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Effects of minocycline on acrylamide-induced neurotoxicity in Wistar rats

Neda Vatankhah [1], Mahboobeh Ghasemzadeh Rahbardar [2], Soghra Mehri [2, 3*], Hossein Hosseinzadeh [2, 3*]

[1] School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[2] Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
[3] Department of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

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ABSTRACT

Objective(s): Acrylamide (ACR) causes neurotoxicity in animals and humans through oxidative stress, inflammation, and apoptosis. Male Wistar rats were used to evaluate the neuroprotective effects of minocycline on ACR-induced neurotoxicity.

Materials and Methods: Animals were distributed into 11 groups: 1. Control (normal saline, 11 days, IP), 2. Normal saline (20 days), 3. ACR (50 mg/kg, 11 days, IP), 4. ACR (11 days, days 11-20 normal saline), 5, 6, 7. (Minocycline 10, 20, 40 mg/kg, IP+ ACR, 11 days) 8. (Minocycline 40 mg/kg, days 6-11), 9. Minocycline (40 mg/kg, days 6-20), 10. Minocycline (40 mg/kg, 11 days), 11. Vitamin E (200 mg/kg, every other day, IP) + ACR. The gait score was assessed at the end of the treatment period. Biochemical markers, including Malondialdehyde (MDA), glutathione (GSH), caspase-3, interleukin-1 beta (IL-1β), and tumor necrosis factor-α (TNF-α) were determined in the cerebral cortex.

Results: Administration of ACR induced movement disorders, reduced GSH levels, and elevated MDA, TNF-α, IL-1β, and cleaved caspase-3 in the cerebral cortex. Co-administration of minocycline 40 mg/kg with ACR ameliorated gait score abnormality. Treatment with minocycline (40 mg/kg), initiated 6 days after ACR administration and continued for 20 days, attenuated movement disorders. Furthermore, intraperitoneal injection of minocycline (40 mg/kg) with ACR reduced the levels of MDA, IL-1β, and caspase-3-cleaved proteins in the cerebral cortex.

Conclusion: Administration of minocycline exhibits both prophylactic and therapeutic properties against ACR-induced neurotoxicity primarily through anti-oxidant, anti-apoptotic, and anti-inflammatory properties.

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Introduction

Acrylamide (ACR), also known as 2-propene amide, is a solid monomer with the formula C_3H_5ON. ACR is an unsaturated α, β-carbonyl molecule that is white in appearance, crystalline, and odorless (1). Polyacrylamide is used in the formulation of pesticides, cosmetics, soil erosion prevention, food packaging, plastic products, and paper production (2). Due to the use of ACR in various industries, humans, especially those working in these industries, are constantly exposed to ACR (3). The monomeric form of ACR is prevalent in food products that are primarily cooked or fried at temperatures above 120 °C. Foods with high carbohydrate content, such as potatoes, chips, and toast, that contain ACR precursors, including free amino acids (primarily asparagine) and reducing sugars, are more likely to release ACR via the Millard reaction (4). Various laboratory studies have shown that ACR exposure causes hepatotoxicity (5), reproductive (6), renal (7), and cardiac toxicity (8) in laboratory animals, similar to those observed in humans.

ACR exposure causes significant damage to the central and peripheral nervous systems in both animals and humans (9). In preclinical studies, ACR administration in laboratory animals leads to neuropathy, numbness of the limbs, skeletal muscle weakness, gait disorders, and ataxia (10). Neurotoxicity of ACR is mediated through various mechanisms. It was discovered that ACR exposure increases malondialdehyde (MDA) content while reducing glutathione (GSH) amount and superoxide dismutase (SOD) activity, thereby inducing oxidative stress (11, 12). Additionally, ACR triggers inflammation by upregulating the expression of tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), interleukin-1β (IL-1β), and nuclear factor κB (NF-κB) genes (13). It also promotes apoptosis by increasing the (BAX)/ Bcl-2 ratio and caspase-3 amounts (14, 15) in the rat brain.

*_**Corresponding authors:**_ Soghra Mehri. Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran. Tel: +98-51-31801179, Email: mehris@mums.ac.ir; Hossein Hosseinzadeh. Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran. Tel +98-51-31801193, Fax: +98-51-38823251, Email: hosseinzadehh@mums.ac.ir

Given the widespread industrial and dietary exposure to ACR, it is essential to find effective compounds that can inhibit the neurotoxicity caused by this agent. Various natural compounds, including grape seed extract (16), selenium (17), and silymarin (18), exhibited protective effects against ACR-induced neurotoxicity.

Minocycline ($C_{23}H_{27}N_3O_7$) is a semi-synthetic antibiotic from the second-generation tetracycline family. Minocycline is used to treat infections and rheumatoid arthritis (19). Minocycline is considered the most effective tetracycline derivative with neuroprotective properties because it readily crosses the blood-brain barrier. The neuroprotective effect of minocycline has been highlighted in animal models of neuropathic pain, brain injury, and ischemia. Minocycline has also been studied in several neurodegenerative diseases, including multiple sclerosis and Alzheimer's disease (20, 21). The anti-oxidant, anti-inflammatory, and inhibition of apoptosis are the main mechanisms responsible for the minocycline neuroprotective effect (22-25).

It was observed that intraperitoneal injection of minocycline decreased MDA levels, nitrite amounts, and increased SOD enzyme function in the spinal cord of rats with diabetic neuropathy (26). Also, intravenous administration of minocycline significantly reduced the amounts of caspase-3 and caspase-7 proteins and amplified the level of Bcl-2 protein in rat nerve tissue, 3 days after obstructive stroke (27). Besides, oral administration of minocycline decreased the amounts of inflammatory factors, including IL-4, IL-1β, IL-10, and TNF-α, in mice (28).

Regarding the critical roles of inflammation, oxidative stress, and apoptosis in ACR-induced neurotoxicity, and the fact that minocycline enhances free radical scavenging, promotes cellular viability, and suppresses both pro-inflammatory cytokines and apoptotic pathways, this study was designed to elucidate further the neuroprotective effects of minocycline in ACR-induced neurotoxicity in rats.

Materials and Methods

Animals

Adult male Wistar rats (240–250 g) were housed in a standard environment (22-25 °C and a 12-hour light/dark cycle) with free access to food and water. The experiments were confirmed by the Mashhad University of Medical Sciences animal ethics committee (ethical number: IR.MUMS.REC 1398.103).

Materials

Acrylamide was acquired from Merck (Germany). Minocycline was purchased from Tinab Shimi. Thiobarbituric acid (TBA) and DTNB [5, 5 ′ di thiobis-(2-nitrobenzoic acid)] were obtained from Sigma-Aldrich (Germany). The polyvinylidene fluoride (PVDF) membrane was provided by BioRad (USA).

Experimental design

Administration of ACR (50 mg/kg, intraperitoneally) was mentioned to produce neurotoxicity in rats (29). To assess the ameliorative effects of minocycline on ACR-induced neurotoxicity, minocycline (10, 20, and 40 mg/kg) was injected intraperitoneally. The selected doses of minocycline were based on previous investigations (30, 31). All injections were administered daily at the designated time. Therapeutic doses were administered to animals 30 min before ACR injection. ACR and minocycline were dissolved in normal saline.

Seventy-seven animals were divided into 11 groups at random (n = 7) as follows:

1: Negative control group, male rats receiving vehicle (normal saline) for 11 days
2: Negative control group, male rats receiving vehicle (normal saline) for 20 days
3: Rats were administered ACR (50 mg/kg, IP) for 11 days (32)
4: Rats were administered ACR (50 mg/kg, 11 d, IP), and received normal saline (IP) from day 11 to 20
5: Rats were administered ACR (50 mg/kg, 11 d, IP) along with minocycline (10 mg/kg, 11 d, IP)
6: Rats were administered ACR (50 mg/kg, 11 d, IP) along with minocycline (20 mg/kg, 11 d, IP)
7: Rats were administered ACR (50 mg/kg, 11 d, IP) along with minocycline (40 mg/kg, 11 d, IP)
8: Rats were administered ACR (50 mg/kg, 11 d, IP) and minocycline (40 mg/kg), the most effective dose, from day 6 to 11
9: Rats were administered ACR (50 mg/kg, 11 d, IP) and minocycline (40 mg/kg), the most effective dose, from day 6 to 20
10: Rats were administered minocycline (40 mg/kg, 11 d, IP)
11: Positive control group, rats were administered ACR (50 mg/kg, 11 d, IP) along with vitamin E (200 mg/kg, every other day, IP) from day 1 to 11 (32)

Groups 8 and 9 were considered to define the therapeutic effects of minocycline.

Gait score test

At the end of the treatment time, the animals' movements were assessed for 3 min in a transparent box (90 × 90 cm). The movement was graded on a scale of one to four (33, 34):

1. Gaiting and taking steps are normal.
2. Gaiting and taking steps are only partially changed (slight weakness of the lower extremities).
3. Gaiting and taking steps are both impaired in a moderate way (modest weakness of the lower extremities).
4. Gaiting and taking steps are severely changed, and lower extremity paralysis has developed (33).

It should be noted that gait score assessments were blinded.

Collecting brain tissue samples

After performing the behavioral examination, the animals were sacrificed, and brain samples (cerebral cortex) were obtained. Liquid nitrogen was used to freeze the samples immediately, and the tissues were then stored in a -80 °C freezer (35).

Determination of lipid peroxidation in brain tissue

The generation of MDA as a byproduct of fatty acid peroxidation was used to determine lipid peroxidation. The TBA reactivity test was used to evaluate MDA levels in brain homogenates, and the data were represented as nmol/g tissue (36, 37). Briefly, brain tissue was homogenized in 1.15% cold KCl. Then, 0.5 ml of the 10% homogenate was mixed with 1 ml of 1% phosphoric acid and 1 ml of 0.6% thiobarbituric acid (TBA) solution. The mixture was boiled in a water bath for 45 min. After cooling, 4 mL of n-butanol was added, the mixture was vortexed for 1 minute, and then centrifuged at 3000 g for 10 min. The absorbance of the pink organic phase was measured at 535 nm.

Determination of reduced glutathione (GSH) content in brain tissue

The GSH concentration in tissue homogenates was defined using the previously established method. The optical density was measured at 412 nm using a spectrophotometer. The data were presented in nmol/g tissue (36, 38, 39). In summary, to prepare a 10% homogenate, brain samples were homogenized in a phosphate buffer solution (pH 7.4). The homogenate-trichloroacetic acid (TCA) mixture was centrifuged at 2500 g for 10 min. The supernatant was carefully collected and mixed with 2.5 ml of phosphate buffer (pH 8.0). Then, 0.5 ml of DTNB reagent was added to each sample, and the absorbance was recorded at 412 nm.

Western blot assay

The tissues were collected and lysed in a lysis buffer (1 mM phenylmethylsulfonyl fluoride, 10 mM sodium azide, 1 mM sodium orthovanadate (Na_3VO_4), 50 mM Tris–HCl (pH 7.4), 2 mM EGTA, 2 mM EDTA, 10 mM β-glycerophosphate, 0.2% W/V sodium deoxycholate, and complete protease inhibitor cocktail) and then centrifuged at 10000 rpm for 10 min at 4 °C (17). SDS-PAGE was used to separate equal amounts of total protein from each sample, which were then transferred to PVDF membranes. The blots were blocked with 5% skim milk for 2 hr at room temperature and then incubated for 2 hr at room temperature with primary antibodies: caspase-3 antibody (Cell Signaling, # 9665), TNF-α antibody (Cell Signaling, #3707), IL-1β antibody (Abcam # 9722), and β-Actin antibody (Cell Signaling, # 3700). Membranes were washed three times in Tris-buffered saline with Tween 20 (TBST). Subsequently, the membranes were incubated with rabbit or mouse horseradish peroxidase-conjugated anti-IgG (Cell Signaling #7074, 1:3000; #7076, 1:3000, respectively) for 2 hr at room temperature. ECL reagent was used to visualize the signal. Integrated optical densities of the bands were quantified using the Alliance 4.7 Gel Doc system and analyzed with UVTec software. All protein levels were normalized to β-actin, which served as the control protein.

Statistical analysis

GraphPad Prism 10.0 (GraphPad Prism Software Inc., San Diego, CA, USA) was used for statistical analysis. Data normality was evaluated using the Shapiro-Wilk test. The findings of the MDA and GSH content tests, as well as the Western blot analysis, were presented as mean ± SD, and one-way ANOVA with Tukey–Kramer *post hoc* test were used for statistical analysis. A *P*-value<0.05 was considered statistically significant. Data on gait disorders were reported as medians with interquartile ranges for each group, and statistical analysis was performed using the Kruskal–Wallis nonparametric test followed by Dunn's Multiple Comparisons test. To compare the two groups, the Mann-Whitney test was utilized.

Results

Effect of minocycline on ACR-related gait disorders

Figure 1A shows that administration of ACR (50 mg/kg, 11 days) resulted in gait abnormalities in rats compared with the control group (*P*<0.001). Administration of minocycline (40 mg/kg) along with ACR for 11 days markedly reduced this disorder compared with the ACR group (*P*<0.05), whereas injection of minocycline (10 or 20 mg/kg) along with ACR did not reduce the gait abnormality compared with the ACR group. Furthermore, administration of minocycline (40 mg/kg) during days 6–11, concurrently with ACR exposure, did not yield a statistically significant improvement compared with the ACR group. However, co-administration of vitamin E (200 mg/kg) with ACR significantly attenuated locomotor abnormalities (*P*<0.01).

Figure 1B was assessed to determine whether the rats would ameliorate if ACR administration were stopped and what the results of administration of minocycline from day 6 to 20 would be. Intraperitoneal injection of ACR for 11 days induced substantial changes in the motor activity of rats. In the other group, ACR injections continued until day 11; thereafter, until day 20, rats received intraperitoneal normal saline and were re-examined on day 20. As can be seen in the graph, the gait scores are reduced, and this change is significant in comparison to the ACR-treated animals from days 1-11 (*P*<0.05). Additionally, animals exposed to ACR from day 1 and receiving minocycline (40 mg/kg) from day 6 to 20 exhibited a significant reduction in gait abnormality compared with animals that received ACR from days 1-11 and were studied up to day 20 (*P*<0.05). Administration of minocycline up to day 20 significantly reduced ACR-related disorder relative to the group that received minocycline up to day 11 (*P*<0.05).

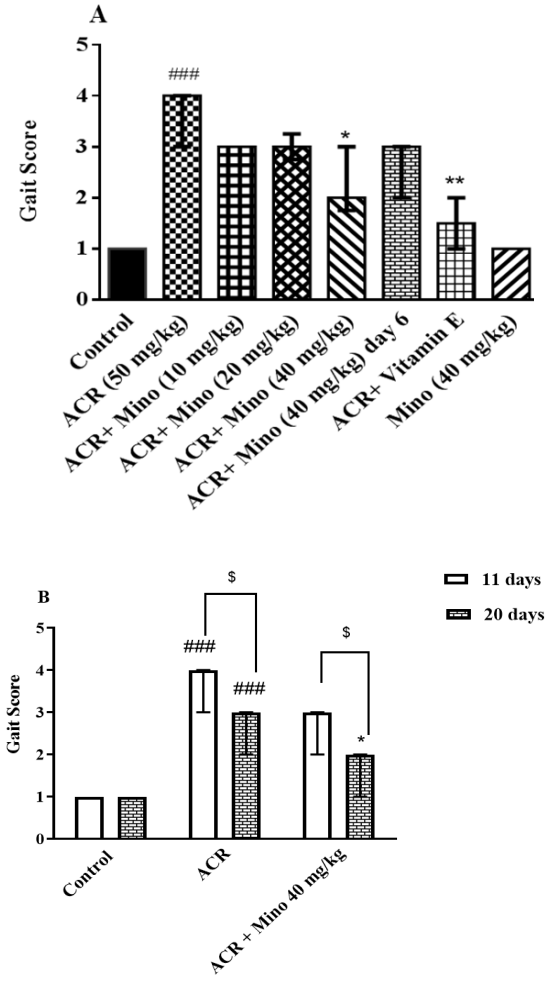

Figure 1. Effect of minocycline on rat ACR-induced gait abnormalities
(A): ACR (50 mg/kg, IP) and minocycline (10, 20, and 40 mg/kg, IP) were administered to rats. The behavioral index was determined after 11 days of administration. (B): ACR (50 mg/kg, IP) and minocycline (40 mg/kg, IP) were administered to rats. The behavioral index was measured after 11 and 20 days. In both A and B graphs, the data are median with an interquartile range (n = 7). Kruskal-Wallis non-parametric tests, Dunn's multiple comparison test, and Mann-Whitney test were used to determine the statistical difference. ### *P*<0.vs control group; * *P*<0.05 and ** *P*<0.01 vs ACR group; $ *P*<0.05 comparing the groups on days 11 and 20. ACR: Acrylamide and Mino: Minocycline.

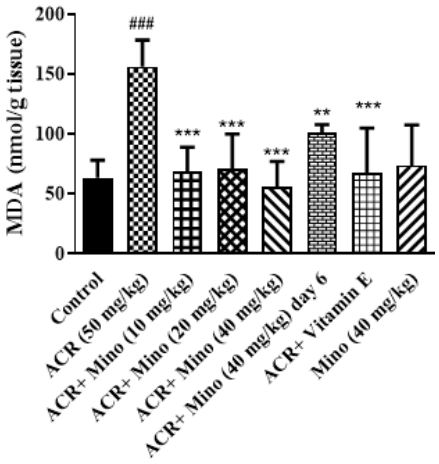

Figure 2. Effect of minocycline and ACR on the MDA level in the rat cerebral cortex

ACR (50 mg/kg, IP), minocycline (10, 20, and 40 mg/kg, IP), and vitamin E (200 mg/kg, IP) were administered to rats. The data are presented as Mean ± SD (n =5-7). ANOVA test and Tukey-Kramer post-test were used to determine statistical differences. ### P<0.01 vs control group; ** P<0.01 and *** P<0.001 vs ACR group. ACR: Acrylamide and Mino: Minocycline.

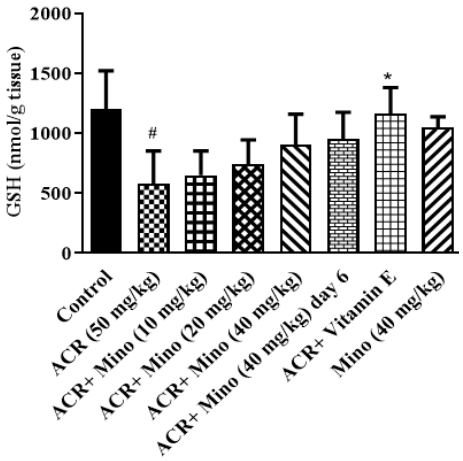

Figure 3. Effect of minocycline and ACR on the GSH content in the rat cerebral cortex

ACR (50 mg/kg, IP), minocycline (10, 20, and 40 mg/kg, IP), and vitamin E (200 mg/kg, IP) were administered to rats. The data are presented as Mean ± SD (n =5). ANOVA and Tukey-Kramer *post hoc* tests were used to assess statistical differences. # P<0.01 in vs control group; * P<0.01 vs ACR group. ACR: Acrylamide and Mino: Minocycline.

Impact of minocycline on ACR–related oxidative stress in brain tissues

Compared to the control animals, injection of ACR (50 mg/kg) for 11 days significantly increased MDA levels in cerebral cortex tissue (P<0.001). In comparison to the ACR group, intraperitoneal injections of minocycline (10, 20, and 40 mg/kg) with ACR significantly lowered MDA levels in cortical tissue (P<0.001). Furthermore, initiating minocycline (40 mg/kg) on day 6, with ACR, resulted in a significant reduction in MDA levels compared with the ACR group (P<0.0084). Compared with the control group, minocycline (40 mg/kg) did not alter brain MDA levels. However, when vitamin E (200 mg/kg) was administered with ACR, the MDA content in the cerebral cortex tissue was significantly attenuated relative to the ACR group (P<0.001) (Figure 2).

Effect of minocycline on ACR–induced GSH reduction in brain tissues

Figure 3 indicates that ACR (50 mg/kg) treatment for 11 days markedly decreased the GSH levels of the cerebral cortex (P<0.0107 vs the control group). Co-administration of minocycline (10, 20, and 40 mg/kg) with ACR did not alter GSH levels (vs the ACR group). Similarly, minocycline (40 mg/kg) administered from day 6 alongside ACR had no significant effect compared with ACR alone. Compared to ACR-treated mice, administration of minocycline (40 mg/kg) from day 6 plus ACR caused no significant change in GSH levels. In addition, GSH content did not differ significantly between the minocycline-only group (40 mg/kg) and the control group. GSH content was significantly elevated following administration of both Vitamin E (200 mg/kg) and ACR (P<0.0177 vs ACR group).

Impact of minocycline and ACR on the TNF-α and IL-1β protein levels in the cerebral cortex

ACR injection resulted in a notable elevation of TNF-α (P<0.0068) and IL-1β levels (P<0.0312) in the cerebral cortex compared with controls. Surprisingly, co-administration of minocycline at a dose of 40 mg/kg with ACR significantly reduced the IL-1β protein level (P<0.0333 vs ACR group), whereas the amount of TNF-α protein remained unchanged in the minocycline (40 mg/kg) with ACR group. Furthermore, combined administration of vitamin E and ACR significantly lowered the quantity of TNF-α (P<0.0019 vs ACR) and IL-1β proteins (P<0.0020 vs ACR) (Figures 4 and 5).

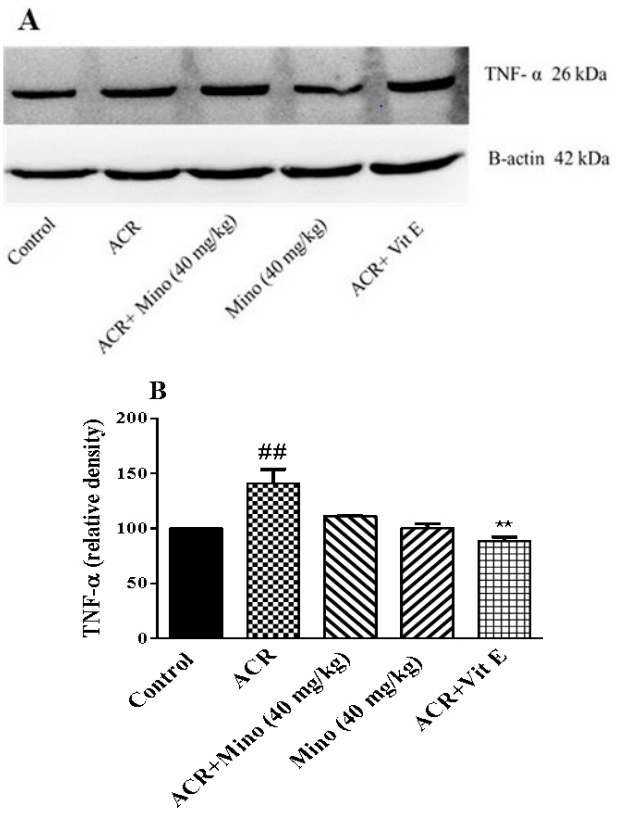

Figure 4.. Effect of minocycline and ACR on the level of TNF-α protein in the rat cerebral cortex

A: Specific bands related to the level of TNF-α were evaluated by Western blotting. B: TNF-α protein level data by densitometric analysis. The data are presented as mean ± SD (n=3-4). ANOVA and Tukey-Kramer *post hoc* tests were used to assess statistical differences. ## P<0.01 vs control group; ** P<0.01 vs ACR group. ACR: Acrylamide, Mino: Minocycline, and Vit E: Vitamin E.

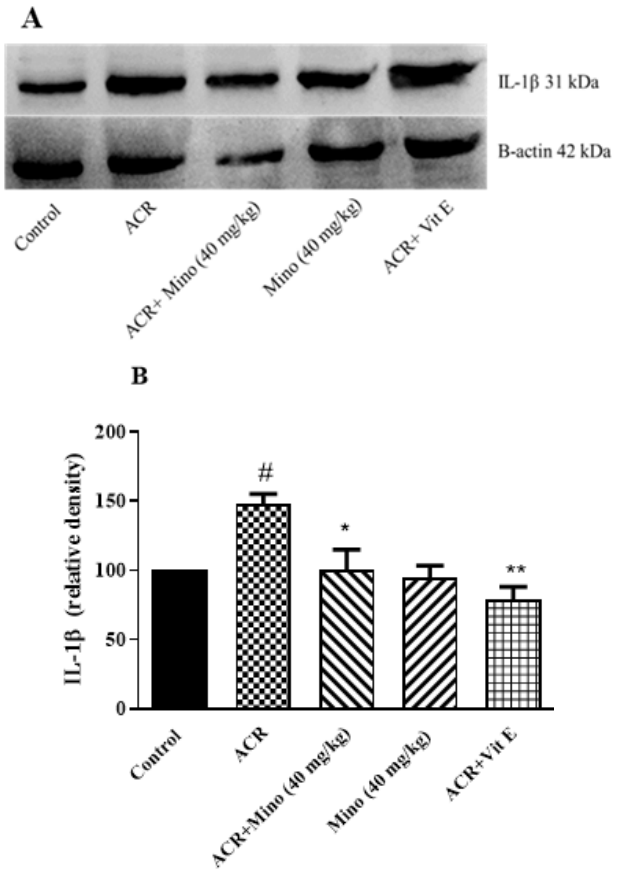

Figure 5. Effect of minocycline and ACR on the level of IL-1β protein in the rat cerebral cortex

A: Specific bands related to the level of IL-1β were evaluated by western blotting. B: IL-1β protein level data by densitometric analysis. The data are presented as mean ± SD (n=3-4). ANOVA test and Tukey-Kramer post-test were used to investigate the statistical difference. # P<0.05 vs control group; * P<0.05 and ** P<0.01 vs ACR group. ACR: Acrylamide, Mino: Minocycline, and Vit E: Vitamin E.

Effect of minocycline and ACR on the caspase-3 protein levels in the cerebral cortex

As demonstrated in Figure 6, ACR significantly increased caspase-3-cleaved protein levels in the cerebral cortex (P<0.0064 vs control). In contrast, combined administration of minocycline (40 mg/kg) alongside ACR significantly decreased caspase-3-cleaved protein levels (P<0.0151 vs ACR group). Minocycline alone did not affect the amount of this protein (vs the control group). In addition, when comparing vitamin E (200 mg/kg) with the ACR group to the ACR group, the level of caspase-3-cleaved protein did not alter. The administration of ACR and/or minocycline caused no notable difference in the levels of procaspase-3 protein in various groups.

Discussion

The protective effects of minocycline against ACR-induced neurotoxicity in rats were evaluated. The results showed that injection of ACR into rats (IP, 11 days) caused significant impairment of locomotion and induced oxidative stress, inflammation, and apoptosis in cerebral cortex tissue. Administration of minocycline (40 mg/kg) with ACR reduced gait score and lowered MDA levels in cortical tissues. The levels of IL-1β and caspase 3-cleaved proteins in cortical tissue were significantly decreased after administration of minocycline. Additionally, treatment of animals with minocycline initiated 6 days after ACR exposure provided protection against ACR neurotoxicity.

ACR, a classic toxicant, is widely formed in baked and fried foods (40). It has been shown to induce neurotoxicity in both the central and peripheral nervous systems of animals and humans, which is identified by some indications such as skeletal muscle weakness, weight loss, and ataxia (41, 42). Additionally, ACR exposure induces motor impairment by causing neuronal degeneration (43, 44).

n the current study, ACR (50 mg/kg, IP, for 11 days) administration induces paralysis of the rat limbs. The intoxicated animals dragged their hind limbs as they walked. In the other protocol, when ACR injection was discontinued on day 11, and animals were assessed on day 20, the gait score decreased, and the improvement was significant compared with the ACR-treated animals from days 1-11. (45). Interestingly, co-administration of minocycline (40 mg/kg, from day 1-11) with ACR markedly inhibited paralysis in rats' limbs. To evaluate the beneficial effect of minocycline, animals received the agent 6 days after initiation of ACR and continued exposure for 20 days. The results showed that minocycline significantly reduced gait abnormalities in this protocol.

Oxidative stress as a key factor in ACR neurotoxicity has been discussed in previous reports. Administration of ACR (50 mg/kg, IP, 11 days) to animals decreased GSH content and increased MDA levels in brain tissue (15, 36). In our research, intraperitoneal injection of ACR reduced GSH content and increased MDA levels in the cerebral cortex. Interestingly, administration of minocycline at

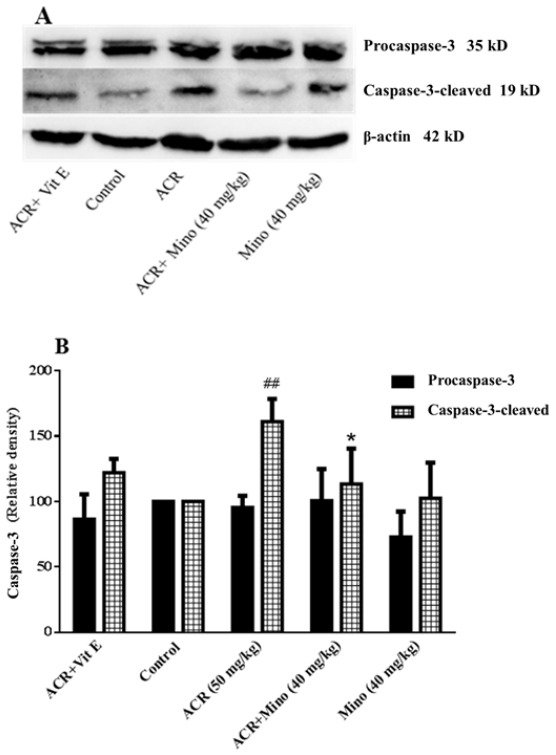

Figure 6. Effect of minocycline and ACR on the level of caspase-3 protein (pro and cleaved) in the rat cerebral cortex

A: Specific bands related to the level of caspase-3 (pro and cleaved) were examined by Western blotting. B: Caspase-3- 3 (pro and cleaved) protein level data by densitometric analysis. The data are presented as mean ± SD (n=3-4). ANOVA and Tukey-Kramer *post hoc* tests were used to assess statistical differences. ## P<0.01 vs control group; * P<0.05 vs ACR group. ACR: Acrylamide, Mino: Minocycline, and Vit E: Vitamin E.

different doses, starting on the first day of ACR injection and at the highest dose from day 6, reduced brain MDA levels in ACR-injected animals. However, minocycline treatment, regardless of dose or administration protocol, showed no significant effect on GSH levels in brain tissue. As minocycline did not alter GSH levels but produced a significant reduction in MDA levels in the cerebral cortex, it can be suggested that minocycline may alter the activity of anti-oxidant enzymes and thus inhibit oxidative stress. The lack of measurement of anti-oxidant enzyme activity is a limitation of the current study and should be addressed in future research. Consistent with our findings, administration of minocycline (20 mg/kg, twice daily, PO) to burned rats did not increase GSH levels in liver and kidney tissues (46). In a separate study, the administration of minocycline (45 mg/kg, 1-3 times) after traumatic brain injury reduced inflammatory markers but did not increase GSH levels (47). Research has reported that administration of minocycline (30, 90 mg/kg, PO) to male Wistar rats during the first and 24th hr after spinal cord injury reduced MDA content while reducing SOD activity in the spinal cord tissue (48). Evidence indicated that exposure of rats to minocycline (10, 30 mg/kg, IP) for 21 days reduced neuropathy due to chronic cerebral obstruction by lowering the content of MDA and increasing the activity of SOD in the rat spinal cord (49).

ACR-induced oxidative stress can cause the overproduction of inflammatory mediators, including TNF-α and IL-1β (50). Additionally, in various studies, ACR-induced neurotoxicity was partly mediated by inflammation (13, 51). Administration of ACR (20 mg/kg, oral, 30 days) elevated TNF-α and IL-1β levels in the cerebral cortex (52). Another study has shown that exposure to ACR increases TNF-α and IL-1β expression (53). In the current study, consistent with previous reports, administration of 50 mg/kg ACR for 11 days resulted in a notable elevation in the levels of inflammatory factors (TNF-α and IL-1β) in the cerebral cortex. Interestingly, in this study, treatment with minocycline (40 mg/kg, 11 days, IP) did not significantly alter TNF-α protein levels but reduced IL-1β protein levels in the cerebral cortex. The anti-inflammatory effects of minocycline have been mentioned in several studies. Injection of minocycline (40 or 80 mg/kg) for 2 weeks reduced TNF-α and IL-β levels in the spinal cord of diabetic rats (26). In another study, peritoneal injection of minocycline (30 or 100 mg/kg as a single dose) 30 min before acetic acid administration in rats significantly increased TNF-α and IL-1β levels in the rat sciatic nerve (54).

Another primary mechanism implicated in ACR neurotoxicity is apoptosis (55, 56). Oral administration of ACR (50 mg/kg, for 11 days) in rats significantly increased caspase-3 and caspase-9 levels in brain tissue (55). In another investigation, ACR (20 and 40 mg/kg, IP) induced neurotoxicity by triggering apoptosis, as evidenced by elevated Bax and procaspase-3 levels and reduced Bcl-2 expression in the rat spinal cord (42). Our results showed that after administration of ACR (50 mg/kg, 11 days, IP), procaspase-3 protein levels in cortical tissue were unchanged, whereas ACR markedly increased the level of caspase-3-cleaved protein. In addition, administration of minocycline (40 mg/kg, 11 days, IP) with ACR significantly decreased the level of caspase-3-cleaved protein in cortical tissue relative to the ACR group. Previous research reported that minocycline (5 mg/kg, 6 weeks, IP) treatment in mice with Huntington's disease attenuated the levels of caspase-1 and caspase-3 proteins in brain samples (57). Administration of minocycline (10 mg/kg, 2 weeks, IP) to mice prevents the release of pro-apoptotic markers, such as apoptosis-inducing factor (AIF), the second mitochondria-derived activator of caspases (SMAC)/Diablo protein, and cytochrome c, by controlling mitochondrial permeability (58, 59).

Vitamin E was selected as a positive control in the present research. Vitamin E is known as one of the most effective anti-oxidants (60). In our research, vitamin E and ACR co-administration recovered gait abnormality, lowered MDA levels, elevated GSH content, and attenuated TNF-α and IL-1β levels in the cerebral cortex. Furthermore, vitamin E (200 mg/kg) reduced ACR neurotoxicity via anti-oxidant and anti-apoptosis effects (55). In our study, minocycline at a dose of 40 mg/kg in rats exerted notable neuroprotective effects. Based on the standard body surface area (BSA) method to translate animal doses to humans, this dose corresponds to a human equivalent dose (HED) of approximately 6.5 mg/kg, or around 454 mg/day for a 70-kg adult. It seems that the calculated dose is higher than the typical clinical doses of minocycline (100–200 mg/day) (61). Therefore, for potential clinical applications, a safety factor and pharmacokinetic and pharmacodynamic differences between species must be considered. Following the application of these factors, the resulting calculated dose appears to be reasonable.

Conclusion

As illustrated in Figure 7, administration of ACR induces severe movement disorders in animals by triggering oxidative stress, inflammation, and apoptosis in the rat cerebral cortex. Minocycline demonstrates both preventive and therapeutic effects against ACR-induced neurotoxicity. Its anti-oxidant, anti-inflammatory, and anti-apoptotic properties play a key role in counteracting ACR

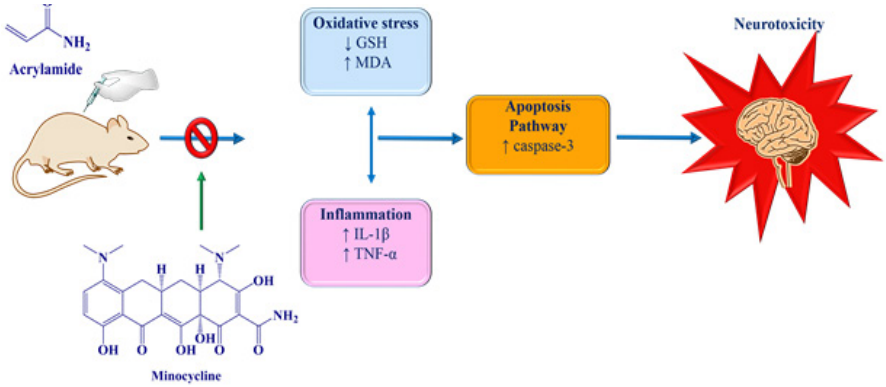

Figure 7. Mechanism of minocycline in protection against acrylamide (ACR) neurotoxicity

neurotoxicity (Figure 7).

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Ethical Approval

All examinations were approved by the Animal Care and Use Committee of Mashhad University of Medical Sciences (ethical number: IR.MUMS.REC 1398.103) and conducted in accordance with the Internationally Accepted Principles for Animal Use and Care.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' Contributions

N V conducted the research and analyzed the data. M GH R conducted the research and wrote the paper. S M supervised, verified the analytical methods, and reviewed the entire procedure and paper. H H conceived the original idea and supervised and reviewed the entire procedure and paper. The authors declare that all data were generated in-house and that no paper mill was used. During the preparation of this work, the authors used AI-assisted technologies to rephrase and reduce plagiarism, improving language and grammar. After using this tool/service, the authors critically reviewed and edited the content to ensure accuracy and accept full responsibility for the publication's content.

Conflicts of Interest

The authors declare no conflicts of interest.

Declaration

We acknowledge the use of GPT-5.1 to rephrase and reduce plagiarism while improving language and grammar.

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