

Carvedilol attenuates acrylamide-induced brain damage through inhibition of oxidative, inflammatory, and apoptotic mediators

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ABSTRACT

Objective(s): Acrylamide is a potent neurotoxic compound and has harmful effects on brain cells. Acrylamide promotes oxidative, inflammatory, and apoptotic mediators in the CNS leading to neurological disorders. The goal of the current study was to examine the potential protective effect of carvedilol and its underlying mechanisms in a mouse model of acrylamide-induced brain injury.

Materials and Methods: Mice were treated with acrylamide (50 mg/kg/day, IP) and carvedilol (5 and 10 mg/kg/day, oral) for 11 continuous days. At the end of the experiment, mice were subjected to gait assessment. They were sacrificed and brain tissues were collected for histological and biochemical analysis.

Results: The results showed that treatment of mice with carvedilol decreased acrylamide-induced bodyweight loss, abnormal gait, and histopathological damage in the brain tissue. Carvedilol treatment significantly reduced the levels of malondialdehyde (MDA) and carbonyl protein and increased the levels of glutathione (GSH), catalase, superoxide dismutase (SOD), nuclear factor erythroid 2-related factor 2 (Nrf2), and heme oxygenase-1 (HO-1). Carvedilol treatment also decreased myeloperoxidase (MPO) activity, expression of nuclear factor kappa B (NF-κB), inducible nitric oxide synthase (iNOS), overproduction of nitric oxide (NO) and proinflammatory cytokines tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 in the brain of mice exposed to acrylamide. Furthermore, administration of carvedilol significantly decreased the levels of bax, cytochrome-c, and caspase-3 as markers of apoptosis in acrylamide-treated mice.

Conclusion: These findings indicate that carvedilol is able to attenuate acrylamide-induced damage to the CNS by inhibition of oxidative stress, inflammation, and apoptosis.

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Introduction

Acrylamide (CH₂=CHCONH₂) is an organic compound that is extensively used to make various acrylamide copolymers, plastics, paper, cosmetics, textiles, and polyacrylamide gel. Acrylamide is a toxic compound to humans and can be harmful to various organs especially to the nervous and reproductive systems. Occupational exposure to acrylamide is a major cause of acrylamide-induced toxicity however the most common sources of acrylamide to humans are foods and tobacco. Acrylamide is produced during the cooking of carbohydrate-rich foods at high temperatures (>120 °C). After absorption and distribution, acrylamide is oxidized to a toxic metabolite, glycidamide, by cytochrome P450 2E1 in the liver. Acrylamide and glycidamide can react with DNA and cause mutations in cells which may lead to cancer. Acrylamide-induced toxicity has been reported in various organs including the brain, reproductive tissues, kidney, liver, gastrointestinal tract, and thyroid (1-3). The CNS is the most important target for toxicity caused by acrylamide. Indeed acrylamide acts as a potent neurotoxin and is able to induce oxidative and inflammatory damage in any region of the brain. Acrylamide causes neurodegeneration and impaired neurotransmission in the central and peripheral nervous systems. Clinical and experimental studies have shown

that acrylamide-induced neurotoxicity may lead to fatigue, ataxia, numbness of the extremities, muscle weakness, and mental impairment (4, 5).

Acrylamide has been reported to induce the generation of reactive oxygen species (ROS) and decrease the levels of anti-oxidants in the brain tissue of experimental animals. Neuronal apoptosis is another important mechanism involved in neurotoxicity caused by acrylamide. Elevation in the levels of caspases-3 and Bax in the brain tissue has been reported following acrylamide exposure (2, 6, 7). Nrf-2 and HO-1 play an indispensable role in the protection against oxidative injury and cell death induced by acrylamide (8, 5). Several studies have shown that expression of transcription factor NF-κB and inflammatory cytokines such as IL-1β, IL-6, and TNF-α was increased in the CNS of animals exposed to acrylamide (8, 9).

Carvedilol is a lipophilic nonselective β-adrenergic receptor antagonist that is generally used in the management of cardiovascular diseases including hypertension and heart failure. Carvedilol has been demonstrated to exhibit potent anti-oxidant, anti-inflammatory, and anti-apoptotic effects. Previous experimental studies have shown that carvedilol exhibits a protective effect against hepatotoxicity (10), paraquat-induced lung injury (11), and pancreatic β-cell damage (12). Neuroprotection is another important effect of

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carvedilol that has been revealed in some studies. Carvedilol has been suggested as a protective agent against Alzheimer's disease (13), neuroleptic-induced dyskinesia (14), peripheral neuropathy (15), cerebral ischemia (16, 17) neurotoxicity (18), and hypoxia-induced neuroinflammation (19).

We postulated that activation of anti-oxidant pathways and inhibition of inflammatory and pro-apoptotic mediators could protect CNS against acrylamide-induced neurotoxicity. Given this background, the present work aimed to study the potential protective effect of carvedilol and its possible mechanisms in acrylamide-induced brain damage.

Materials and Methods

Animals

The experiments were performed on male Swiss albino mice weighing 21–23 g (6–7 weeks of age). Mice were housed and acclimatized in polypropylene cages with a wire mesh top under controlled conditions (22 ± 2 °C temperature, 12 hr light and dark cycle) for one week before starting the experiments. Animals were allowed free access to water and a standard chow diet. All experiments on animals were performed according to NIH guidelines for Care and Use of Laboratory Animals and were approved by our institutional ethics committee (IR.ARUMS.REC.1399.413).

Experimental protocol

Mice were randomly divided into four groups (7–8 mice in each group). Group I (control) received normal saline; Group II (ACR) received acrylamide (50 mg/kg/day, IP) for 11 days; Group III (ACR+Car 5) received acrylamide (50 mg/kg/day, IP) and carvedilol (5 mg/kg/day, orally) for 11 days; Group IV (ACR+Car 10) received acrylamide (50 mg/kg/day, IP) and carvedilol (10 mg/kg/day, orally) for 11 days. The dose of acrylamide was selected according to the results of previous studies (5, 7, 20, 21) and our initial experiments. Acrylamide was dissolved in normal saline and injected intraperitoneally. Carvedilol was suspended in saline and used orally by gavage. At the end of the experiments, mice were subjected to behavioral assessment. After being anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg), the mice were sacrificed and the brain tissues were collected. A portion of each brain tissue was fixed in formalin (10%) and another portion was frozen in liquid nitrogen and stored at -70 °C for use in biochemical and gene expression analysis.

Bodyweight changes and behavioral test

The bodyweight of mice in each group was monitored daily for 11 days. Gait analysis was used to assess the toxic effects of acrylamide on the brain of mice and subsequent locomotion abnormality. Each mouse was placed in a clear plexiglass box and monitored for 3 min. The gait score was assigned from 1 to 4 as follows: 1 = normal gait; 2 = slightly abnormal gait (slight ataxia and foot splay); 3 = moderately abnormal gait (obvious ataxia and foot splay with limb abduction during ambulation); and 4 = severely abnormal gait (inability to support bodyweight and foot splay) (20).

Assessment of oxidant and anti-oxidant markers

Brain tissues were homogenized in cold Trizma buffer (50 mM, pH 7.4) containing protease inhibitors. Homogenates were centrifuged at 30,000 g for 20 min at 4 °C and the supernatants were separated and used for biochemical analysis.

The concentration of MDA was measured using a method based on the reaction of thiobarbituric acid with MDA in the presence of trichloroacetic acid at the temperature of 95 °C. The absorbance of the resulting red-pink color was measured at 532 nm (22). The content of carbonyl proteins was assessed according to the method based on the reaction of 2,4-dinitrophenylhydrazine with carbonylated proteins to produce the corresponding hydrazones which have a maximum absorbance at 370 nm. The concentration of GSH was measured using a method based on the reaction of 5,5'-dithiobis-2-nitrobenzoic acid (Ellman's reagent) with GSH, producing a yellow color with a maximum absorbance at 412 nm. The activity of the catalase enzyme was assessed spectrophotometrically by the degradation of hydrogen peroxide molecules to water and oxygen. The rate of reaction was measured at the absorbance of 240 nm and catalase activity was defined as one unit of enzyme that degrades 1 micromole of hydrogen peroxide per minute (23). The activity of the SOD enzyme was measured using a commercial SOD assay ELISA kit (Cayman Chemical, No. 706002).

Assessment of NO, Nrf2, and HO-1

The level of nitrate/nitrite as an index of nitric oxide content was assessed by a commercial NO assay ELISA kit (Cayman Chemical, No. 780001). The levels of Nrf2 (ZB-11367C-M9648) and HO-1 (ZB-10266C-M9648) in the supernatants of brain tissue were evaluated by mouse ELISA kits (ZellBio GmbH, Germany) according to the manufacturer's instructions.

Real-time PCR analysis of iNOS, NF- κ B, Nrf2, and HO-1

To evaluate mRNA expressions, total RNA was extracted from brain tissues by Trizol reagent. First-Strand cDNA was synthesized using a cDNA Synthesis Kit (Thermo Scientific, USA). Real-time PCR reactions were done with SYBR Green using the LightCycler instrument (Roche). GAPDH was used for normalization of relative gene expression. The sequences of primers were as follows: iNOS forward, GCCTTGGCTCCAGCATGTACCCTCAG and reverse, CCTGCCACTGAGTTCGTCCCCTTC; NF- κ B forward, ACCTTTGCTGGAAACACACC and reverse, ATGGCCTCGGAAGTTTCTTT; Nrf2 forward, TCTCCTCGCTGGAAAAAGAA and reverse, AATGTGCTGGCTGTGCTTTA; HO-1 forward, GTGGAGACGCTTTACGTAGTGC and reverse, CTTTCAGAAGGGTCAGGTGTCC; GAPDH forward, CTGCCACCCAGAAGACTGTG and reverse, GGTCTCAGTGTAGCCCAAG.

Assessment of MPO and inflammatory cytokines

The collected supernatants of brain tissue were used to assess inflammatory markers. The activity of MPO was measured using a spectrophotometric method based on hydrogen peroxide-dependent oxidation of tetramethylbenzidine by myeloperoxidase. The absorbance of the resulting blue color was measured at 370 nm. The levels of cytokines IL-1 β (LOT168882032), IL-6 (LOT161537017), and TNF- α (ZB-10117C-M9648) in brain tissue were assessed with commercial ELISA kits (IBL and ZellBio) specific for mice following the manufacturer's instructions.

Assessment of apoptotic markers

The levels of apoptotic markers Bax (ZB-11612C-M9648), caspase-3 (ZB-10445C-M9648), and cytochrome-c

(ZB-10499C-M9648) in brain tissue were assessed with commercial ELISA kits (ZellBio GmbH, Germany) specific for mice according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using SigmaPlot 12.0 (Systat Software Inc. 2011). Comparisons among groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. Gait scores were examined by non-parametric Kruskal–Wallis test followed by Dunn's *post hoc* test. Data were expressed as the mean \pm standard deviation (SD). $P < 0.05$ was considered statistically significant.

Results

Effect of carvedilol on acrylamide-induced bodyweight loss and behavioral impairment

The bodyweight of mice exposed to acrylamide (50 mg/kg/day) was gradually diminished during 11 days of experiment as compared to the normal control mice (Figure 1). Co-treatment with carvedilol at the dose of 10 mg/kg/day significantly inhibited the bodyweight loss induced by acrylamide. The effect of carvedilol at the dose of 5 mg/kg/day on acrylamide-induced bodyweight reduction was not significant. The results of our study revealed that administration of acrylamide at the dose of 50 mg/kg/day for 11 continuous days produced neurological signs in mice including ataxia, skeletal muscle weakness, and hind limb paralysis. As shown in Figure 1, mice exposed to acrylamide (50 mg/kg/day) showed remarkably higher gait scores in comparison with the control group. Treatment of mice with carvedilol (10 mg/kg/day) significantly reduced the gait score as compared with the ACR group. There was no significant difference in gait scores between the carvedilol group at the dose of 5 mg/kg/day and the ACR group.

Effect of carvedilol on acrylamide-induced histopathological changes in the brain

Hematoxylin and Eosin (H&E)-stained brain sections from the cortex and cerebellum were examined by a pathologist. As shown in Figure 2, the brain tissue of control mice showed normal neuronal architecture. Acrylamide intoxication (50 mg/kg) caused histopathological changes in neurons characterized by swelling of the perinuclear space, hyperchromatic nuclei, and pyknosis. Treatment with carvedilol reduced these histological alterations induced by acrylamide.

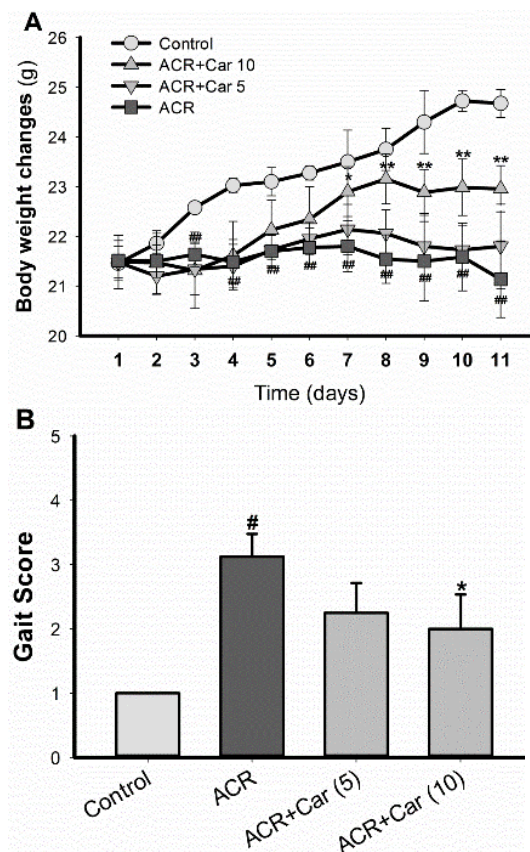


Figure 1. Effect of acrylamide exposure and carvedilol treatment on bodyweight (A) and gait score (B) in mice. Administration of acrylamide (50 mg/kg/day) caused bodyweight loss and increased gait score while carvedilol treatment (10 mg/kg/day) significantly reduced acrylamide-induced bodyweight loss and gait score. The data were expressed as mean \pm SD, (n=7-8). # $P < 0.05$ and ## $P < 0.001$ compared with control group; * $P < 0.05$ and ** $P < 0.001$ compared with ACR group

Effect of carvedilol on oxidant and anti-oxidant markers

Administration of acrylamide significantly increased the content of MDA and carbonyl proteins compared with those in the control group while carvedilol at the dose of 10 mg/kg significantly inhibited the effect of acrylamide on the levels of MDA and carbonyl proteins as markers of oxidative stress (Figure 3). Exposure to acrylamide significantly decreased the levels of anti-oxidants (GSH, SOD, and catalase) in brain tissue as compared with those in the control group while

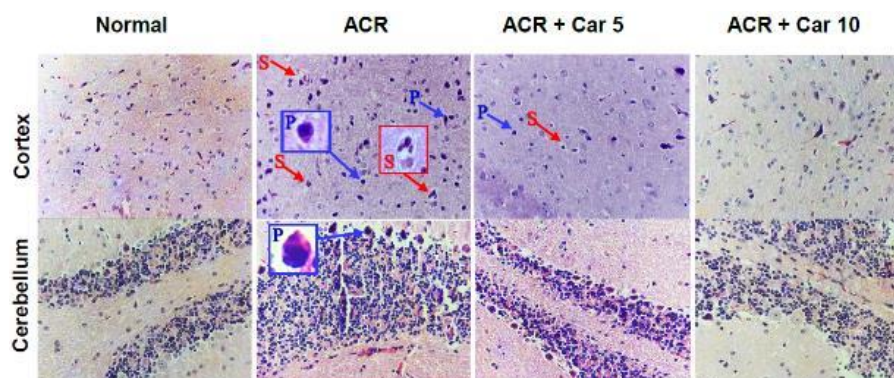


Figure 2. Histological appearance of the cerebral cortex (up) and cerebellum (down) from four experimental groups. Acrylamide intoxication (50 mg/kg) caused swelling of the perinuclear space (S), hyperchromatic nuclei, and pyknosis (P). Treatment with carvedilol at the dose of 10 mg/kg/day significantly reduced these histological alterations induced by acrylamide. (H & E, 400 \times)

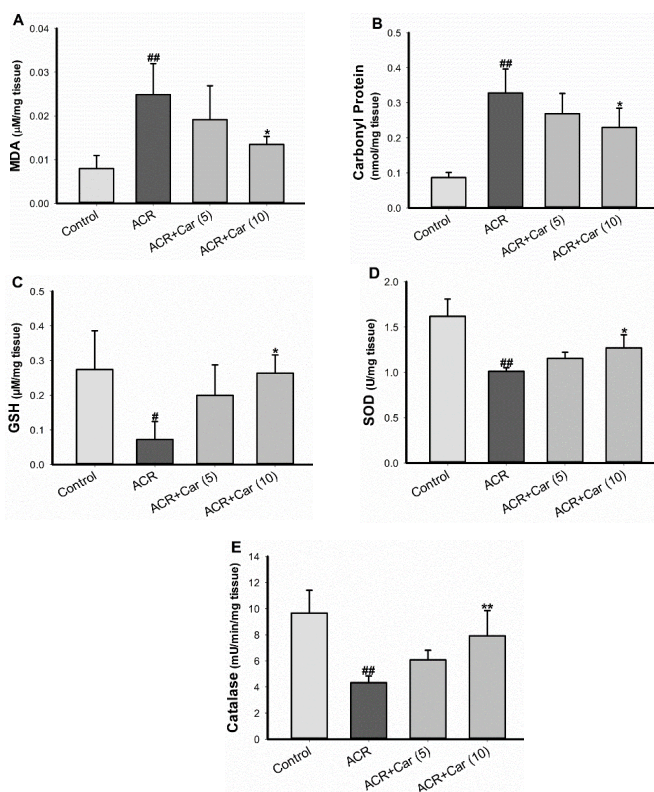


Figure 3. Effect of acrylamide and carvedilol treatment on the levels of MDA (A), carbonyl proteins (B), GSH (C), SOD (D), and catalase (E) in the brain of mice. Administration of acrylamide (50 mg/kg/day) increased the levels of MDA and carbonyl proteins, and decreased the levels of GSH, SOD, and catalase. Carvedilol treatment (10 mg/kg/day) significantly reduced the effects of acrylamide on these oxidant and anti-oxidant mediators. [#] $P < 0.05$ and ^{##} $P < 0.001$ compared with control group; ^{*} $P < 0.05$ and ^{**} $P < 0.01$ compared with ACR group

carvedilol treatment (10 mg/kg) significantly increased these antioxidants compared with mice treated only with acrylamide (Figure 3). Administration of carvedilol at the lower dose of 5 mg/kg did not significantly affect the levels of these oxidant and anti-oxidant markers.

Effect of carvedilol on the expression and levels of Nrf2 and HO-1

As shown in Figure 4, administration of acrylamide decreased the mRNA expression and the levels of Nrf2 and HO-1 in brain tissue of mice as compared with the normal control group. Nevertheless, treatment with carvedilol at the dose of 10 mg/kg significantly increased the expression and the levels of Nrf2 and HO-1 in brain tissue as compared with those in acrylamide-only-treated mice.

Effect of carvedilol on acrylamide-induced expression of iNOS and the production of NO

Intoxication of mice with acrylamide resulted in an increase in the mRNA expression of iNOS and subsequent overproduction of NO in brain tissue as compared with those in the normal control group (Figure 5). Carvedilol treatment (10 mg/kg) reduced acrylamide-induced iNOS expression and NO over-production in brain tissue.

Effect of carvedilol on acrylamide-induced inflammatory mediators

As shown in Figure 6, administration of acrylamide

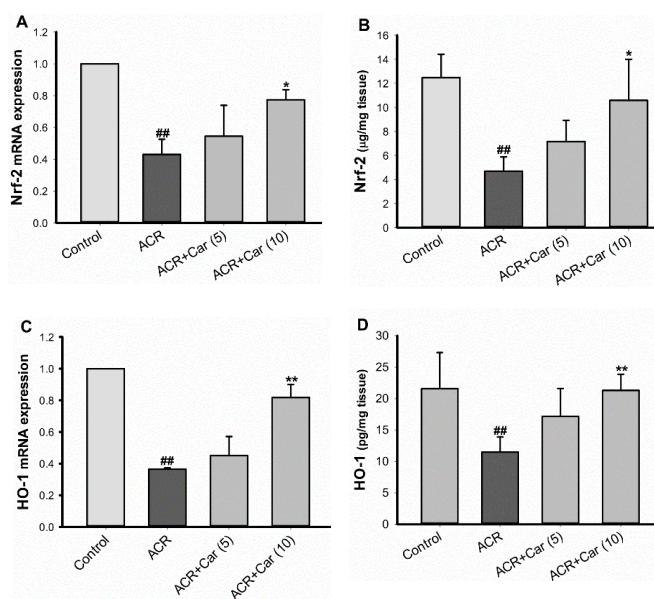


Figure 4. Effect of acrylamide and carvedilol treatment on gene expression and content of Nrf2 (A and B) and HO-1 (C and D) in the brain of mice. Administration of acrylamide (50 mg/kg/day) decreased gene expression and content of Nrf2 and HO-1 in brain tissue of mice. Carvedilol treatment (10 mg/kg/day) significantly inhibited the effects of acrylamide on the levels of Nrf2 and HO-1. [#] $P < 0.01$ and ^{##} $P < 0.001$ compared with control group; ^{*} $P < 0.05$ and ^{**} $P < 0.01$ compared with ACR group

increased the expression of NF- κ B as an inflammatory transcription factor in brain tissue as compared with the control group whereas carvedilol treatment (10 mg/kg) significantly decreased acrylamide-induced NF- κ B expression. As shown in Figure 6, the activity of MPO and the levels of proinflammatory cytokines TNF- α , IL-1 β , and IL-6 in brain tissue of acrylamide-poisoned mice were significantly higher than those of control normal mice. Treatment of mice with carvedilol (10 mg/kg) significantly decreased MPO activity and production of TNF- α , IL-1 β , and IL-6 in brain tissue as compared with those in mice treated with acrylamide alone.

Effect of carvedilol on acrylamide-induced apoptosis

The levels of cytochrome-c, bax, and caspase-3 as markers of apoptosis were increased in the brain of mice exposed to acrylamide as compared with those in the control mice

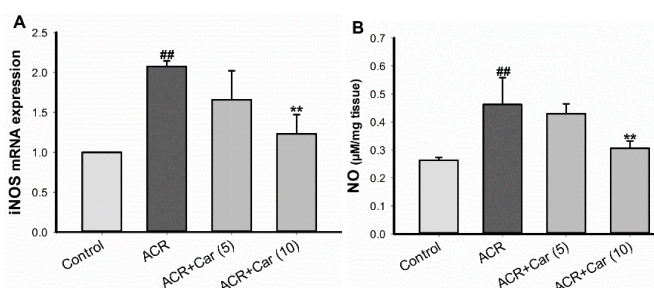


Figure 5. Effect of acrylamide and carvedilol treatment on gene expression of iNOS (A) and levels of NO (B) in the brain of mice. The expression of iNOS and the level of NO were significantly increased by acrylamide (50 mg/kg/day) exposure and decreased by carvedilol treatment (10 mg/kg/day). ^{##} $P < 0.001$ compared with control group; ^{**} $P < 0.01$ compared with ACR group

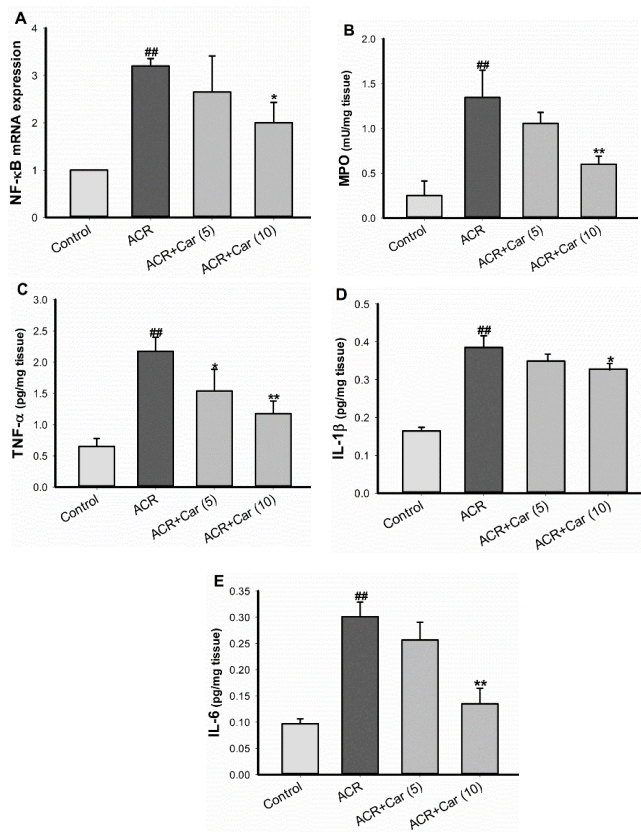


Figure 6. Effect of acrylamide and carvedilol treatment on expression of NF-κB (A), MPO activity (B), and the levels of cytokines TNF-α (C), IL-1β (D), and IL-6 (E) in the brain of mice. The expression of NF-κB and the levels of MPO, TNF-α, IL-1β, and IL-6 were significantly increased by acrylamide (50 mg/kg/day) exposure and decreased by carvedilol treatment (10 mg/kg/day). ## $P < 0.001$ compared with control group; * $P < 0.05$ and ** $P < 0.01$ compared with ACR group

(Figure 7). Carvedilol treatment at both doses (5 and 10 mg/kg) significantly reduced acrylamide-induced raise in the levels of bax, cytochrome-c, and caspase-3 in brain tissue. These results revealed the potent anti-apoptotic activity of carvedilol against acrylamide toxicity on the brain cells.

Discussion

Neurotoxicity is an important and potentially dangerous consequence of acrylamide exposure. In the present study, the beneficial effect of carvedilol against acrylamide-induced CNS toxicity was demonstrated.

Acrylamide is a small and reactive molecule that can enter the brain and impair the functions of the blood-cerebrospinal fluid barrier. Experimental studies have shown that acrylamide exposure causes progressive damage to the brain cells leading to neurotoxic symptoms including ataxia, muscle weakness, and paralysis (3, 24). In line with previous studies (5, 7, 20), the results of our study revealed that subacute exposure to acrylamide (50 mg/kg/day, IP) for 11 days resulted in weight reduction, muscle weakness, ataxia, and increased gait score in mice. Carvedilol treatment significantly ameliorated these alterations induced by acrylamide.

Various hypotheses have been proposed to clarify molecular mechanisms by which acrylamide induces its neurotoxicity. It has been shown that acrylamide and its

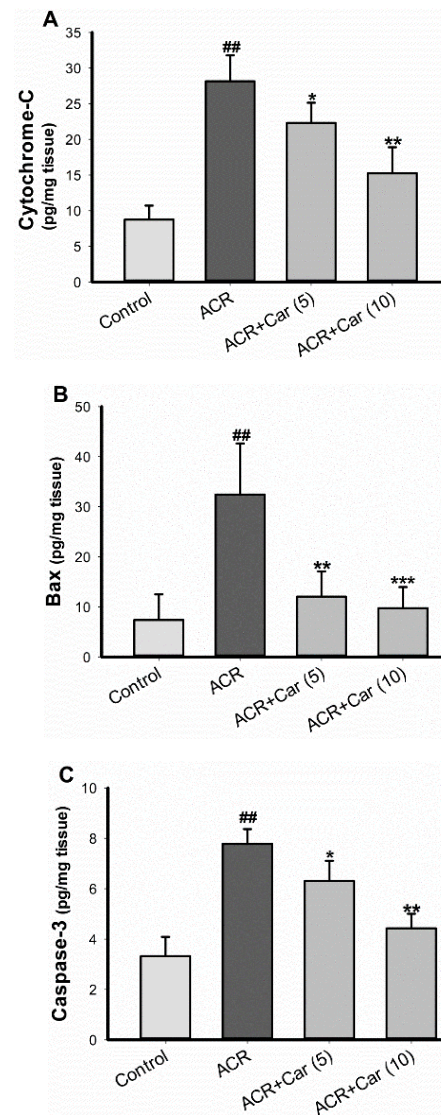


Figure 7. Effect of acrylamide and carvedilol treatment on the levels of cytochrome-c (A), bax (B) and caspase-3 (C) in brain of mice. The levels of cytochrome-c, bax and caspase-3 were increased by acrylamide (50 mg/kg/day) exposure and significantly decreased by carvedilol treatment dose dependently. ## $P < 0.001$ compared with control group; * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ compared with ACR group

metabolite glycidamide induce neurotoxicity through activation of protein kinase C (PKC) and inhibition of the AMP-activated protein kinase (AMPK) pathway. Moreover, acrylamide exposure decreases brain neurotransmitters and acetylcholinesterase activity (25, 26).

Oxidative stress is an essential mechanism of acrylamide-induced neurotoxicity. Exposure of cells to acrylamide causes the generation of ROS and imbalance between oxidant and anti-oxidant factors leading to cell dysfunction and death. Reactive metabolites of oxygen, such as superoxide anion, hydroxyl radicals, NO, and hydrogen peroxide are capable of causing damage to cellular nucleic acids, proteins, and lipids. Oxygen radicals may initiate the lipid peroxidation process which generates reactive aldehydes such as MDA. Oxygen radicals may also oxidize the amino acid residues of proteins and increase protein carbonyls in cells (1, 12). The brain cells have effective anti-oxidant defense mechanisms

to protect against damage induced by oxidants nevertheless these mechanisms are limited and may be overwhelmed by excessive production of ROS. GSH is an essential thiol-containing anti-oxidant that can be found in nearly every cell and has a key role in the neutralization of ROS and DNA repair pathways. Conjugation with GSH is an important detoxification mechanism involved in the metabolism of acrylamide and its metabolite glycidamide. Experimental studies have demonstrated that the level of MDA as a product of lipid peroxidation was increased during acrylamide-induced neurotoxicity. The SOD enzyme is an anti-oxidant that converts the superoxide anions into hydrogen peroxide, which is then detoxified to water by the enzyme catalase. Several studies have confirmed that acrylamide is able to decrease the levels of these anti-oxidants in cells (1-3, 7, 27, 28). In the current study, we also observed that acrylamide exposure increased MDA and protein carbonyl levels as biomarkers of oxidative damage and decreased the anti-oxidants GSH, SOD, and catalase in the brain tissue. This effect might result from further consumption of GSH and other anti-oxidants in reactions with oxygen-free radicals and conjugation with acrylamide. Treatment with carvedilol significantly decreased the levels of MDA and protein carbonyl and restored the levels of GSH, SOD, and catalase as an anti-oxidant defense mechanism in the brain tissue. These results exhibit the anti-oxidant property of carvedilol and can elucidate its protective effect against acrylamide-induced neurotoxicity.

Nrf2 is an important transcription factor that regulates the expression of anti-oxidant defense mechanism of cells against oxidative damage. Nrf2 increases anti-oxidant molecules such as HO-1 and phase II enzymes which play an important role in detoxifying xenobiotics. HO-1 is an enzyme that protects cells from injury induced by ROS and inflammation. The Nrf2/HO-1 pathway can be activated by various therapeutic agents with anti-oxidative properties. It has been reported that carvedilol is able to induce the Nrf2/HO-1 pathway in several experimental models including testicular toxicity (29), cardiotoxicity (30), and oxidative stress-induced neuronal death (31, 32). In the present study, we found that acrylamide intoxication reduced gene expression and the levels of Nrf2 and HO-1 in the brain tissue. Treatment with carvedilol significantly inhibited acrylamide-induced reduction in the levels of Nrf2 and HO-1. It seems that carvedilol may protect brain cells from acrylamide-induced oxidative damage by preventing the depletion of the anti-oxidant defense pathways such as Nrf2 and HO-1.

NO, synthesized by the enzyme NOS, is a major biological mediator that regulates various physiologic functions in the CNS. However, increased NO production and particularly its reactive metabolites such as peroxynitrite (ONOO^-) are very toxic to neurons by damaging proteins, lipids, and DNA. Over-production of NO plays an important role in acrylamide-induced toxicity. It has been shown that acrylamide induces the expression of iNOS and triggers the release of NO by microglial cells in the brain (28, 33, 34). Our study also confirmed that acrylamide increased the iNOS expression and subsequently NO production in the brain tissue. Treatment with carvedilol decreased the acrylamide-induced iNOS/NO pathway which can indicate the neuroprotective effect of carvedilol.

MPO is a heme-containing peroxidase involved in neuroinflammatory and neurodegenerative disorders

including ischemic stroke, Alzheimer's and Parkinson's diseases. Besides inflammatory cells, the brain cells including neurons, microglia, and astrocytes highly express MPO in inflammatory and oxidative processes. This enzyme catalyzes the reaction of hydrogen peroxide molecule with chloride ion to create hypochlorous acid, which is a potent oxidant (35, 36). It has been shown that the activity of MPO was increased in acrylamide-induced brain toxicity (28, 37, 38). In the present study, we also showed that acrylamide exposure significantly increased the MPO activity in the brain tissue. Carvedilol treatment inhibited the increase of MPO as a marker of oxidative stress and inflammation.

It has been well known that the immune response and inflammation play an essential role in the pathogenesis of neurotoxicity caused by toxic agents. The immunological balance of the CNS may be disrupted by various neurotoxicants such as acrylamide and lead to the activation of pro-inflammatory signaling pathways. NF- κ B is a transcription factor that can be activated in response to oxidative and inflammatory stimuli and plays an important role in acrylamide-induced toxicity (5, 39, 40). In the CNS, NF- κ B activation is responsible for the production and release of proinflammatory cytokines leading to neuroinflammation and impairment of cognitive behavior (9). NF- κ B regulates the expression of various genes including apoptotic proteins, adhesion molecules, SOD, and iNOS (41). NF- κ B is also associated with the activation of NLRP3 inflammasome which is involved in the neurotoxicity mechanisms of acrylamide (5). The release of inflammatory cytokines plays a crucial role in acrylamide-induced toxicity in various organs of the body (1, 27, 42). Several studies have found that the inflammatory cytokines such as IL-1 β , IL-6, and TNF- α were increased in the brain during acrylamide intoxication (9, 28, 40). TNF- α and IL-1 β are potent members of cytokines that can induce other cytokines and chemokines and aggravate inflammation-induced damage. There is positive feedback between TNF- α and NF- κ B signaling, thereby TNF- α is able to induce NF- κ B and production of further proinflammatory mediators (1, 43).

In the present study, our findings exhibited that expression of NF- κ B and production of proinflammatory cytokines IL-1 β , IL-6, and TNF- α were significantly increased in the brain tissue after acrylamide poisoning. Treatment with carvedilol reduced acrylamide-induced NF- κ B expression and production of these cytokines. Our results are in agreement with previous studies indicating the neuroprotective effect of carvedilol through inhibition of expression of NF- κ B, TNF- α , IL-1 β , and IL-6 (16, 19).

It has been well shown that the apoptosis process is another mechanism involved in neurotoxicity caused by acrylamide. Acrylamide is able to disrupt the blood-brain barrier and easily enter the brain cells and trigger apoptotic pathways by induction of oxidative stress. Mitochondria play a key role in the initiation of the intrinsic pathway of apoptosis. Acrylamide-induced ROS and DNA damage can lead to mitochondrial dysfunction. Bax is a pro-apoptotic protein that may increase mitochondrial permeability and accelerate apoptosis. Cytochrome-c is a protein located in the intermembrane space of mitochondria and can be released from mitochondria into the cytosol during apoptosis. Cytochrome-c activates a family of protease enzymes known as caspases (such as caspase-3) that are involved in apoptosis, necrosis, and inflammation. Numerous studies have shown

that the levels of bax, cytochrome-c, and caspase-3 were increased during acrylamide-induced neurotoxicity (2, 6, 7, 28, 34, 44-47). In the present study, intoxication with acrylamide resulted in increased cytochrome-c, bax, and caspase-3 levels in the brain tissue of mice. However, these markers of apoptosis were reduced in animals treated with carvedilol. We suppose that the neuroprotective activity of carvedilol is mediated at least partly by inhibition of apoptosis in the brain cells.

In agreement with our results, previous studies have also shown that carvedilol prevents apoptosis and improves mitochondrial function in various experimental models including neurotoxicity (16, 48), cardiotoxicity (49, 50), and nephrotoxicity (51).

Conclusion

The findings of the present study showed that treatment with carvedilol is able to attenuate acrylamide-induced brain damage in mice. The neuroprotective effect of carvedilol may be due to anti-oxidant, anti-inflammatory, and anti-apoptotic effects of carvedilol. Further investigations are needed to evaluate the mechanisms by which carvedilol may protect CNS cells against acrylamide as an important neurotoxic agent.

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Authors' Contributions

KA Designed the research, conducted experiments, analyzed data, and wrote the manuscript. AA Conducted the experiments.

Conflicts of Interest

There are no conflicts of interest.

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