as during ACR-induced neurotoxic progress (LoPachin and Gavin, 2008). The ROS accumulation, GSH content, and oxidative stress products, such as MDA and 8-OHdG, were measured in the brain homogenate after exposure to all ACR doses. Figure 3A shows significant ROS accumulation ($P < 0.05$), while the GSH content decreased substantially after all ACR treatments in a dose-dependent manner ($P < 0.05$, Fig. 3D). The levels of the oxidative biomarkers, MDA (Fig. 3B) and 8-OHdG (Fig. 3C) were visibly higher than in the control groups. In the other groups, the MDA level in the 50 mg/kg/day ACR group showed a more significant increase ($P < 0.05$). These results suggest that ACR causes oxidative damage, and the accumulated ROS probably induces a downstream signal cascade.

**ACR-induced neurotransmission impairment**

Neurotransmitters are essential for animals to transmit nerve impulses (Grant, 2015). Of these, ACh and DA are particularly important for transmitting nerve impulses between synapses (Faure et al., 2014). The results showed that ACR decreased the ACh content ($P < 0.05$, Fig. 4C) and ChAT activity ($P < 0.05$, Fig. 4A) in a dose-dependent manner, while ACR exposure increased the activity of AChE. More significant AChE activity was promoted only in the high-dose group ($P < 0.05$, Fig. 4B). These findings showed that the ACh-related neurotransmitter was inhibited after ACR treatment and may cause gait abnormality.

An increase in the α-syn levels led to abnormal aggregation, causing neuronal degeneration (Ottolini et al., 2017). The expression levels of α-syn were examined via western blot analysis to further clarify the possible mechanisms involved in gait abnormality. The results indicated that ACR caused a significant increase in α-syn expression after all ACR treatments (Fig. 4G).

The substantia nigra (SN) is rich in dopaminergic neurons, and is a region of the brain that controls movement which is closely related to neurodegenerative diseases (Patil et al., 2014). TH is the rate-limiting enzyme responsible for conversion of L-DOPA to dopamine (DA) (Daubner et al., 2011). To assess the impact of ACR on the functionality of dopaminergic neurons in the SN, the DA content, TH
protein expression, and TH-positive cell levels were investigated (Fig. 4E and Fig. 4G). Compared with the control groups, ACR treatment significantly reduced the DA level in all exposure groups, while the lowest concentration of DA was found in the high-dose exposure group ($P<0.05$, Fig. 4F). The expression and distribution of TH-positive cells were markedly decreased, indicating that dopaminergic neurons were lost in the SN after ACR treatment (Fig. 4E). These results showed that ACR caused damage to the dopaminergic and cholinergic neurons, leading to neurotransmission impairment and gait abnormality.

**ACR increased the NLRP3 priming, promoted neuroinflammation, and activated the NLRP3 inflammasome-related pathway**

NF-κB represents the upstream signaling of the NLRP3-related inflammasome components (Zhong et al., 2016a). The activation of NF-κB (p-p65) was measured using the immunohistochemical method. The ACR stimuli significantly activated NF-κB in the cerebrum and cerebellum, while the levels of p-p65 visibly increased (Fig. 5A and Fig. 5B). The protein expression levels of NLRP3 also increased significantly in all ACR treatment groups (Fig. 6A and Fig. 7A), indicating NLRP3 priming. Furthermore, the NF-κB pathway participated in the inflammatory reaction, regulating various other cytokines, such as TNF-α and IL-6 (Bonizzi and Karin, 2004). The protein levels of TNF-α and IL-6 increased significantly in the cerebrum and cerebellum at all treatment doses ($P<0.05$, Fig. 5C and Fig. 5D).

NLRP3 inflammasome was formed upon activation, consisting of pro-caspase-1, ASC, and NLRP3 (Swanson et al., 2019). This prompted the activation of mature IL-1β/IL-18 cytokines via caspase-1, which were transported from the cell via the pore-forming protein, GSDMD (Zhou et al., 2010). In the cerebrum, ACR treatment at different doses significantly upregulated the ASC, cleaved-caspase 1 (p20), mature IL-1β, and IL-18 protein expression levels. In the high-dose, 50 mg/kg ACR group, the mature IL-1β, mature IL-18, and N-GSDMD exhibited higher levels than the low- and medium-dose groups (Fig. 6). Similar results were observed in the cerebellum (Fig. 7). ACR significantly increased NLRP3 inflammasome formation and activated the related downstream proteins, such as ASC, cleaved-caspase 1 (p20), N-GSDMD, mature IL-18, and mature IL-1β. The results demonstrated that ACR exposure induced neuroinflammation by NLRP3-caspase 1-GSDMD signaling, further contributing to the neurotoxic mechanism.

**Discussion**

Neurotoxicity resulting from ACR causes neuronal degeneration, neuronal energy inactivation, learning and memory alterations, and peripheral motor nerve injury (LoPachin et al., 2002a; Murray et al., 2020; Zhang et al., 2017). However, the underlying mechanism of ACR-induced neurotoxic phenotypes requires further clarification (Zong et al., 2019). This study indicates that neuroinflammation and neurotransmission impairment in the central neuron system contribute to ACR-mediated neurotoxicity at different intoxication periods in a time- and dose-dependent manner. Subsequently, it was confirmed that
an increase in oxidative stress, as well as inflammatory cytokine release, and the activation of NLRP3-caspase 1-GSDMD related pathways represent essential responses to ACR-induced neurotoxicity. Additionally, the results reveal the biomarker of ACR-induced gait abnormality and neuronal survival, including α-syn aggression, a decrease in TH+ cell distribution and DA content, and the disruption of the ACh-related metabolism. This indicated that ACR-induced neurotoxicity was closely associated with the initiation and progression of neurodegenerative disease. Overall, these results enhance the understanding of the potential mechanisms of ACR-induced neurotoxicity. The cellular responses could present a more systematic insight for establishing alternative pathway strategies for assessing the risk associated with ACR.

In the central nervous system, ACR-induced damage is a systematic response that can be attributed to injury to the cerebellum and cerebrum. Gait abnormality, weakness of the skeletal muscles, and hind limb numbness are typical symptoms of ACR toxicity and are closely related to the dysfunction of the cerebellum and axon damage (Erkekoglu and Baydar, 2014). In this study, representative phenotypes, including an increase in the gait score and foot landing splay, started 21 d and 10 d after 25 mg/kg and 50 mg/kg ACR exposure, respectively (Fig. 1E). At the end of the intonation period (54th day), a slight gait abnormality was observed in the 5 mg/kg ACR exposure group. The histological results also confirmed ACR-induced neuron loss, Purkinje cells pyknosis, and inflammatory cell infiltration in cerebellum (Fig. 2G and Fig. 2H). In hippocampus, histological staining showed a loose, disordered cellular arrangement of pyramidal cells in the CA3 regions (Fig. 2A), while the number of Nissl bodies was visibly reduced in the DG regions of the medium- and high-dose ACR-exposed mice (Fig. 2E). CA3 is crucial for working memory processes, as well as retrieving and consolidating short-term memory, while DG is essential for spatial memory encoding (Denny et al., 2014). In line with our results, the ACR-induced injury of the CA3 and DG regions resulted in learning and memory damage during the Morris water maze test conducted by Liu et al. (Liu et al., 2020). Thus, neurological deficits of cerebellum and cerebrum could contribute to ACR-induced gait abnormality.

Evidence has shown that gait abnormality-related neurodegenerative alterations are related to decreased neuron function (Jafarian et al., 2015). The neurobiological markers of cholinergic and dopaminergic neurons were also investigated in this study, showing that ChAT and AChE were the key enzymes involved in the synthesis and metabolism of ACh (Vijayaraghavan et al., 2013). The data showed that ACR decreased the ACh level and inhibited the ChAT activity in a dose-dependent manner while significantly increasing AChE activity (Fig. 4A and Fig. 4B). Furthermore, the rate-limiting enzyme, TH, is responsible for the synthesis of DA, while TH-positive cells represent dopaminergic neurons (Daubner et al., 2011). Immunocytochemistry and Western blot analysis were used to assess the TH-positive cells and the expression of TH protein. Results showed that TH protein expression was significantly decreased, while a loss in dopaminergic neurons was evident in the striatum of the mice subjected to ACR treatment (Fig. 4G and Fig. 4E). Subsequently, all ACR doses reduced the DA levels in the brains of the mice (Fig. 4D), indicating that the dopaminergic neurons represent a primary site for ACR activity. Additionally, Barber and LoPachin (Barber and LoPachin, 2004) indicated that the neurological imperfections affiliated with
ACR exposure are conciliated by damaged peripheral and central synaptic neurotransmission. α-syn is essential for adequately supplying synaptic vesicles in the presynaptic terminals in physiological conditions (Lautenschläger et al., 2017). Aggregated α-syn could induce fibrils and accumulate the pathological hallmark in neurodegenerative diseases (Stefanis, 2012). A high expression of aggregated α-syn was found in the brains of ACR-exposed mice (Fig. 4G). The mechanism by which α-syn aggregation induces neuronal toxicity may occur via α-syn and TH interaction, decreasing TH activity and releasing DA (Pan et al., 2012). Therefore, it is inferred that ACR-induced neurotoxicity occurs due to a decrease in the DA and ACh levels, reduced ChAT activity and TH expression, as well as an increase in AChE activity and α-syn aggregation, ultimately suppressing cholinergic and dopaminergic neuronal functionality.

Oxidative stress and inflammatory response are present during the entire neurological process of ACR intoxication. Exposure to ACR at 5 mg/kg, 25 mg/kg, and 50 mg/kg was associated with significant upregulation in the levels of ROS, MDA, and 8-OHdG, while downregulating the GSH levels in the brains of the mice (P< 0.05, Fig. 3). Unbalanced redox status and pro-inflammatory cytokine release are crucial mediators of neuroinflammation, further contributing to acute and chronic ACR-induced neurodegeneration in the central nervous system. Moreover, this study highlighted the involvement of the NLRP3 inflammasome pathway in ACR-induced neuroinflammation. In both the cerebellum and cerebrum, ACR-induced NF-κB-related NLRP3 priming allowed the assembly of the NLRP3 inflammasome, activating downstream signaling cascades, which included ASC, cleaved caspase-1, N-GSDMD, IL-1β, and IL-18 (Fig. 6 and Fig. 7). These findings were consistent with previous work, which reported the release of IL-1β in vitro (Zhao et al., 2017a, b). The results suggest that the NLRP3-caspase-1-GSDMD enrolled neuroinflammation, which occurred in both the cerebellum and cerebrum, possibly contributed to the pathogenesis of the gait abnormality and neurological deficit induced by ACR.

In recent years, the involvement of NLRP3-related pathways in exogenous chemically-induced neurotoxicity has received significant attention and include cadmium, arsenic trioxide, and molybdenum (Pei et al., 2019; Pi et al., 2021; Zhang et al., 2020), while concerns have been raised due to its association with neuroinflammation and neurodegenerative diseases. A study by Zong et al. in 2019 indicated that the neurotoxic effects of ACR included microglial activation, while neuroinflammation was found in both the BV2 cell model and the cerebral cortex of rats (Zong et al., 2019). Liu's work further reported that chronic exposure to ACR caused microglial activation, causing the release of inflammatory factors. The IL-1β level was enhanced via NLRP3 inflammasome activation, increasing other inflammatory factors that directly caused neuronal damage in the cerebrum of rats (Liu et al., 2020). However, in previous research, the hippocampus and frontal cortex in the cerebrum were mainly considered to be associated with ACR-induced neurotoxicity (Liu et al., 2020). The cerebellum, skeletal muscle, and peripheral nerves are also vulnerable targets and are closely related to exogenous stimuli-induced gait abnormality and neurological injury (De La Monte and Kril, 2014). Here, our study fully demonstrated that neuronal damage, neurotransmission impairment, and neuroinflammation in both of the cerebellums and cerebrums of mice mutually contributed to ACR-induced gait abnormality. Additionally, the endpoints at different times in conjunction with exposure to the low, medium, and high ACR dosages represent acute, subacute, and sub-chronic neurotoxic responses in this study. Therefore, the causal relationship among
behavioral phenomena, such as gait abnormality, and the potential mechanism of oxidative stress, inflammation response, neurotransmission impairment, and neuroinflammation, is more clearly evident in our study.

**Conclusion**

In conclusion, this study demonstrates that neuronal damage, neurotransmission disorder, oxidative stress, and neuroinflammation contribute to ACR-mediated neurotoxicity in a time- and dose-dependent manner. The potential ACR mechanism responsible for inducing gait abnormality and neurological deficit can be correlated with oxidative stress, neurotransmission impairment, and inflammation response-NLRP3 inflammasome signaling cascade activation. These findings may provide a more systematic insight for assessing the risk associated with ACR.

In future research, we will mainly focus on the correlation between oxidative stress and neuroinflammation. In particular, the potential biomarkers like NLRP3 and GSDMD, should be further confirmed *in vitro*. Due to the different functions of microglia, astrocytes, and neurons, further research should compare the different responses in different *in vitro* neurocyte models to confirm the neuroinflammation-related pathway and investigate its role in ACR-induced neurotoxicity. Then, an additional intriguing question relates to realizing cell-cell communication between glia and neurons, especially regarding the microglia-astrocyte-neuron effect on ACR-induced neuroinflammation. A co-cultural model may be established to explain the interaction. Additionally, an appropriate transgenic model should be used to further confirm the NLRP3 related biomarkers and explore the mechanism.

**Abbreviations**

ASC, apoptosis-associated speck-like protein containing CARD;

Caspase-1, cysteiny1 aspartate specific proteinase 1;

H&E, hematoxylin and eosin;

DG, dentate gyrus;

MDA, malondialdehyde;

GSH, glutathione;

8-hydroxy-2-deoxyguanosine, 8-OHdG;

ELISA, enzyme-linked immune sorbent assay;

IL-1, interleukin-1;

NF-κB, nuclear factor-kappa B;
TNF-α, tumor necrosis factor-α;

GSDMD, gasdermin D;

TH, tyrosine hydroxylase;

α-syn, α-synuclein;

**Declarations**

**Author contribution**

**Mengyao Zhao**: Conceptualization, Investigation, Funding acquisition, Writing the original draft. **Linlin Deng**: Methodology, Investigation, Data curation, Formal analysis. **Xiaoxuan Lu**: Validation. **Liqiang Fan**: Methodology. **Yang Zhu**: Writing review and editing. **Liming Zhao**: Funding acquisition, Project administration, Supervision.

**Data availability** All data are available upon request.

**Ethical approval**

All applicable international, national, and institutional guidelines for the care and use of animals were followed. All procedures performed in this study involving animals were in accordance with the ethical standards of the Welfare and Ethical use of Animals Committee of the Institute of Biochemistry and Cell Biology, SIBS, CAS (permit number IACUC-PR01).

**Consent to publication** The manuscript was reviewed by and received consent from all authors.

**Consent to Participate** The manuscript was reviewed by and received consent from all authors.

**Competing interest** The authors declare no competing interests.

**Acknowledgments**

This project was financially supported by the National Natural Science Foundation for Young Scientists of China (No. 31801668), the Fundamental Research Funds for the Central Universities (grant number 222201814036, 22221818014), the Shanghai PuJiang Program (18J1401900), the 111 Project (B18022), and the Open Project Funding of the State Key Laboratory of Bioreactor Engineering, ECUST (ZDXM2019).

**References**

1. Abderrazak A, Syrovets T, Couchie D, Hadri E, Friguet K, Simmet B, Rouis T, M (2015) NLRP3 inflammasome: From a danger signal sensor to a regulatory node of oxidative stress and
inflammatory diseases. Redox Biol 4:296–307. https://doi.org/10.1016/j.redox.2015.01.008

2. Abelli L, Ferri GL, Astolfi M, Conte B, Geppetti P, Parlani M, Dahl D, Polak JM, Maggi CA (1991) Acrylamide-induced visceral neuropathy: Evidence for the involvement of capsaicin-sensitive nerves of the rat urinary bladder. Neurosci 41(1):311–321. https://doi.org/10.1016/0306-4522(91)90220-I

3. Barber DS, LoPachin RM (2004) Proteomic analysis of acrylamide-protein adduct formation in rat brain synaptosomes. Toxicol Appl Pharmacol 201(2):120–136. https://doi.org/10.1016/j.taap.2004.05.008

4. Barber DS, Stevens S, LoPachin RM (2007) Proteomic analysis of rat striatal synaptosomes during acrylamide intoxication at a low dose rate. Toxicol Sci 100:156–167. https://doi.org/10.1093/toxsci/kfm210

5. Bonizzi G, Karin M (2004) The two NF-κB activation pathways and their role in innate and adaptive immunity. Trends Immunol 25(6):280–288. https://doi.org/10.1016/j.it.2004.03.008

6. Chen JH, Yang CH, Wang YS, Lee JG, Cheng CH, Chou CC (2013) Acrylamide-induced mitochondria collapse and apoptosis in human astrocytoma cells. Food Chem Toxicol 51:446–452. https://doi.org/10.1016/j.fct.2012.10.025

7. Cook C, Petrucelli L (2012) Oxidative stress, in: Parkinson’s Disease. CRC Press 2(5):a 009423. https://doi.org/10.1201/b12948

8. Daubner SC, Le T, Wang S (2011) Tyrosine hydroxylase and regulation of dopamine synthesis. Arch Biochem Biophys 508(1):1–2. https://doi.org/10.1016/j.abb.2010.12.017

9. De La Monte SM, Kril JJ (2014) Human alcohol-related neuropathology. Acta Neuropathol 127:71–90. https://doi.org/10.1007/s00401-013-1233-3

10. Denny CA, Kheirbek MA, Alba EL, Tanaka KF, Brachman RA, Laughman KB, Tomm NK, Turi GF, Losonczy A, Hen R (2014) Hippocampal memory traces are differentially modulated by experience, time, and adult neurogenesis. Neuron 83(1):189–201. https://doi.org/10.1016/j.neuron.2014.05.018

11. Dias FFG, Bogusz Junior S, Hantao LW, Augusto F, Sato HH (2017) Acrylamide mitigation in French fries using native L-asparaginase from Aspergillus oryzae CCT 3940. LWT - Food Sci Technol 76(B):222–229. https://doi.org/10.1016/j.lwt.2016.04.017

12. Edwards PM, Parker VH (1977) A simple, sensitive, and objective method for early assessment of acrylamide neuropathy in rats. Toxicol Appl Pharmacol 40:589–591. https://doi.org/10.1016/0041-008X(77)90083-7

13. Elblehi SS, Euony E, El-Sayed OI, Y.S (2020) Apoptosis and astrogliosis perturbations and expression of regulatory inflammatory factors and neurotransmitters in acrylamide-induced neurotoxicity under ω3 fatty acids protection in rats. Neurotoxicol 76:44–57. https://doi.org/10.1016/j.neuro.2019.10.004

14. Erkekoglu P, Baydar T (2014) Acrylamide neurotoxicity. Nutr Neurosci 17:49–57. https://doi.org/10.1179/1476830513Y.0000000065

15. Exon JH (2006) A review of the toxicology of acrylamide. J Toxicol Environ Heal - Part B Crit Rev 5:397–412. https://doi.org/10.1080/10937400600681430
16. Faure P, Tolu S, Valverde S, Naudé J (2014) Role of nicotinic acetylcholine receptors in regulating dopamine neuron activity. Neurosci 282:86–100. https://doi.org/10.1016/j.neuroscience.2014.05.040

17. Friedman M (2003) Chemistry, biochemistry, and safety of acrylamide. A review. J Agric Food Chem 51(16): 4506–4526. https://doi.org/10.1021/jf030204+

18. Grant P (2015) Neurotransmitters. International Encyclopedia of the Social & Behavioral Sciences: Second Edition. 15: 749–754. https://doi.org/10.1016/B978-0-08-097086-8.55040-5

19. Guan Q, Su B, Wei X, Wang S, Wang M, Liu N, Jiang W, Xu M, Yu S (2018) Protective effect of calpeptin on acrylamide-induced microtubule injury in sciatic nerve. Toxicol 409:103–111. https://doi.org/10.1016/j.tox.2018.08.002

20. Hagmar L, Törnqvist M, Nordander C, Rosén I, Bruze M, Kautiainen A, Magnusson AL, Malmberg B, Aprea P, Granath F, Axmon A (2001) Health effects of occupational exposure to acrylamide using hemoglobin adducts as biomarkers of internal dose. Scand J Work Environ Heal 27(4):219–226. https://doi.org/10.5271/sjweh.608

21. He Y, Tan D, Mi Y, Zhou Q, Ji S (2017) Epigallocatechin-3-gallate attenuates cerebral cortex damage and promotes brain regeneration in acrylamide-treated rats. Food Funct 8:2275–2282. https://doi.org/10.1039/c6fo01823h

22. Heneka MT, Kummer MP, Latz E (2014) Innate immune activation in neurodegenerative disease. Nat Rev Immunol 14:467–477. https://doi.org/10.1038/nri3705

23. Jafarian M, Karimzadeh F, Alipour F, Attari F, Lotfinia AA, Speckmann EJ, Zarrindast MR, Gorji A (2015) Cognitive impairments and neuronal injury in different brain regions of a genetic rat model of absence epilepsy. Neurosci 298:161–170. https://doi.org/10.1016/j.neuroscience.2015.04.033

24. Kaufmann W, Bolon B, Bradley A, Butt M, Czasch S, Garman RH, George C, Gröters S, Krinke G, Little P, McKay J, Narama I, Rao D, Shibutani M, Sills R (2012) Proliferative and nonproliferative lesions of the rat and mouse central and peripheral nervous systems. Toxicol Pathol 40:87–157. https://doi.org/10.1177/019263312439125

25. Koszucka A, Nowak A, Nowak I, Motyl I (2019) Acrylamide in human diet, its metabolism, toxicity, inactivation and the associated European Union legal regulations in food industry. Crit Rev Food Sci Nutr 3:1–16. https://doi.org/10.1080/10408398.2019.1588222

26. Lautenschläger J, Kaminski CF, Kaminski Schierle GS (2017) α-Synuclein – Regulator of exocytosis, endocytosis, or both? Trends Cell Biol 27(7):468–479. https://doi.org/10.1016/j.tcb.2017.02.002

27. Lebda MA, Gad SB, Rashed RR (2015) The effect of lipoic acid on acrylamide-induced neuropathy in rats with reference to biochemical, hematological, and behavioral alterations. Pharm Biol 53(8):1207–1213. https://doi.org/10.3109/13880209.2014.970288

28. Lee JG, Wang YS, Chou CC (2014) Acrylamide-induced apoptosis in rat primary astrocytes and human astrocytoma cell lines. Toxicol Vitr 28(4):562–570. https://doi.org/10.1016/j.tiv.2014.01.005

29. Lehning EJ, Balaban CD, Ross JF, Reid MA, LoPachin RM (2002) Acrylamide neuropathy I. Spatiotemporal characteristics of nerve cell damage in rat cerebellum. Neurotoxicol 23(3):39–414.
30. Libby P, Everett BM (2019) Novel Antiatherosclerotic Therapies. Arterioscler Thromb Vasc Biol 39:538–545. https://doi.org/10.1161/ATVBAHA.118.310958

31. Liu Y, Zhang X, Yan D, Wang Y, Wang N, Liu, Yufan, Tan A, Chen X, Yan H (2020) Chronic acrylamide exposure induced glia cell activation, NLRP3 infl-ammasome upregulation and cognitive impairment. Toxicol Appl Pharmacol 393:114949. https://doi.org/10.1016/j.taap.2020.114949

32. LoPachin RM (2004) The changing view of acrylamide neurotoxicity. NeuroToxicology 25(4):617–630. https://doi.org/10.1016/j.neuro.2004.01.004

33. LoPachin RM, Balaban CD, Ross JF (2003) Acrylamide axonopathy revisited. Toxicol Appl Pharmacol 188(3):135–153. https://doi.org/10.1016/S0041-008X(02)00072-8

34. LoPachin RM, Barber DS, He D, Das S (2006) Acrylamide inhibits dopamine uptake in rat striatal synaptic vesicles. Toxicol Sci 89(1):224–234. https://doi.org/10.1093/toxsci/kfj005

35. LoPachin RM, Gavin T (2012) Molecular mechanism of acrylamide neurotoxicity: Lessons learned from organic chemistry. Environ Health Perspect 120(12):1650–1657. https://doi.org/10.1289/ehp.1205432

36. LoPachin RM, Gavin T (2008) Acrylamide-induced nerve terminal damage: Relevance to neurotoxic and neurodegenerative mechanisms. J Agric Food Chem 56(15):5994–6003. https://doi.org/10.1021/jf703745t

37. LoPachin RM, Ross JF, Lehning EJ (2002a) Nerve terminals as the primary site of acrylamide action: A hypothesis. Neurotoxicol 23(1):43–59. https://doi.org/10.1016/S0161-813X(01)00074-2

38. LoPachin RM, Ross JF, Reid ML, Das S, Mansukhani S, Lehning EJ (2002b) Neurological evaluation of toxic axonopathies in rats: Acrylamide and 2,5-hexanedione. Neurotoxicol 23:96–110. https://doi.org/10.1016/S0161-813X(02)00003-7

39. Mottram DS, Wedzicha BL, Dodson AT (2002) Food chemistry: Acrylamide is formed in the Maillard reaction. Nature 419:448–449. https://doi.org/10.1038/419448a

40. Murray SM, Waddell BM, Wu CW (2020) Neuron-specific toxicity of chronic acrylamide exposure in C. elegans. Neurotoxicol Teratol 77:106848. https://doi.org/10.1016/j.ntt.2019.106848

41. Ottolini D, Calì T, Szabò I, Brini M (2017) Alpha-synuclein at the intracellular and the extracellular side: Functional and dysfunctional implications. Biol Chem 398(1):77–100. https://doi.org/10.1515/hsz-2016-0201

42. Pan T, Zhu J, Hwu WJ, Jankovic J (2012) The role of alpha-synuclein in melanin synthesis in melanoma and dopaminergic neuronal cells. PLoS One 7(9):e45183. https://doi.org/10.1371/journal.pone.0045183

43. Pan X, Yan D, Wang D, Wu X, Zhao W, Lu Q, Yan H (2017) Mitochondrion-mediated apoptosis induced by acrylamide is regulated by a balance between Nrf2 antioxidant and MAPK signaling pathways in PC12 Cells. Mol Neurobiol 54:4781–4794. https://doi.org/10.1007/s12035-016-0021-1
44. Patel M (2016) Targeting oxidative stress in central nervous system disorders. Trends Pharmacol Sci 37(9):768–778. https://doi.org/10.1016/j.tips.2016.06.007

45. Pei P, Yao X, Jiang L, Qiu T, Wang N, Yang L, Gao N, Wang Z, Yang G, Liu X, Liu S, Jia X, Tao Y, Wei S, Sun X (2019) Inorganic arsenic induces pyroptosis and pancreatic β cells dysfunction through stimulating the IRE1α/TNF-α pathway and protective effect of taurine. Food Chem Toxicol 125:392–402. https://doi.org/10.1016/j.fct.2019.01.015

46. Pepeu G, Grazia Giovannini M (2017) The fate of the brain cholinergic neurons in neurodegenerative diseases. Brain Res 1670:173–184. https://doi.org/10.1016/j.brainres.2017.06.023

47. Pi S, Nie G, Wei Z, Yang F, Wang C, Xing C, Hu G, Zhang C (2021) Inhibition of ROS/NLRP3/Caspase-1 mediated pyroptosis alleviates excess molybdenum-induced apoptosis in duck renal tubular epithelial cells. Ecotoxicol Environ Saf 208:111528. https://doi.org/10.1016/j.ecoenv.2020.111528

48. Pirzada RH, Javaid N, Choi S (2020) The roles of the NLRP3 inflammasome in neurodegenerative and metabolic diseases and in relevant advanced therapeutic interventions. Genes (Basel) 11(131):2–20. https://doi.org/10.3390/genes11020131

49. Pundir CS, Yadav N, Chhillar AK (2019) Occurrence, synthesis, toxicity and detection methods for acrylamide determination in processed foods with special reference to biosensors: A review. Trends Food Sci Technol 85:211–225. https://doi.org/10.1016/j.tifs.2019.01.003

50. Patil SP, Jain PD, Sancheti JS, Ghumatkar RT, Sathaye S (2014) Neuroprotective and neurotrophic effects of Apigenin and Luteolin in MPTP induced parkinsonism in mice. Neuropharmacology 86:192–202. https://doi.org/10.1016/j.neuropharm.2014.07.012

51. Qamar MA, Sauerbier A, Politis M, Carr H, Loehr P, Chaudhuri KR (2017) Presynaptic dopaminergic terminal imaging & non-motor symptoms assessment of Parkinson’s disease: Evidence for dopaminergic basis? Parkinsons. Dis 3(5):1–19. https://doi.org/10.1038/s41531-016-0006-9

52. Rannou C, Laroque D, Renault E, Prost C, Sérot T (2016) Mitigation strategies of acrylamide, furans, heterocyclic amines and browning during the Maillard reaction in foods. Food Res Int 90:154–176. https://doi.org/10.1016/j.foodres.2016.10.037

53. Salim S (2017) Oxidative stress and the central nervous system. J Pharmocol Exp Ther 360(1):201–205. https://doi.org/10.1124/jpet.116.237503

54. Santhanasabapathy R, Vasudevan S, Anupriya K, Pabitha R, Sudhandiran G (2015) Farnesol quells oxidative stress, reactive gliosis and inflammation during acrylamide-induced neurotoxicity: Behavioral and biochemical evidence. Neuroscience 308:212–227. https://doi.org/10.1016/j.neuroscience.2015.08.067

55. Sarkar S, Malovic E, Harishchandra DS, Ghaisas S, Panicker N, Charli A, Palanisamy BN, Rokad D, Jin H, Anantharam V, Kanthasamy A, Kanthasamy AG (2017) Mitochondrial impairment in microglia amplifies NLRP3 inflammasome proinflammatory signaling in cell culture and animal models of Parkinson’s disease. npj Park Dis 3(30):1–15. https://doi.org/10.1038/s41531-017-0032-2

56. Song L, Pei L, Yao S, Wu Y, Shang Y (2017) NLRP3 inflammasome in neurological diseases, from functions to therapies. Front Cell Neurosci 11(63):1–17. https://doi.org/10.3389/fncel.2017.00063
57. Spencer PS, Schaumburg HH (1974) A review of acrylamide neurotoxicity Part I. Properties, uses and human exposure. Can J Neurol Sci / J Can des Sci Neurol 1(2):143–150. https://doi.org/10.1017/S0317167100019739

58. Stadler RH, Blank I, Varga N, Robert F, Hau J, Guy PA, Robert MC, Riediker S (2002) Food chemistry: acrylamide from Maillard reaction products. Nature 419:449–450. https://doi.org/10.1038/419449a

59. Stefanis L (2012) α-Synuclein in Parkinson's disease. Cold Spring Harb Perspect Med 16(7):1383–1391. https://doi.org/10.1101/cshperspect.a009399

60. Swanson KV, Deng M, Ting JPY (2019) The NLRP3 inflammasome: molecular activation and regulation to therapeutics. Nat Rev Immunol 19:477–489. https://doi.org/10.1038/s41577-019-0165-0

61. Tan X, Ye J, Liu W, Zhao B, Shi X, Zhang C, Liu Z, Liu X (2019) Acrylamide aggravates cognitive deficits at night period via the gut-brain axis by reprogramming the brain circadian clock. Arch Toxicol 93:467–486. https://doi.org/10.1007/s00204-018-2340-7

62. Tareke E, Rydberg P, Karlsson P, Eriksson S, Törnqvist M (2002) Analysis of acrylamide, a carcinogen formed in heated foodstuffs. J Agric Food Chem 50(17):4998–5006. https://doi.org/10.1021/jf020302f

63. Vijayaraghavan S, Karami A, Aeinehband S, Behbahani H, Grandien A, Nilsson B, Ekdahl KN, Lindblom RPF, Piehl F, Darreh-Shori T (2013) Regulated extracellular choline acetyltransferase activity- the plausible missing link of the distant action of acetylcholine in the cholinergic anti-inflammatory pathway. PLoS One 8(6):e65936. https://doi.org/10.1371/journal.pone.0065936

64. Xu F, Oruna-Concha MJ, Elmore JS (2016) The use of asparaginase to reduce acrylamide levels in cooked food. Food Chem 210:163–171. https://doi.org/10.1016/j.foodchem.2016.04.105

65. Yan D, Yao J, Liu Y, Zhang X, Wang Y, Chen X, Liu L, Shi N, Yan H (2018) Tau hyperphosphorylation and P-CREB reduction are involved in acrylamide-induced spatial memory impairment: Suppression by curcumin. Brain Behav Immun 71:66–80. https://doi.org/10.1016/j.bbi.2018.04.014

66. Yousef MI, El-Demerdash FM (2006) Acrylamide-induced oxidative stress and biochemical perturbations in rats. Toxicology 219(1):133–141. https://doi.org/10.1016/j.tox.2005.11.008

67. Zamani E, Shokrzadeh M, Fallah M, Shaki F (2017) A review of acrylamide toxicity and its mechanism. Pharm Biomed Res 3(1):1–7. https://doi.org/10.18869/acadpub.pbr.3.1.1

68. Zhang M, Meng T, Zhao WJ, Li B (2017) Effect and mechanism of acrylamide on learning and memory and long-term potential in female Wistar rats. Chinese J Pharmacol Toxicol 31(1):87–93. https://doi.org/10.3867/j.issn.1000-3002.2017.01.011

69. Zhang Y, Liu Q, Yin H, Li S (2020) Cadmium exposure induces pyroptosis of lymphocytes in carp pronephros and spleens by activating NLRP3. Ecotoxicol Environ Saf 202:e110903. https://doi.org/10.1016/j.ecoenv.2020.110903

70. Zhao M, Wang L, Hu FS, Chen X, Chan F, H.M (2017a) Acrylamide-induced neurotoxicity in primary astrocytes and microglia: Roles of the Nrf2-ARE and NF-κB pathways. Food Chem Toxicol 106(A):25–33. https://doi.org/10.1016/j.fct.2017.05.007
71. Zhao M, Wang FSL, Hu XS, Chen F, Chan HM (2017b) Effect of acrylamide-induced neurotoxicity in a primary astrocytes/microglial co-culture model. Toxicol Vitr 39:119–125. https://doi.org/10.1016/j.tiv.2016.11.007

72. Zhong Z, Umemura A, Sanchez-Lopez E, Liang S, Shalapour S, Wong J, He F, Boassa D, Perkins G, Ali SR, McGeough MD, Ellisman MH, Seki E, Gustafsson AB, Hoffman HM, Diaz-Meco MT, Moscat J, Karin M, 2016b. NF-κB restricts inflammasome activation via elimination of damaged mitochondria. Cell. 164(5): 896–910. https://doi.org/10.1016/j.cell.2015.12.057

73. Zhou K, Shi L, Wang Y, Chen S, Zhang J (2016) Recent advances of the NLRP3 inflammasome in central nervous system disorders. J. Immunol. Res. 2016: e9238290. https://doi.org/10.1155/2016/9238290

74. Zhou R, Tardivel A, Thorens B, Choi I, Tschopp J (2010) Thioredoxin-interacting protein links oxidative stress to inflammasome activation. Nat Immunol 11:136–140. https://doi.org/10.1038/ni.1831

75. Zong C, Hasegawa R, Urushitani M, Zhang L, Nagashima D, Sakurai T, Ichihara S, Ohsako S, Ichihara G (2019) Role of microglial activation and neuroinflammation in neurotoxicity of acrylamide in vivo and in vitro. Arch Toxicol 93:2007–2019. https://doi.org/10.1007/s00204-019-02471-0

Figures
Exposure to ACR caused physiological performance and gait abnormalities in the mice. (A) The flow chart of the test. Different colored lines represent different doses and times. (B) Representative symptoms of gait abnormalities. (C) Bodyweight. (D) Organ coefficients. (E) The gait scores. (F) Landing foot spread of the hind limbs. Low-dose (5 mg/kg, 60 d), medium-dose (25 mg/kg, 30 d), and high-dose (50 mg/kg, 15 d) ACR treatment exposure. The control group received physiological saline and served as a control. The
results are expressed as mean ± SD (n =10). Different lowercase letters denote significant differences (P < 0.05) between the groups after multiple comparisons.

Figure 2

Exposure to ACR caused neuron lesions and neuron loss in the cerebrum and cerebellum. (A-B) The representative histopathological H&E staining of the hippocampus (A) and dentate nucleus (B). The black arrow denotes the nucleus condensation and pycnosis, while the blue arrow indicates changes in the
neuron morphology; (C-D) The representative histopathological Nissl staining of the hippocampus (C) and dentate nucleus (D); (E-G) The number of Nissl bodies in the hippocampus (E) and the number of Purkinje cells in the dentate nucleus (G); (F-H) The size of neurons in the hippocampus (F) and the size of the Purkinje cells in the dentate nucleus (H). The exposed mice received low-dose (5 mg/kg, 60 d), medium-dose (25 mg/kg, 30 d), and high-dose (50 mg/kg, 15 d) ACR treatment. The control group received physiological saline that served as a control. The results are presented as mean ± SD (n = 5), Scale bar = 100 μm, * P < 0.05.

Figure 3

Exposure to ACR caused oxidative stress in the brain tissues of the mice. Oxidative stress markers of (A) ROS, (B) MDA, (C) 8-OHdG, and (D) GSH in the brain tissue were detected using oxidative stress kits. The exposed mice received low-dose (5 mg/kg, 60 d), medium-dose (25 mg/kg, 30 d), and high-dose (50 mg/kg, 15 d) ACR treatment, while the control group received physiological saline that served as a control. The results are presented as mean ± SD (n = 5). Different lowercase letters denote significant differences (P<0.05) between the groups after multiple comparisons.
Figure 4

Exposure to ACR caused neurotransmission impairment in the brain tissue. (A) ChAT, (B) AChE, (C) ACh, and (D) DA in the brain, detected using a commercial kit. (E) Representative image of the TH-positive cells (dopaminergic neuronal) in the SN of each group. The nuclei were stained with Hoechst in blue. Scale bar=50 μm. (F) The number of TH-positive neurons in the SN of each group. (G) A representative image and expression levels of the TH and α-syn in each group, where β-actin served as a loading control. The
results are presented as mean ± SD (n = 5). Different lowercase letters denote significant differences (P < 0.05) between the groups after multiple comparisons.

**Figure 5**

Exposure to ACR promoted the activation of the NF-κB pathway and the release of inflammatory cytokines in the cerebrum and cerebellum. Immunohistochemical staining of p-p65 (tan) in (A) the cerebrum and (B) the cerebellum zone region. Inflammatory markers, including (C) IL-6 and TNF-α in the cerebrum, and (D) IL-6 and TNF-α in the cerebellum, were detected using ELISA. The results are presented as mean ± SD (n = 5). Different lowercase letters denote significant differences (P < 0.05) between the groups after multiple comparisons.
Exposure to ACR enhanced NLRP3 inflammasome activation in the cerebrum. The representative image (A) and expression levels of NLRP3 (B), ASC (C), cleaved caspase 1 (D), IL-1β (E), IL-18 (F), GSDMD (G), and N-GSDMD (H) in the cerebrums of each group were obtained via Western blot analysis, while β-actin served as a loading control. The results are presented as mean ± SD (n =5). Different lowercase letters denote significant differences (P < 0.05) between the groups after multiple comparisons.
Exposure to ACR enhanced NLRP3 inflammasome activation in the cerebellum. The representative image (A) and expression levels of NLRP3 (B), ASC (C), cleaved caspase 1 (D), IL-1β (E), IL-18 (F), GSDMD, (G), and N-GSDMD (H) in the cerebellums of each group were determined via Western blot analysis, while β-actin served as a loading control. The results are presented as mean ± SD (n = 5). Different lowercase letters denote significant differences (P < 0.05) between the groups after multiple comparisons.