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Article

The Effect of Anakinra on Acrylamide-induced Peripheral Neuropathy and Neuropathic Pain in Rats

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Acrylamide is a neurotoxic compound. Moreover, anakinra is an interleukin-1 (IL-1) receptor antagonist used in rheumatoid arthritis treatment. This study investigated the effect of anakinra on acrylamide-related neuropathy and neuropathic pain. Acrylamide exposure caused a significant decrease in the pain threshold; an increase in malondialdehyde (MDA), tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β) levels; and a decrease in total glutathione (tGSH) values in the sciatic nerve. This indicates hyperalgesia presence, oxidative stress, and peripheral nerve tissue inflammation. Anakinra treatment significantly reduced the MDA, IL-1β, and TNF-α levels, and increased the pain threshold and mean tGSH values. The analgesic effect of anakinra was 67.9% at the first hour, increasing to 74.9% and 76.7% at the second and third hours, respectively. The group receiving acrylamide exhibited histopathological changes (e.g., swollen and degenerated axons, hypertrophic and hyperplasic Schwann cells, and congested vessels). The use of anakinra significantly improved these morphological changes. Anakinra is concluded to reduce neuropathic pain and prevent neurotoxic effect of acrylamide on peripheral nerves due to its analgesic, antioxidant, and anti-inflammatory properties.

Keywords: Acrylamide. Anakinra. Oxidative stress. Inflammation. Sciatic nerve injury. Neuropathic pain.

INTRODUCTION

Acrylamide is an amide containing double bonds that can bind to small reactive molecules, such as urea [$CO(NH_2)_2$] and formaldehyde (HCHO) or glyoxal [$(CHO)_2$], aldehydes (RCHO), amines (R_2NH), and thiols (RSH) (Lingnert *et al,* 2002; Blasiak *et al*., 2004). Tareke *et al.* (2002) disproved the idea that acrylamide was not present in nature by showing that a large amount of acrylamide was formed when cooking at high temperatures. Acrylamide was later discovered to be released during the burning of tobacco and taken into the body through the inhaled smoke (Scherer *et al.,* 2007). Acrylamide is used in research laboratories in molecular biology methods (e.g., electrophoresis and chromatography) and is also utilized as an additive in printing and textile industries, wastewater purification, and preparation of many different cosmetic products, including lotions and deodorants (Smith, Oehme, 1991). Many studies have shown that acrylamide causes neurotoxic effects in humans and animals (Shipp *et al*., 2006; Semla *et al*., 2017; Guo *et al*., 2020; Xu, Wu, Chen, 2020; Ahmad Bainmahfouz *et al.,* 2021; Farag *et al.,* 2021). Construction workers applying acrylamide joint material has been determined to experience

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tingling and numbness in their hands and feet, and excessive fatigue, ataxia, and peripheral neuropathy may occur in longer exposures (Hagmar *et al*., 2001). Muscle weakness and sensory loss are the main signs of peripheral neuropathies. However, pain is one of the most disturbing symptoms (Marchettini *et al.,* 2006). Neuropathic pain is known to be caused by a somatosensory system lesion, including peripheral fibers and central neurons (Colloca *et al.,* 2017). Acrylamide neuropathy is associated with increased malondialdehyde (MDA) and decreased glutathione (GSH) contents in the sciatic nerve, brain, and spinal cord tissue (Tabeshpour *et al*., 2019; Elblehi, El Euony, El-Sayed, 2020). Furthermore, proinflammatory cytokines have been suggested to play a role in the pathogenesis of acrylamide neuropathy as well as reactive oxygen species (ROS) and oxidant products (Yan *et al.,* 2019; Elblehi, El Euony, El-Sayed, 2020). Moreover, Vanitha *et al.* (2015) showed that acrylamide-related neuropathic pain and damage were reduced with antioxidant treatment. Goudarzi *et al.* (2019) found that ellagic acid protected nerve tissue from the acrylamide oxidative and proinflammatory damage by inhibiting the acrylamide-induced increase of oxidants, tumor necrosis factor (TNF-α), and interleukin-1 beta (IL-1β) in the cerebral cortex. The TNF-α and IL-1β antagonists and antioxidant therapy may be useful in treating acrylamide-induced neuropathy and neuropathic pain according to these data.

Anakinra is an IL-1 receptor antagonist, which has been shown to inhibit IL-1 proinflammatory effects and is used as a biological response-modifying agent for rheumatoid arthritis treatment (Cvetkovic, Keating, 2002). Hasturk *et al.* (2015a) showed in an animal experiment that anakinra reduced MDA content, which is a lipid peroxidation product, and increased the activity of enzymatic antioxidants in case of damage to the spinal cord. In addition, anakinra has been noted to protect brain tissue from inflammatory damage by inhibiting IL-β level increase (Hasturk *et al*., 2015b). This study aims to conduct a biochemical and histopathological investigation of the effect of anakinra on acrylamide-induced neuropathy and neuropathic pain in rats.

MATERIAL AND METHODS

Ethics approval

The study protocol was approved by the Ethics Committee of Ataturk University, Erzurum, Turkey (number 254, date: 26.12.2019). All the stages of the experiment were performed following the ethical rules and guidelines of the same university.

Experimental animals

This study included 24 albino Wistar male rats weighing 280–290 g. All experimental animals were obtained from the Medical Experimental Application and Research Center of Ataturk University, Erzurum, Turkey. The animals were maintained at normal room temperature (22°C) in a 12-h light/dark cycle for environmental adaptation. Moreover, they were fed a standard rat chow diet and provided *ad libitum* access to water.

Chemicals

Chemicals such as acrylamide, thiobarbituric acid (TBA), sodium dodecyl sulfate, *n*-butanol, Tris, hydrogen chloride (HCl), ethylenediaminetetraacetic acid (EDTA), and 2-nitrobenzoic acid were supplied from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Moreover, anakinra (Kineret) was from Sobi (Waltham, Sweden), and thiopental sodium was from IE Ulagay (Istanbul, Turkey).

Experimental groups

The experimental animals were randomly divided into three groups with eight animals each: a group exposed to acrylamide alone (ACR), a group treated with anakinra plus acrylamide (AAC), and a control group that was only given distilled water (CG).

Experimental protocol

The experimental protocol scheme is shown in Figure 1. First, the rats in the AAC group were

intraperitoneally injected with anakinra at a dose of 50 mg/kg (Kuyrukluyıldız *et al*., 2016). Simultaneously, distilled water, as a solvent, was similarly administered to the animals in the ACR and CG groups. The animals in the ACR and AAC groups were given acrylamide at a dose of 20 mg/kg by gavage an hour after the administration of distilled water and anakinra (Goudarzi *et al.,* 2019). This procedure was repeated once a day for 30 days.

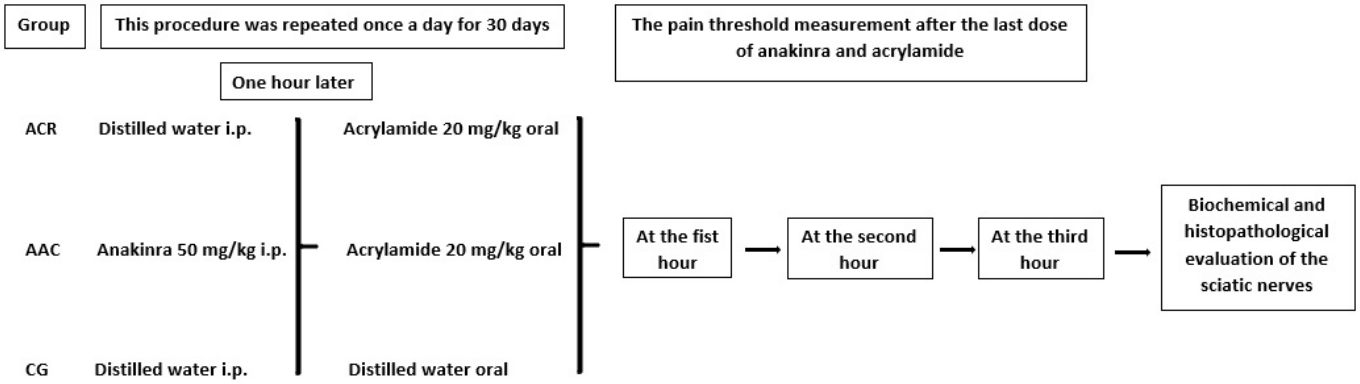

FIGURE 1 - Schematic description of the experimental protocol. *CG* control group, *ACR* acrylamide-treated group, *AAC* anakinra + acrylamide-treated group.

The pain threshold in the hind paws of all animal groups was measured using a Basile algesimeter to measure mechanical hyperalgesia (Ugo Basile SRL, Comerio, VA, Italy) at the first, second, and third hours after the last dose of anakinra and acrylamide (Randall, Selitto, 1957; Cadirci *et al.,* 2010; Kuyrukluyıldız *et al.,* 2016). Thus, the hind paw of each rat was placed on a press pad. The mechanical stimulation (in grams) applied to the paw increased at a constant rate until the rat withdrew the paw. The pressure was immediately stopped, and the value of this force was recorded.

The rats were sacrificed by high-dose thiopental anesthesia, and sciatic nerve tissues were extracted after the third hour of assessment. The MDA, total glutathione (tGSH), TNF-α, and IL-1β levels were determined in the removed nerve tissues, and a histological examination was also performed. All biochemical and histopathological results obtained from the ACR and AAC groups were compared with each other and those in CG. The analgesic activity of anakinra was calculated at the first, second, and third hours after the administration of the last dose of anakinra and acrylamide using the formula:

Analgesic activity (%) = 100 − [100 × paw pain threshold measured after acrylamide administration (g)/ paw pain threshold measured after the administration of anakinra plus acrylamide (g)] (Ince *et al*., 2015). Moreover, the simultaneously obtained paw pain threshold values of the ACR and AAC groups were used in the formula.

Biochemical analysis

Sample preparation

The MDA level in the nerve tissue was determined using 1.15% potassium chloride solution. Other measurements were carried out with phosphate buffer at pH 7.5. The nerve tissue was homogenized in ice and mixed with phosphate buffer, completed to a total of 2 mL. The mixture was then centrifuged at +4°C at 10,000 rpm for 15 min. The supernatant portion was collected for use in the analysis.

MDA analysis

The MDA measurement was performed using the method described by Ohkawa *et al*. (1979), which is based on the spectrophotometric measurement of a pink-colored complex formed by MDA with TBA heated at a 95°C

temperature and absorbed at 532 nm. The homogenates were centrifuged at 5,000 ×g for 20 min, and the supernatants were extracted. Moreover, 250 µL of the homogenate was vortexed into capped test tubes with 100 µL of 8% sodium dodecyl sulfate, 750 µL of 20% acetic acid, 750 µL of 0.08% TBA, and 150 µL of pure water to determine the MDA level. The mixture was incubated at 100°C for 60 min, and 2.5 mL of *n*-butanol was added. For spectrophotometric measurement, 3-mL cuvettes at 532 nm were used to define the red saturation. The MDA level in the samples was measured using standard graphs created in advance from a stock of MDA solution prepared following the dilution coefficients.

tGSH analysis

The tGSH quantity in the specimens was determined according to the modified method of Sedlak and Lindsay (1968). The samples were weighed and homogenized in 2 mL of 50 mmol/L Tris–HCl buffer containing 20 mmol/L EDTA and 0.2 mmol/L sucrose at pH 7.5. The homogenate was immediately precipitated with 0.1 mL of 25% trichloroacetic acid and centrifuged at 4,200 rpm at 4°C for 40 min. The obtained precipitate was removed, and the amount of tGSH was determined in the remaining supernatant. A tube was filled with 1,500 µL of the measurement buffer (200 mmol/L Tris–HCl buffer containing 0.2 mmol/L EDTA at pH 7.5) and vortexed with 500 µL supernatant, 100 µL of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB; 10 mmol/L), and 7,900 µL methanol. The content was then incubated for 30 min at 37°C and mixed with DTNB, which resulted in a yellow complex with sulfhydryl groups forming a chromogen. The absorbance was measured at 412 nm with a spectrophotometer (Beckman DU 500, Ramsey, MN, USA). The reduced glutathione was used to determine the standard curve.

IL-1β and TNF-α analysis in tissue

A rat-specific sandwich enzyme-linked immunosorbent assay (rat interleukin 1β ELISA) kit (Cat no: YHB0616Ra, Shanghai, China) was used to determine the quantity of tissue-homogenate IL-1β. Moreover, a rat TNF-α ELISA kit (Cat no: YHB1098Ra, Shanghai, China) was used to measure the tissue-homogenate TNF-α concentrations. All the measurements were carried out following the manufacturers' instructions. First, the monoclonal antibodies specific to rat IL-1β and TNF-α were coated onto the microplate wells. Second, the tissue homogenate, standards, specific biotinylated monoclonal antibody, and streptavidin–horseradish peroxidase were pipetted into these wells. Third, the content was incubated at 37°C for 60 min and washed. The chromogen reagents A and B, which produced color in the presence of bound enzymes, were then added. Moreover, the stop solution was added after the incubation of microplates at 37°C for 10 min. The intensity of this colored product as well as the concentration of rat IL-1β and TNF-α in the original samples were directly proportional. A microplate reader (Bio-Tek, USA) at 450 nm was used to estimate the well plates. The absorbance of the samples was evaluated according to the formulas and standard graphics.

Histopathological examination

The tissue samples were fixed in a 10% formaldehyde solution for a light microscope assessment, and the specimens were washed under tap water in cassettes for 24 h. The tissue samples were then passed through a series of increasing concentrations of alcohol to dehydrate tissues and kept for 10 min in each alcohol solution (70%, 80%, 90%, and 100%) to gradually dehydrate the tissue. The obtained samples were passed through xylol and embedded in paraffin. The latter was cut into sections of 4–5 µm thickness, stained with hematoxylin-eosin dye, and photographed using an Olympus DP2-SAL firmware program (Olympus® Inc., Tokyo, Japan). The histopathological assessment of the slices was performed by a pathologist blinded to the study groups. Six sections were taken from the sciatic nerve for each rat. The severity of histopathological findings in each section was graduated as 0 (normal), 1 (mild damage), 2 (moderate damage), and 3 (severe damage). The mean of six measurements was used as the value of that rat. The mean and median were then calculated for all groups.

Statistical analysis

IBM SPSS v. 22 (IBM Corp., released 2013; IBM SPSS Statistics for Windows, version 22.0; Armonk, NY, USA) was used for statistical analyses. The results of the continuous variables were presented as mean ± standard deviation. The Kolmogorov–Smirnov test was used to confirm the normality of oxidant/antioxidant and inflammatory markers. One-way analysis of variance (ANOVA) was applied for between-groups comparison. After ANOVA, Tukey's honestly significant difference or the Games–Howell test was used as post hoc according to the homogeneity of variances. The measurements of the pain threshold obtained at three different times were analyzed with repeated-measures ANOVA. Kruskal–Wallis test was used for quantitative examination of the histopathological data. Post hoc Dunn's test was performed, and adjusted *p* values were reported. Furthermore, the statistical level of significance (*p*) for all tests was <0.05.

RESULTS AND DISCUSSION

The antioxidant/oxidant status of the sciatic nerve tissue was determined by measuring the MDA and tGSH contents, while inflammatory changes were assessed by evaluating the TNF-α and IL-1β values. The MDA, tGSH, IL-1β, and TNF-α levels differed significantly between all groups ($p < 0.001$ for all).

A binary comparison was performed to determine the difference between group pairs. The MDA values in the ACR group differed significantly compared to CG ($p < 0.001$). MDA values were 4.17 ± 0.37 and 2.18 ± 0.32 µmol/g protein in the acrylamide and control groups, respectively. The anakinra treatment significantly reduced the MDA levels to 2.36 ± 0.32 µmol/g protein in the AAC group ($p < 0.001$). However, the difference between the AAC group and CG was not significant ($p = 0.649$; Figure 2).

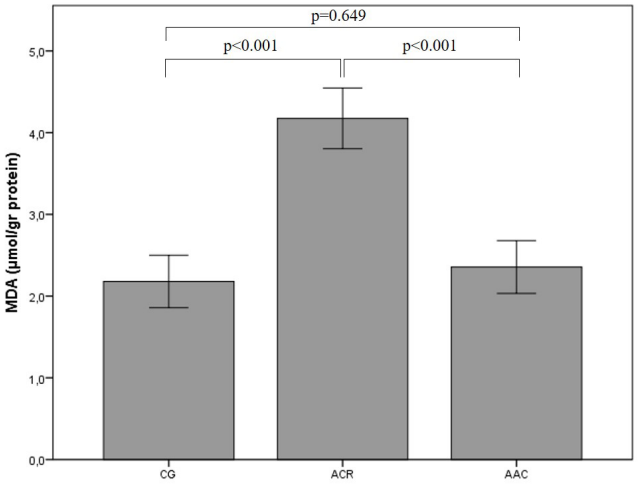

FIGURE 2 - Comparison of the malondialdehyde (MDA) levels between study groups. *Bars* represent mean ± standard deviation. *CG* control group, *ACR* acrylamide-treated group, *AAC* anakinra + acrylamide-treated group.

In contrast, the tGSH levels were 1.22 ± 0.09 µmol/g protein in acrylamide-exposed rats which were significantly lower than those in the CG group (3.79 ± 0.07 µmol/g protein; $p < 0.001$). The use of anakinra significantly increased the tGSH level to 3.05 ± 0.12 µmol/g protein ($p < 0.001$). However, their difference remained significant despite the mean values of the AAC and CG groups approaching each other ($p < 0.001$; Figure 3).

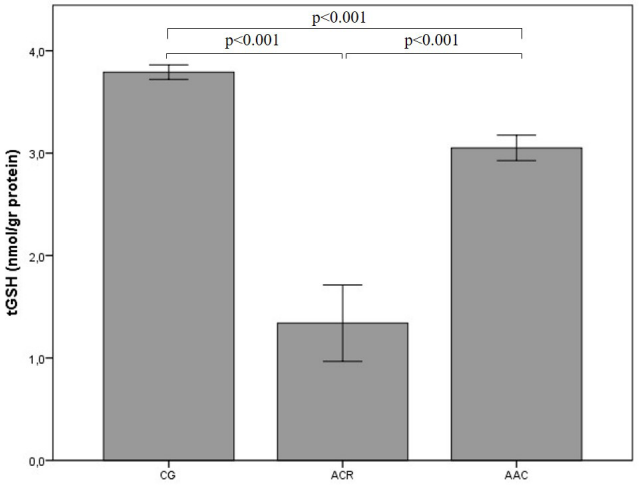

FIGURE 3 - Comparison of the tGSH levels between the study groups. *Bars* represent mean ± standard deviation. *CG* control group, *ACR* acrylamide-treated group, *AAC* anakinra + acrylamide-treated group.

Concerning proinflammatory markers, the IL-1β and TNF-α levels significantly differed in the group exposed to acrylamide compared to CG ($p < 0.001$ for both). Moreover, IL-1β and TNF-α values were 6.26 ± 0.11 and 5.64 ± 0.43 pg/mL in the acrylamide group, respectively, and were 3.00 ± 0.20 and 2.15 ± 0.09 pg/mL in the control group, respectively. The IL-1β contents decreased to 2.83 ± 0.28 pg/mL when anakinra was administered, and TNF-α contents decreased to 2.52 ± 0.33 pg/mL in AAC group ($p < 0.001$ for both). However, no significant difference in these levels was noted between the AAC and CG groups ($p = 0.471$ and $p = 0.086$, respectively; Figures 4 and 5).

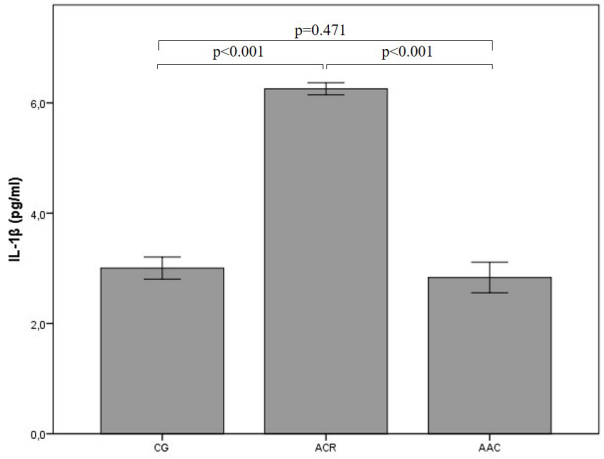

FIGURE 4 - Comparison of the IL-1β levels between the study groups. *Bars* represent mean ± standard deviation. *CG* control group, *ACR* acrylamide-treated group, *AAC* anakinra + acrylamide-treated group.

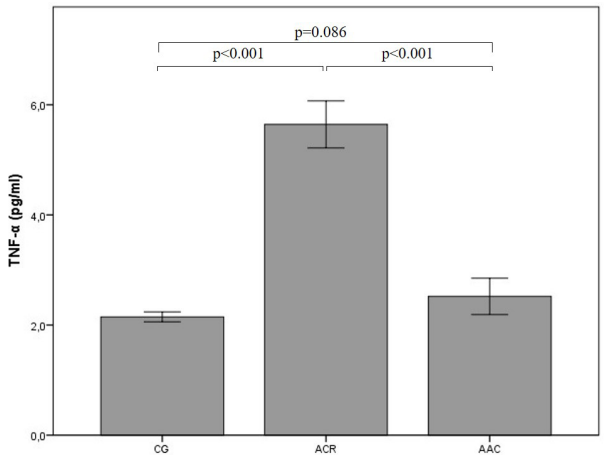

FIGURE 5 - Comparison of the TNF-α levels between the study groups. *Bars* represent mean ± standard deviation. *CG* control group, *ACR* acrylamide-treated group, *AAC* anakinra + acrylamide-treated group.

The pain threshold levels and their changes over time are shown in Figure 6. At the end of the experiment, the first evaluation (first hour) showed that the pain threshold in the ACR group was 9.8 ± 1.5 g, which is significantly lower than in the CG group where the pain threshold was 47.8 ± 3.5 g ($p < 0.001$). The pain threshold increased significantly to 30.5 ± 1.6 g but did not reach the level of the CG group ($p < 0.001$ for both) with anakinra administration. No significant change in the pain threshold in the second (9.2 ± 1.5 g) and third (8.8 ± 1.2 g) hours in the ACR group were noted compared with the initial assessment, and their mean values were significantly lower than in CG in which the pain threshold was 45.2 ± 3.4 and 45.8 ± 1.9 g, respectively. A significant increase in the pain threshold to 36.7 ± 1.9 g was noted in the AAC group in the second hour compared to the first assessment. However, this was significantly different from CG ($p < 0.001$ for both). In the AAC group, the mean values of the pain threshold at the third hour compared to the second hour showed a slight increase to 37.8 ± 1.2 g without a significant difference. Moreover, the pain threshold values significantly differed between the AAC and CG groups in the third hour. The analgesic effect of anakinra was 67.9% at the first hour and increased to 74.9% and 76.7% at the second and third hours, respectively.

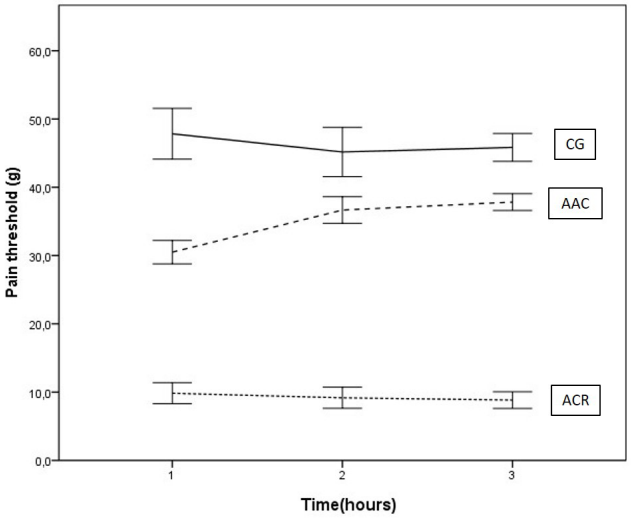

FIGURE 6 - Graphic representation of the threshold pain values (in grams) of the experimental groups according to the examination time. *CG* control group, *ACR* acrylamide-treated group, *AAC* anakinra + acrylamide-treated group.

In histopathological examination, the myelinated fibers of the sciatic nerve were observed to be normal in CG with the axon in the center and Schwann cells surrounding it, and the vessels were in a normal histological arrangement, together with the connective tissue surrounding the myelinated nerve fiber bundle (Figure 7A). The examination of the acrylamide-treated sections showed that the axons of the myelin nerve fibers noticeably swelled and degenerated and lost their central location. The Schwann cells forming the myelin sheath were hypertrophic and hyperplasic, and the vessels were congested (Figure 7B–C). In the sections belonging to the AAC group, the myelinated nerve fibers were mostly observed to have a normal structure, and the axons were in normal appearance and centrally located. The Schwann cells had normal morphology, and no congestion was observed in the vessels (Figure 7D). The quantitative data of histopathological changes in sciatic nerves shown in Figure 7A–D are presented in Table I.

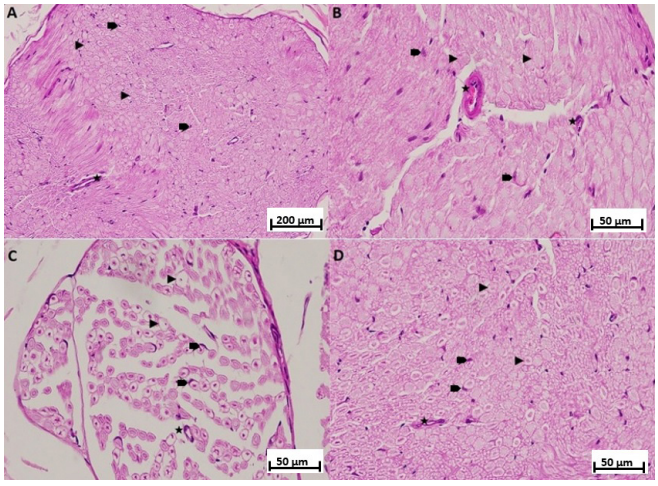

FIGURE 7 - Hematoxylin-eosin staining of the sciatic nerve tissue. **A** healthy nerve tissue in CG; ▶: myelinated axons, ➡: Schwann cells, ★ blood vessels, ×200. **B**–**C** Damaged nerve tissue in the ACR group; ▶: degenerated myelinated axons, ➡: hypertrophic and hyperplasic Schwann cells, ★: congested blood vessels, ×400. **D** AAC appearance is similar to normal nerve tissue: ▶: myelinated axons, ➡: Schwann cells, ★ blood vessels, ×400.

TABLE I - Quantitative analysis of histopathological examination

	CG		ACR		AAC	
	Mean ± SD	**Median (Min-Max)**	**Mean ± SD**	**Median (Min-Max)**	**Mean ± SD**	**Median (Min-Max)**
Axon destruction	0.0 ± 0.0	0(0-0)	2.6 ± 0.2	2.7(2.3-2.8) [a]	0.6 ± 0.3	0.5(0.3-1.2) [b]
Schwann cell degeneration	0.0 ± 0.0	0(0-0)	2.7 ± 0.2	2.7(2.3-3.0) [a]	0.6 ± 0.4	0.6(0.2-1.0) [b]
Vessel congestion	0.0 ± 0.0	0(0-0)	2.5 ± 0.2	2.5(2.2-2.7) [a]	0.3 ± 0.1	0.3(0.2-0.5) [b]

[a] Statistically significant when compared with CG. [b] Statistically significant when compared with ACR.
CG – control group, ACR – acrylamide-treated group, AAC – acrylamide + anakinra-treated group.

This is the first study to evaluate acrylamide-related proinflammatory cytokine levels in the sciatic nerve and mechanical hyperalgesia, as well as the effect of anakinra on neuropathic pain and biochemical and histopathological changes in the sciatic nerve tissue of acrylamide-treated rats. Acrylamide is a well-known environmental toxin with central and peripheral neurotoxicities. The most intense exposure of acrylamide occurs among people working in the industry, construction, laboratories, photography, and so on. The general population ingests acrylamide through water, diet, tobacco smoking, and cosmetics (Smith, Oehme, 1991; Erkekoglu, Baydar, 2014). The severity of the toxic effect of acrylamide depends on the total dose and duration of toxin contact. The prolonged intake of acrylamide in small doses can lead to a cumulative effect, thereby

creating a neurotoxicity risk, while exposure to episodic daily small doses does not cause any clinical change (LoPachin, 2004; Erkekoglu, Baydar, 2014).

Acrylamide neurotoxicity is manifested as central/peripheral distal axonopathies with a primary lesion in the nerve terminals of large-myelinated fibers (Schaumburg, Wisniewski, Spencer, 1974). Brismar *et al*. (1987) showed that secondary nonspecific nodal–paranodal changes were also observed in the proximal parts of the peripheral nerves, thereby confirming the idea of a *dying-back* progression of neuropathy caused by acrylamide. Furthermore, many human and animal studies have examined the development of acrylamide-associated peripheral neuropathy. Most human studies have been conducted with groups of people that are occupationally in contact with acrylamide. The examination of chemical factory and tunnel workers exposed to acrylamide revealed the presence of numbness and sweating of the hands and feet; hypoactive reflexes; difficulty grasping; gait disturbance; and loss of pain, vibration, and touch sensation (Calleman *et al.,* 1994; Hagmar *et al*., 2001).

Previous studies have reported that small-diameter nerves can also be damaged by acrylamide exposure (Ralevic, Aberdeen, Burnstock, 1991; Navarro *et al.,* 1993). Ko *et al.* (2002) demonstrated that acrylamide could affect the small sensory nerves of the skin responsible for nociception. Furthermore, Seale *et al.* (2012) found that acrylamide inhibited the expression of the opioid receptor gene (Oprk 1) in the sciatic nerve, which regulates neuropathic pain. This explains how acrylamide intoxication can lead to painful peripheral neuropathy. Thus, the paw pain threshold of acrylamide-treated rats in the present study was significantly reduced, indicating an impairment in the nociceptive system. The current findings are also in line with the results of a rat study by Vanitha *et al*. (2015), in which the intraperitoneal administration of acrylamide at a dose of 30 mg/kg for 24 days resulted in sciatic neuropathy manifested in the development of thermal hyperalgesia and mechanical allodynia with a decreased hind paw pain threshold. Similarly, Ling *et al.* (2005) demonstrated that low doses of acrylamide (max, 30 mg/kg/day) and a cumulative dose (<250 mg/kg) that did not cause motor dysfunction led to mechanical and thermal allodynia and hyperalgesia. Thus, the authors suggested that the use of sensory tests, especially the detection of allodynia, could help diagnose acrylamide-induced polyneuropathy at an early stage (Ling *et al.,* 2005). However, the nociceptive effect of acrylamide may be associated not only with a toxic effect on the peripheral nerve axon but also on the dorsal root ganglion and the gray matter of the spinal dorsal horn, which suggests the possible involvement of the central nervous system, in particular the spinal cord, in the development of acrylamide-induced neuropathic pain (Xiao *et al*., 2011; Sun *et al*., 2018; Elblehi, El Euony, El-Sayed, 2020).

Anakinra is the first recombinant human-specific and competitive antagonist of the IL-1 receptor that inhibits the binding of both subtypes (IL-1α and IL-1β) of the agonists to this receptor (Cvetkovic, Keating, 2002). Anakinra was shown to have antioxidant and analgesic properties, along with anti-inflammatory effects (Baamonde *et al*., 2007; Hasturk *et al*., 2015b). Further, a study by Hsieh *et al.* (2020) showed that anakinra can exert a central analgesic effect by reducing painful hypersensitivity, oxidative stress, and inflammation in the spinal cord caused by systemic lipopolysaccharides. An increase in paw pain threshold with anakinra treatment was noted in the current study. Furthermore, anakinra was found to have a significant analgesic effect at the first hour after the experiment. This effect was intensified at the second hour but did not lead to complete analgesia, which may be due to an insufficient dose of anakinra used in the current study. These results correspond to the findings of a study conducted by Kuyrukluyildiz *et al*. (2016), who showed that anakinra had analgesic properties in a dose-dependent manner. In that study, anakinra administered at a dose of 100 mg/kg had a more significant effect on paw pain caused by paclitaxel, an alkaloid chemotherapy drug used to treat several cancers, compared to a dose of 50 mg/kg (Kuyrukluyıldız *et al*., 2016). Also, the insufficiency of anakinra's analgesic effect could be due to severe nerve damage caused by acrylamide, which remains the cause of neuropathic pain despite the histochemical improvement after treatment with anakinra described below.

Previous studies have shown that MDA levels are inversely correlated with the paw pain threshold

and increased during neuropathic pain, indicating an oxidative imbalance (Ince *et al*., 2015; Amin, Poureshagh, Hosseinzadeh, 2016; Kuyrukluyıldız *et al*., 2016). However, acrylamide has been found in many studies to cause oxidative stress in the sciatic nerve tissue by increasing MDA and thiobarbituric acid-reactive substances as well as decreasing superoxide dismutase, GSH, GPx, GR, and anti-ROS levels (Zhu *et al*., 2008; Vanitha *et al*., 2015; Tabeshpour *et al.,* 2019). In the rats treated with acrylamide in the current study, the MDA level in the sciatic nerve was increased and the tGSH level was decreased, while the threshold of pain was reduced, which is consistent with the results of the aforementioned studies. In addition, exposure to acrylamide was found to increase the IL-1β and TNF-α levels in the sciatic nerve, suggesting an inflammatory process along with oxidative stress. It is believed that the levels of proinflammatory cytokines in the sciatic nerve of the animals exposed to acrylamide have not been studied in the literature. However, acrylamide exposure has been previously shown to increase the levels of proinflammatory cytokines (e.g., IL-1β, TNF- α, and IL-6) and decrease the level of the anti-inflammatory cytokine IL-10 in the serum (Zhang *et al*., 2013; Elblehi, El Euony, El-Sayed, 2020), which is in line with the current findings.

Treatment with anakinra in the present study significantly reduced the MDA, TNF-α, and IL-1β contents and increased the tGSH level in the sciatic nerve of rats that were exposed to acrylamide. The incomplete recovery of the tGSH value may be due to the insufficient dose of anakinra and that acrylamide and its metabolite, glycidamide, directly bind to the sulfhydryl groups of glutathione (Smith, Oehme, 1991). The current findings are consistent with the results of Kuyrukluyildiz *et al.* (2016), reporting that the increased MDA and IL-1β levels significantly decreased in the paw tissues of the rats receiving paclitaxel, while the decreased tGSH level significantly increased after anakinra administration at doses of 50 and 100 mg/kg, although a higher dose of anakinra had a more pronounced antioxidant and anti-inflammatory effect.

Several histopathological studies evaluating the effect of acrylamide on peripheral nerves exist. Gold *et al.* (2004) found a decrease in the total number of myelin fibers, swelling, and degeneration of axons in the tibial nerve. Another study showed altered myelin sheaths and axolemma invaginations, along with severe axon damage (Perez-Saad *et al.,* 2017). In addition, Vanitha *et al*. (2015) detected the derangement of the nerve fibers of the sciatic nerve. Furthermore, acrylamide increased the immunoexpression of the apoptotic marker caspase-3 and inducible nitric oxide synthase, the marker of inflammation, in the sciatic nerve (Ahmad Bainmahfouz *et al*., 2021). The literature data are consistent with the results of the current study which showed that exposure to acrylamide caused edema and degeneration of myelinated axons, as well as location change. Moreover, pathological changes also affected the Schwann cells and blood vessels. Nevertheless, these abnormalities were significantly restored by the anakinra administration. The effect of anakinra on histopathological changes in the peripheral nerves caused by acrylamide had not been studied before the current study. However, Gonçalves *et al.* (2015) found that the anakinra administration in mice with a model of the familial amyloid polyneuropathy reduced the expression of transthyretin, a protein found in the plasma and cerebrospinal fluid and associated with amyloidosis, in the Schwann cells and its extracellular accumulation after nerve damage, which indicates the ability of anakinra to play a role in the protection and restoration of peripheral nerves similar to the current study.

CONCLUSIONS

Based on the results of the current study, exposure to acrylamide leads to oxidative and inflammatory damage of the peripheral nervous system, which is confirmed by biochemical and histopathological changes in the sciatic nerve and is accompanied by a decrease in the pain threshold as evidenced by neuropathic pain. Anakinra treatment significantly reduced the level of oxidants and proinflammatory markers, increased the mean values of antioxidants, caused an improvement in histopathological changes, increased the pain threshold, and, consequently, decreased neuropathic pain. Thus, anakinra was suggested to be used for treating peripheral neuropathy and neuropathic pain caused by acrylamide.

LIMITATIONS

Unstudied enzyme activities (e.g., catalase and superoxide dismutase) and reactive species levels as well as the lack of histopathological examination of unmyelinated sciatic nerve fibers are the limitations of this study.

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