

An investigation of the effect of acrylamide on fracture healing in rats

Özhan Pazarcı, M.D.,¹ Erkan Gumus, M.D.,² Ayca Taş, M.D.,³ Neşe Keklikcioğlu Çakmak, M.D.,⁴
Seyran Kılınç, M.D.,¹ Şeyma Nur Yıldız, M.D.,⁵ Okay Bulut, M.D.¹

¹Department of Orthopaedics and Traumatology, Sivas Cumhuriyet University Faculty of Medicine, Sivas-Turkey

²Departments of Histology and Embryology, Aydın Adnan Menderes University Faculty of Medicine, Aydın-Turkey

³Department of Nutrition and Diet, Sivas Cumhuriyet University Faculty of Health Sciences, Sivas-Turkey

⁴Department of Chemical Engineering, Sivas Cumhuriyet University Faculty of Engineering, Sivas-Turkey

⁵Department of Biochemistry, Sivas Cumhuriyet University Faculty of Medicine, Sivas-Turkey

ABSTRACT

BACKGROUND: The aim of this study was to investigate the effects of acrylamide (AA) on fracture healing histologically, biochemically, and radiologically in a rat femur fracture model.

METHODS: Scanning electron microscopy imaging, Fourier transform infrared spectroscopy, and ultraviolet-visible spectrophotometer examination were performed for AA characterization. In this study, after the femur fracture model was created, the groups were formed to include eight rats in each group (G) as follows: G1: 15th-day control, G2: 15th-day AA, G3: 30th-day control, and G4: 30th-day AA. In G2 and G4, 5 mg/kg AA was administered 3 times a week by gastric gavage. The fracture was evaluated radiologically according to Lane-Sandhu scoring and histologically according to Huo scoring. The weight changes of the rats were recorded. Albumin, total protein, cholesterol, high-density lipoprotein, low-density lipoprotein, triglyceride, alkaline phosphatase, lactate dehydrogenase, vit. D, parathormone, calcium, phosphorus (P), white blood cell (WBC), hemoglobin (Hb), and Platelet values were examined in the blood samples.

RESULTS: The characterization properties of AA were confirmed. No significant weight change was observed in the rats during the study. When blood values were compared, a statistically significant difference was determined between albumin, total protein, P, WBC, and Hb groups ($p=0.41$, $p=0.00$, $p=0.003$, $p=0.019$, and $p=0.017$, respectively). According to the histological score comparisons, G3 was significantly different from G1, G2, and G4 ($p<0.05$), and G4 was significantly different from G1 and G2 ($p<0.05$). According to Lane-Sandhu scoring, there was a significant difference between G2 and G3 and G4 ($p=0.0$, $p=0.034$), G1 and G3 ($p=0.001$), respectively.

CONCLUSION: AA adversely affects fracture healing even at low doses, as in the present study. According to the results of this study, the authors recommend a diet poor in AA during fracture treatment. Therefore, further human studies are required to find out the complex effect of AA on bone healing and the body.

Keywords: Acrylamide; bone; fracture; fracture healing; rat.

INTRODUCTION

Fracture healing is a complex physiological process in which many local and systemic pathways play roles.^[1] The fact that this process is adversely affected may result in nonunion.

Nonunion of fracture is associated with additional complications and increased treatment costs.^[2] Bone health and the optimization of local and systemic conditions are important for fracture healing.^[3] The relationship between the dietary pattern and fracture healing is well known. A healthy diet is

Cite this article as: Pazarcı Ö, Gumus E, Taş A, Keklikcioğlu Çakmak N, Kılınç S, Yıldız ŞN, et al. An investigation of the effect of acrylamide on fracture healing in rats. *Ulus Travma Acil Cerrahi Derg* 2022;28:570-578.

Address for correspondence: Özhan Pazarcı, M.D.

Sivas Cumhuriyet Üniversitesi Tıp Fakültesi, Ortopedi ve Travmatoloji Anabilim Dalı, Sivas, Turkey

Tel: +90 346 - 219 11 56 E-mail: dr.pazarci@gmail.com

Ulus Travma Acil Cerrahi Derg 2022;28(5):570-578 DOI: 10.14744/tjtes.2021.94848 Submitted: 23.09.2020 Accepted: 14.02.2021

Copyright 2022 Turkish Association of Trauma and Emergency Surgery



required both for reducing the risk of fracture and accelerating the post-fracture healing process.^[4] Diet is a modifiable risk factor in fracture healing.^[4]

In 2002, the heat-induced formation of acrylamide (AA) was determined in dietary foods we consumed.^[5] AA represents a soluble compound with low molecular weight formed during thermal processing as an intermediate product of the Maillard reactions, primarily through the reaction between the amino acid asparagine and a number of reducing sugars.^[6] AA exposure increases with dietary products. The consumption of foods such as fried potatoes, chips, chicken nuggets, onion rings, breakfast cereals, biscuits, crackers, and instant coffee increases the intake of AA into the body.^[7,8]

The carcinogenic effect of AA in rat models has been demonstrated. However, this effect is controversial in humans.^[9,10] Moreover, it has neurotoxic and apoptosis-enhancing effects.^[11,12] In the literature, the effects of AA on bone metabolism were emphasized in various studies. Raju et al.^[13] showed that AA leads to an increase in the oxidative stress and peroxidation of lipids. Oxidative stress is characterized by an increased level of reactive oxygen species, which improve bone resorption^[14] and osteoclastogenesis.^[15] Furthermore, a biochemical link between increased lipid peroxidation and reduced bone mineral density was described.^[16] Furthermore, AA was shown to decrease the blood levels of calcium (Ca), which is essential for bone.^[13] According to the best of our current knowledge, although the harmful effects of AA were discussed in the literature, its effects on fracture healing were not previously investigated. In this context, the aim of this study was to present the effects of AA on fracture healing by investigating them histologically, biochemically, and radiologically in a rat fracture model.

MATERIALS AND METHODS

AA and Characterization

AA (Cas 79-06-1) ($\text{CH}_2=\text{CHCONH}_2$), a white, dry, and odorless crystal solid with a chemical purity of 99%, was obtained from Sigma-Aldrich Chemical Co. (St. Louis, USA). The morphology of AA was investigated by scanning electron microscopy (SEM: TESCAN MIRA3 XMU, Brno-Kohoutovice, Czech Republic). The pressing of all specimens into potassium bromide pellets was performed, and they were utilized for the examination of the functional groups of the samples by a Fourier transform infrared spectroscopy (FTIR: Bruker: Tensor II) spectrophotometer in the range of 3500–750 cm^{-1} . A ultraviolet (UV)-visible (Vis) spectrophotometer (UV-2600, Shimadzu, Japan) was utilized for the purpose of recording the spectra of the prepared samples in the range from 200 to 300 nm.

Animal Material

Local animal ethics committee approval was obtained for 32

adult Wistar albino male rats weighing 210–250 g included in this study (Date/no:17.09.2019/65202830-050.04.40-240). The feed required for the feeding of rats was obtained from our university's laboratory of experimental animals. The rats were cared and fed by providing water and feed ad libitum in metal cages with a base, at room temperature of 22–24°C and humidity of 55%, in a 12-h light/12-h dark environment.

Fracture Model

After the general anesthesia combined with xylazine and the intraperitoneal ketamine administration, the rat was placed on the operating table, and a single dose of antibiotic prophylaxis was administered (cefazolin sodium, 5 mg). After the left thigh of the rat was shaved, it was prepared with Povidone-iodine solution. After a 1-cm long skin incision was made along the lateral aspect of the left thigh, soft tissues were dissected to expose the femoral shaft. A standard transverse fracture was made from the middle part of the femur by multiple drilling and then osteotomy. Afterward, the fracture was detected by inserting retrograde intramedullary Kirschner wire (0.8 mm diameter steel K-wire) from the knee joint. The skin layers were sutured properly. No loading restriction was performed.

Experimental Stage

The experimental stage of the study and sampling was conducted in our university's experimental animal unit. In this study, the groups were formed to include eight rats in each group (G) as follows: G1:15th-day control, G2: 15th-day AA, G3: 30th-day control, and G4: 30th-day AA. On the 1st day, a fracture model was created for all rats, as stated above. G2 and G4, which constituted the experimental group, were administered with AA at a dose of 5 mg/kg 3 times a week by gastric gavage. It was administered 3 times a week to avoid esophageal irritation. Afterward, the rats in 15th-day G1 and G2 and 30th-day G3 and G4 were sacrificed, and the bones removed were taken for examination. The macroscopic and radiological examinations of the samples were performed. Radiological fracture classification was performed according to Lane-Sandhu scoring.^[17]

Biochemical Analysis

Before the rats were sacrificed, 3 ml of blood samples were taken from the groups into sterile tubes. Plasma was obtained from the blood samples collected (centrifuged 1000×g, 15 min). These plasmas were transferred to Eppendorf tubes and stored at -80°C until analysis measurements were performed. In plasma samples, alkaline phosphatase (ALP), parathormone (PTH), and Vitamin D (Dvit) concentrations were analyzed by the enzyme-linked immunosorbent assay (ELISA) technique. All measurements were recorded blindly by the same person. All samples and standards were analyzed twice. Repeated readings were averaged for each standard and sample. The detectable ranges of human ALP (Catalog no: 201-11-0346 Sunred Biological Technology Co., Ltd., Shanghai, CHINA),

PTH (Catalog no: 201-11-0334 Sunred Biological Technology Co., Ltd., Shanghai, CHINA), and Dvit (Catalog no: 201-11-0614 Sunred Biological Technology Co., Ltd., Shanghai, CHINA) were 0.8–200 ng/mL, 0.5–60 ng/mL, and 0.8–200 ng/mL, respectively.

Complete blood count analyses were performed using an automated hemoanalyzer (Mindray BC6800; Mindray, Shenzhen, China). The plasma levels of total protein, albumin, Ca, total cholesterol, high-density lipoprotein cholesterol, triglyceride, low-density lipoprotein cholesterol, phosphorus (P), and the activity of lactate dehydrogenase were determined by spectrophotometric methods (Roche COBAS 8000 Modular; Roche Diagnostics, Mannheim, Germany).

Histopathological Examination

For the histopathological evaluation of the tissues of the control and study groups, the femur samples containing the fracture site were fixed with 10% neutral buffered formalin for at least 2 weeks, and then, decalcification was performed. After the samples were washed under running tap water, they were subjected to parafinization by passing through the series of graded alcohols and xylol. Afterward, 5- μ m-thick longitudinal sections were taken from the samples placed in paraffin blocks using a rotary microtome (Leica, RM2135, Germany). These sections were stained with Hematoxylin-Eosin and examined under the light microscope (Olympus BX51, Tokyo, Japan).

The changes observed in the control and study groups were examined in four sections containing the fracture site from each sample at 100- μ m thickness intervals. The changes were based on the histological scoring described by Huo et al.^[18]

Statistical Analysis

Statistical analysis was conducted using SPSS ver 23.0 (SPSS Inc., IBM, NY, USA). Continuous variables were given as mean and standard deviations. A comparison of four independent groups was performed by the one-way analysis of variance test. The post hoc comparison of groups was performed by the Bonferroni test. P-values lower than 0.05 were considered as statistically significant.

RESULTS

AA and Characterization

The SEM image of AA demonstrates a continuous and non-ordered microporous mesh-like network. As shown in Figure 1, the structure of AA is in the form of chain binding. Due to its AA structure, it is easily soluble in water.

In Figure 2, the IR spectra of AA are presented. The peak at 1650 cm^{-1} represented the carbonyl double bond C=O stretching, that is, ν C=O. The band at 1650–1580 cm^{-1} was assigned to ν N-H bending vibration. The peak in the region

of 960–920 cm^{-1} suggested the δ NH bending vibration of amines. The peak at 985 cm^{-1} represented the C=C bending. Furthermore, the peak at 1423 cm^{-1} indicated the C-H bending.^[19]

The UV-Vis absorbance spectrum of AA is shown in Figure 3. For a spectrophotometric measurement, AA was prepared at a low concentration in water and subjected to the probe sonicator for 15 min to dissolve in a short time. The peak at 198 nm is observed in Figure 3. The peak in the figure is the characteristic peak of AA.^[20]

Experimental Groups

The results of the rat groups with AA administration and the control rat group were examined by comparing (Table

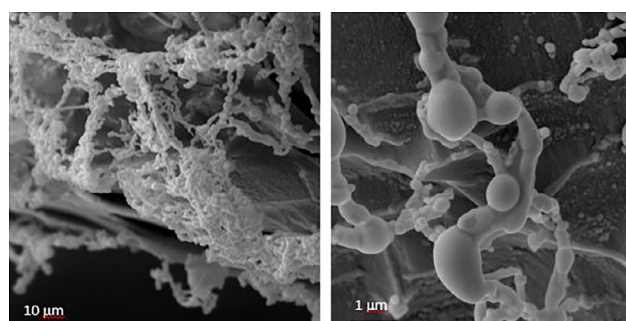


Figure 1. The scanning electron microscopy images of acrylamide.

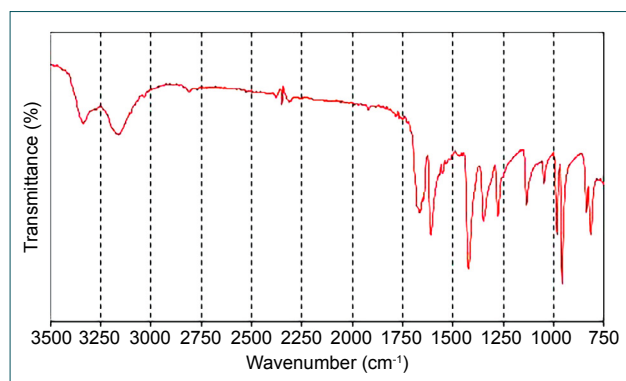


Figure 2. Infrared spectra of acrylamide.

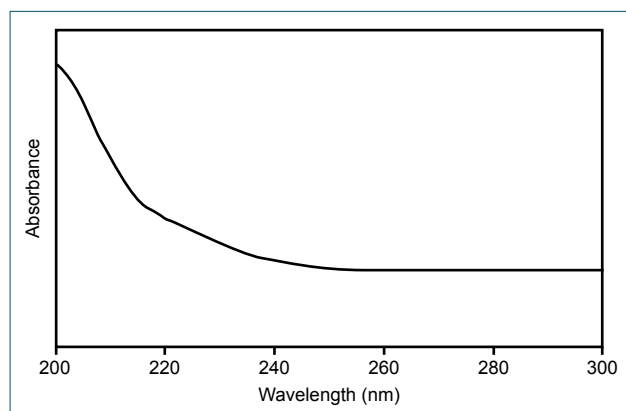


Figure 3. Ultraviolet-visible spectrum of acrylamide in water.

Table 1. Comparative display of averages for all parameters and groups

	Group 1 15 th -day control		Group 2 15 th -day acrylamide		Group 3 30 th -day control		Group 4 30 th -day acrylamide		p-value*
	n=8	Std. Err	n=8	Std. Err	n=8	Std. Err	n=8	Std. Err	
Weights of rats on the first day (gram)	228.87	5.68	230.62	2.52	228.0	3.91	244.12	4.72	0.050
Weights during sampling (gram)	217.12	4.91	209.50	4.76	218.12	4.85	226.37	5.18	0.173
Weight change (gram)	12.5	5.22	21.12	3.73	12.75	2.61	17.75	3.03	0.299
Albumin (g/dL)	41.78	1.42	40.98	2.46	43.42	1.48	48.22	1.93	0.041
Total protein (g/dL)	62.43	1.15	64.37	0.9	69.20	1.49	71.63	1.08	0.000
Cholesterol (mg/dL)	62.62	2.98	64.63	5.38	64.50	4.85	66.62	9.88	0.293
High-density lipoprotein (mg/dL)	47.20	2.69	50.46	5.39	47.62	3.8	60.42	2.33	0.053
Low-density lipoprotein (mg/dL)	10.31	0.89	9.51	0.57	10.83	1.22	10.17	1.11	0.917
Triglyceride (mg/dL)	72.50	10.81	60.0	5.19	80.25	9.21	63.5	8.17	0.434
Alkaline phosphatase (ng/mL)	17.20	2.53	15.51	2.87	17.67	1.63	23.75	2.88	0.181
Lactate dehydrogenase (U/L)	1136.63	235.68	760.63	87.8	760.87	101.68	1003.25	172.42	0.181
D vit (ng/mL)	18.92	2.99	17.16	2.59	23.86	2.86	20.93	2.52	0.305
Parathormone (ng/ml)	7.47	0.37	6.63	0.75	9.1	0.67	7.48	1.33	0.229
Calcium (mg/dL)	10.11	0.09	9.9	0.11	10.1	0.11	10.17	0.07	0.370
Phosphorus (mg/dL)	6.41	0.2	6.78	0.18	5.88	0.16	5.68	0.2	0.003
White blood cell (10 ⁹ /L)	7.52	0.61	6.47	0.36	10.17	1.0	7.59	0.84	0.019
Hemoglobin (g/dL)	13.7	0.47	13.3	0.88	15.13	0.46	15.27	0.62	0.017
Platelets (10 ⁹ /L)	597.62	83.25	664.0	124.14	663.0	76.62	842.62	118.63	0.069
Lane sandhu	2.88	0.44	2.38	0.26	6.25	0.7	3.88	0.44	0.001

*Those with p<0.05 were considered significant.

I). When the first day of the study and the day of sampling by sacrificing were compared, no significant difference was determined between the groups in terms of the rats' weights ($p=0.050$ and $p=0.173$, respectively). No significant difference was found in the weight changes between the groups ($p=0.299$).

Biochemical Analysis

When the blood values of the groups were compared, a statistically significant difference was determined between albumin, total protein, P, white blood cell (WBC), and hemoglobin (Hb) groups ($p=0.41$, $p=0.00$, $p=0.003$, $p=0.019$, and $p=0.017$, respectively) (Fig. 4). No significant difference was observed between other blood parameters. The results and their comparisons are presented in Table 1. A significant difference was observed in albumin values between G1 and G4, G2 and G4 ($p=0.009$ and $p=0.022$), respectively. A significant difference was determined in total protein values between G1 and G3 and G4 ($p=0.005$, 0.000), G2 and G3 and G4 ($p=0.032$, 0.02), respectively. With respect to P, a significant difference was determined between G4 and G1 and G2 ($p=0.031$, 0.001), G3 and G2 ($p=0.004$). With respect to WBC, a difference was observed between G2 and G3 ($p=0.002$). With respect

to Hb level, a significant difference was observed between G2 and G3 and G4, G1, and G4 ($p=0.048$, $p=0.013$, and $p=0.013$) (Table 1).

There was no significant difference between the groups in ALP, PTH, and Dvit levels analyzed by the ELISA method ($p=0.181$, $p=0.22$, and $p=0.305$) (Table 1).

Histopathological Evaluation

All preparations prepared after radiological examination were evaluated histopathologically in line with the scale described by Huo et al.^[18] according to fibrous tissue, cartilage, new bone, and mature bone ratios (Fig. 5). According to the pairwise comparisons of all groups, no significant difference was determined between the 15th-day AA group (G2) and the 15th-day control group (G1) in terms of histological evaluation ($p=0.1244$). The mean scores of histological examination were determined as 2.54 and 2.14, respectively, for G1 and G2 groups (Fig. 6). The difference between the 30th-day control group (G3) and the 30th-day AA group (G4) was determined to be significant in terms of histological evaluation ($p<0.0001$). The mean scores in these groups were determined to be 8.57 and 6.14, respectively (Fig. 6). It was deter-

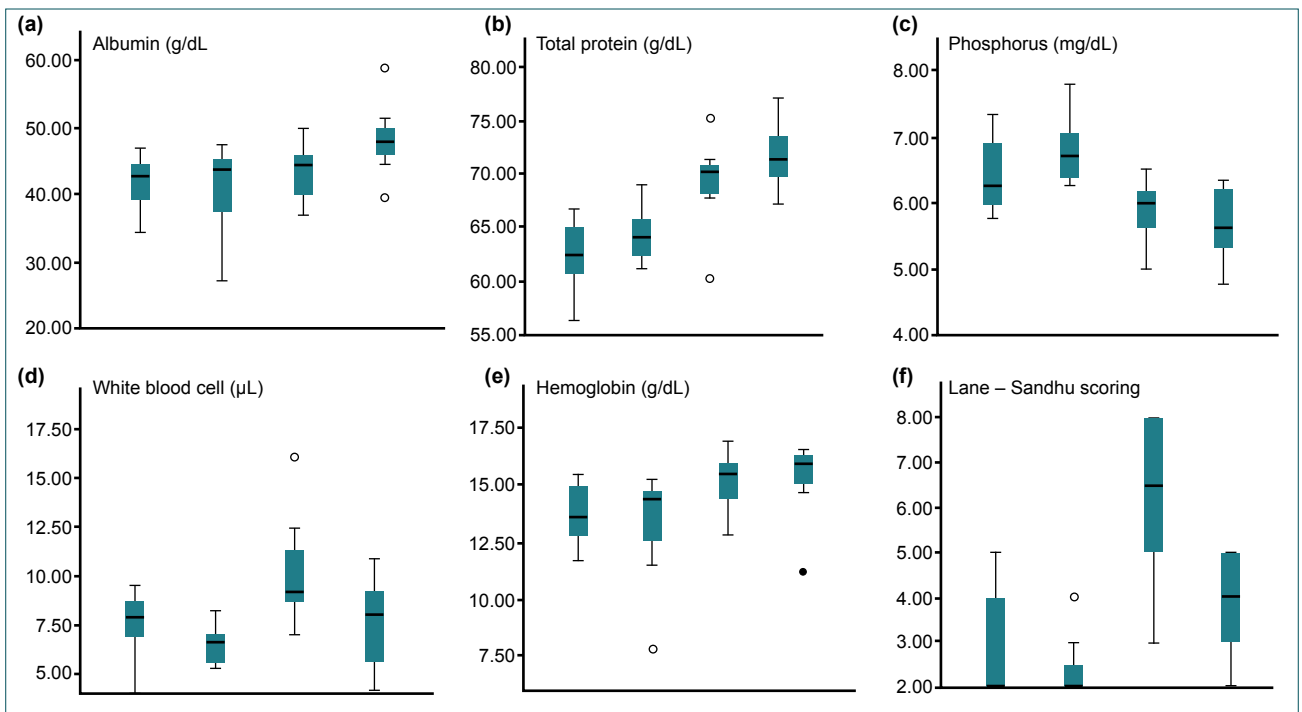


Figure 4. Graphical representation of the parameters with significant differences between the groups*. **(a)** Albumin: A significant difference was observed between G4 and G1 and G2, respectively ($p=0.009$, $p=0.022$). **(b)** Total protein: A significant difference was observed between G1 and G3 and G4 ($p=0.005$, $p=0.000$), respectively, G2 and G3 and G4 ($p=0.032$, $p=0.002$), respectively. **(c)** Phosphorus: A significant difference was observed between G4 and G1 and G2 ($p=0.031$, $p=0.001$), G3 and G2 ($p=0.004$), respectively. **(d)** White blood cell: A significant difference was observed between G2 and G3 ($p=0.002$). **(e)** Hemoglobin: A significant difference was observed between G2 and G3 and G4 ($p=0.48$, $p=0.013$), G1 and G4 ($p=0.013$), respectively. **(f)** Lane-Sandhu score: A significant difference was observed between G2 and G3 and G4 ($p=0.0$, $p=0.034$), G1 and G3 ($p=0.001$), respectively. *(G1: 15th-day control, G2: 15th-day acrylamide, G3: 30th-day control, and G4: 30th-day acrylamide).

mined that G3 had the best histological score and that the difference was statistically significant compared to all other groups ($p<0.05$). It was determined that the histological score of G4 was better compared to G1 and G2 and that the difference was statistically significant (Fig. 6; $p<0.05$).

Radiological Evaluation

In the direct radiographs, the union at the fracture line was evaluated according to Lane-Sandhu scoring. A significant difference was observed between G2 and G3 and G4 ($p=0.0$, $p=0.034$), G1 and G3 ($p=0.001$), respectively (Figs. 4 and 7).

DISCUSSION

The strongest aspect of this study is that it is the first study investigating the effects of AA on fracture healing. AA is an important industrial chemical, which is mainly used in the production of polymers and copolymers.^[21] However, after the formation of AA depending on the preparation conditions of foods was reported, the studies investigating the adverse effects of AA on humans increased in the literature.^[22] Nowadays, AA is one of the most common toxic substances that people are exposed to through diet.^[23] Since there is a close relationship between fracture healing and bone health and diet, the effects of AA on fracture healing were inves-

tigated in this study.^[24] AA can be easily distributed to all tissues and organs after being taken into the body, the main reason for which is that it is water-soluble.^[8] These data were confirmed by AA characterization data in the study. Furthermore, the SEM and FTIR analysis results of AA included in the study have also contributed to the literature (Fig. 1).

AA is a substance that is known to be distributed to all tissues when it is taken into the body.^[8] Nevertheless, the effects of AA on bones are still unknown and unclear, and the literature in this regard is scarce. AA increases oxidative stress and lipid peroxidation in the body.^[13] There are studies claiming that this oxidative stress and lipid peroxidation also increase bone resorption.^[14,16] Furthermore, Sarocka et al.^[25] showed that compact and trabecular bone microarchitecture was also affected in mice exposed to AA (rats were used in this study). AA has effects on possible fracture healing through these mechanisms. Furthermore, our study showed that P level changes in AA exposure. This changing it may effect fracture healing. Furthermore, AA exposure affects cortical bones more than trabecular bones. It was emphasized in the literature that cortical thinning occurred in mice with single and high dose AA exposure compared to the control group. Moreover, the fact that a vasoconstriction of primary osteons' vascular canals was shown in mice exposed to AA

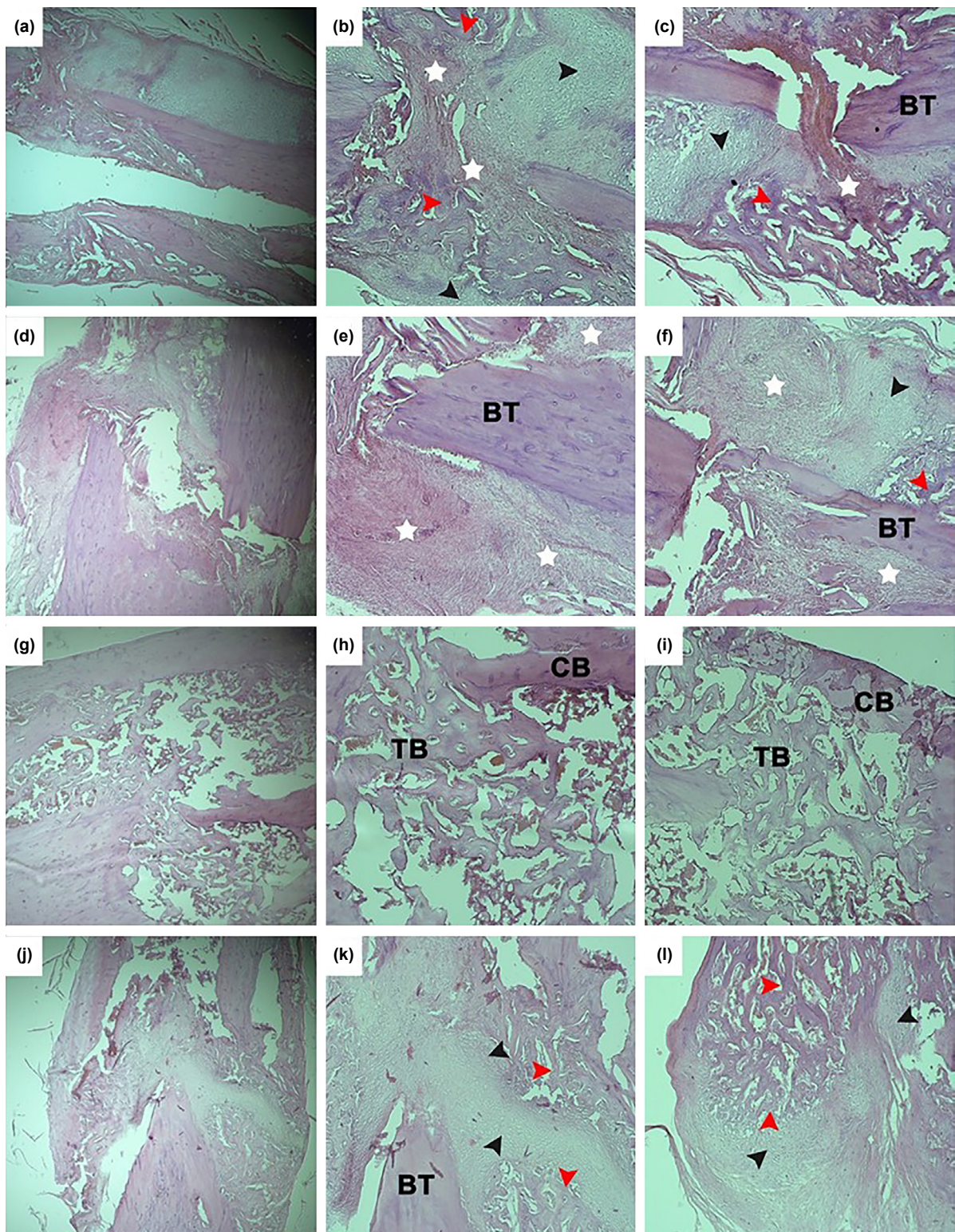


Figure 5. Histopathological view of the groups*: G1 (a-c), G2 (d-f), G3 (g-i), and G4 (j-l). In the images of G1 (a-c), mainly cartilage tissue, a small amount of fibrous tissue and new bone were observed (Grade 4). In the images of G2 (d-f), mainly fibrous tissue, a small amount of cartilage tissue and new bone were observed (Grade 2). In the images of G3 (g-i), mainly trabecular and cortical bone tissue and a small amount of new bone were observed (Grade 10). In the images of G4 (j-l), cartilage and immature bone tissue were observed equally (Grade 7). Star: Fibrous tissue, black arrowheads: Cartilage tissue, red arrowheads: new bone, BT: Bone tissue, CB: Cortical bone, and TB: Trabecular bone. (Hematoxylin-eosin staining a, d, g, and j; $\times 40$ magnification, b-c, e-f, h-i, and k-l; $\times 100$ magnification). *(G1:15th-day control, G2: 15th-day acrylamide, G3: 30th-day control, and G4: 30th-day acrylamide).

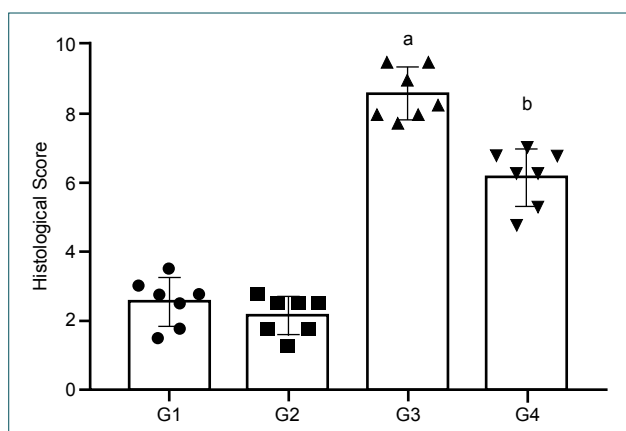


Figure 6. Histological score comparisons of all groups*. G3 was significantly different from G1, G2, and G4 ($^aP<0.05$). G4 was significantly different from G1 and G2 ($^bP<0.05$). *(G1:15th-day control, G2: 15th-day acrylamide, G3: 30th-day control, and G4: 30th-day acrylamide).

is also important for emphasizing the effect of AA on bones.^[25] In the present study, the fact that fracture healing was negatively affected in the group with AA administration was shown for the first time in the literature by histological examination (Fig. 3). Radiologically, it was also determined that fracture healing was more delayed, especially in the group administered with 30th-day AA.

In this study, similar to the literature, a low dose of 5 mg/kg AA was used 3 days a week.^[26] There are studies recommending that the daily dietary intake of AA in humans should not exceed 0.012 mg/kg. Exposure to AA over 0.5 mg/kg/day may also lead to neuropathy.^[27] However, daily exposure is higher in children and those who eat foods rich in AA.^[22,28] In the studies investigating the carcinogenic and neuropathic effects of AA, studies are conducted at higher doses such as 50

mg/kg/day.^[29] It should not be forgotten that the effects of AA are directly associated with “dose X time.”^[30] In this regard, the fact that low-dose and short-term AA exposure may even have adverse effects on fracture healing is important in that it was emphasized in this study.

Human studies indicate that all orally consumed AA is absorbed from the intestinal wall.^[31] Orally consumed AA, which is absorbed into the bloodstream and then distributed to different organs, reacts with DNA, neurons, Hb, and enzymes.^[32] This study is valuable in terms of showing the blood parameters of the groups besides fracture healing. No significant difference was observed between the groups in the lipid profile. Raju et al.^[13] achieved the same results at a dose of 5 mg/kg. It was reported that there was an increase in lipid profile in the blood due to the toxic effect caused by higher doses of AA in the liver.^[33] Another prominent aspect of this study is that Ca, PTH, and Vitamin D levels, which are effective in bone metabolism, were also investigated. No significant difference was observed in the AA dose administered in the study between the groups. The change in Ca and Dvit levels in AA exposure may be encountered in cases accompanied by liver dysfunction. Furthermore, it was shown in the literature that there was a decrease in bone mineral density in acute exposure to AA.^[25] A significant decrease was observed in the P level on the 30th day in the group administered with AA. Raju et al.^[13] reported a decrease in P at a higher dose of AA, similarly to this study.

In this study, a significant elevation was observed in total protein and albumin levels in the AA group, especially on the 30th day. A change in total protein and albumin levels in rats exposed to AA was reported in the literature, similarly to this study.^[33] Furthermore, in this study, a significant difference was observed between the 15th day and 30th-day Hb values of

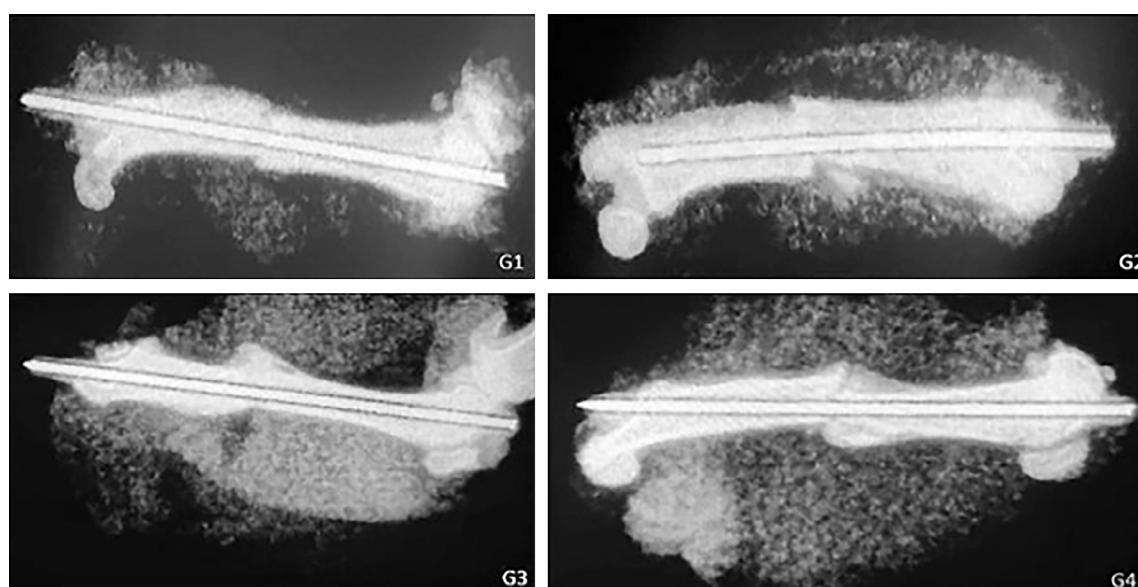


Figure 7. Radiological imaging samples of the groups*. *(G1:15th-day control, G2: 15th-day acrylamide, G3: 30th-day control, and G4: 30th-day acrylamide).

the groups. Acute exposure to AA may also lead to early erythrocyte membrane damage.^[34] Moreover, an early surgical operation may explain decreased Hb levels and a subsequent increase. Benziane et al.^[35] added AA at similar doses to this study to the drinking water of rats and did not observe a significant difference in Hb values. In long-term exposure to AA, changes in the immune system and liver and kidney failure can be observed depending on the dose. If the exposure continues, toxic effects can be observed in all systems related to AA. In a study investigating low-dose long-term exposure in rats, increased tumor incidence was observed in the scrotum, adrenal gland, thyroid, breast, and uterus. Testicular atrophy and low sperm count were also determined in mice administered with low dose gavage for 8 weeks.^[30] It is known that many foods in our daily consumption have different degrees of AA contamination. A diet rich in AA has adverse effects on metabolism.^[36] Yousef et al.'s^[37] results showed that different doses of AA exerted deterioration effects on enzyme activities and lipid peroxidation in a dose-dependent manner. Moreover, we have also known hypophosphatemia leads to rickets and osteomalacia, the latter of which results in decreased biomechanical integrity of bones, accompanied by poor fracture healing. Phosphate plays an important role in the skeleton that extends beyond mineralized matrix formation and growth plate maturation and is critical for endochondral bone repair.^[38] Ali et al.'s^[39] reported that serum albumin level is correlated with tibial fracture healing and may affect the final outcome of healing. All these reports support our findings and AA adverse bone healing effects. However, this area needs new researches.

The present study has a number of limitations. First, only one dose of AA was used, the main reason for which was to show the results of exposure to low dose AA close to the amount of daily dietary intake. Second, fracture healing was not evaluated by biomechanical tests. Instead, fracture healing was evaluated radiologically and histologically.

Conclusion

In summary, AA adversely affects fracture healing even at low doses, as in the present study. It is necessary to avoid AA exposure during fracture healing. According to the results of this study, the authors recommend a diet poor in AA during fracture treatment. Therefore, further human studies are required to find out the complex effect of AA on bone healing and the body.

Ethics Committee Approval: This study was approved by the Cumhuriyet University Animal Experiments Local Ethics Committee (Date: 17.01.2019, Decision No: 65202830-050.04.04-240).

Peer-review: Internally peer-reviewed.

Authorship Contributions: Concept: Ö.P.; Design: Ö.P.; Supervision: Ö.P.; Resource: Ö.P., E.G., A.T., N.K.Ç., Ş.N.Y.; Materials: Ö.P., E.G., A.T., N.K.Ç., Ş.N.Y.; Data: Ö.P., E.G.,

A.T., N.K.Ç., Ş.N.Y.; Analysis: S.K.; Literature search: Ö.P.; Writing: Ö.P.; Critical revision: O.B.

Conflict of Interest: None declared.

Financial Disclosure: This work was supported by the Turkish Orthopedics Research Council (TORC) and the Turkish Society of Orthopedics and Traumatology (TSOT).

REFERENCES

- Dimitriou R, Tsiridis E, Giannoudis PV. Current concepts of molecular aspects of bone healing. *Injury* 2005;36:1392–404. [CrossRef]
- Kanakaris NK, Giannoudis PV. The health economics of the treatment of long-bone non-unions. *Injury* 2007;38 Suppl 2:S77–84. [CrossRef]
- Einhorn TA. The science of fracture healing. *J Orthop Trauma* 2005;19:S4–6. [CrossRef]
- Karpouzou A, Diamantis E, Farmaki P, Savvanis S, Troumpis T. Nutritional aspects of bone health and fracture healing. *J Osteoporos* 2017;2017:4218472. [CrossRef]
- Tareke E, Rydberg P, Karlsson P, Eriksson S, Törnqvist M. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *J Agric Food Chem* 2002;50:4998–6. [CrossRef]
- Stadler RH, Blank I, Varga N, Robert F, Hau J, Guy PA, et al. Acrylamide from Maillard reaction products. *Nature* 2002;419:449–50. [CrossRef]
- Cengiz MF, Pelin C, Gündüz B. Acrylamide exposure among Turkish toddlers from selected cereal-based baby food samples. *Food Chem Toxicol* 2013;60:514–9. [CrossRef]
- Kumar J, Das S, Teoh SL. Dietary acrylamide and the risks of developing cancer: Facts to ponder. *Front Nutr* 2018;5:14. [CrossRef]
- Lipworth L, Sonderman JS, Tarone RE, McLaughlin JK. Review of epidemiologic studies of dietary acrylamide intake and the risk of cancer. *Eur J Cancer Prev* 2012;21:375–86. [CrossRef]
- Klaunig JE. Acrylamide carcinogenicity. *J Agric Food Chem* 2008;56:5984–8. [CrossRef]
- Tian S, Ma Y, Shi J, Lou T, Liu S, Li G. Acrylamide neurotoxicity on the cerebrum of weaning rats. *Neural Regen Res* 2015;10:938–43. [CrossRef]
- Yu D, Xie X, Qiao B, Ge W, Gong L, Luo D, et al. Gestational exposure to acrylamide inhibits mouse placental development in vivo. *J Hazard Mater* 2019;367:160–70. [CrossRef]
- Raju J, Roberts J, Taylor M, Patry D, Chomyshyn E, Caldwell D, et al. Toxicological effects of short-term dietary acrylamide exposure in male F344 rats. *Environ Toxicol Pharmacol* 2014;39:85–92. [CrossRef]
- Bai X, Lu D, Liu A, Zhang Z, Li X, Zou Z, et al. Reactive oxygen species stimulates receptor activator of NF-kappa B ligand expression in osteoblast. *J Biol Chem* 2005;280:17497–506. [CrossRef]
- Garrett R, Boyce BF, Oreffo RC, Bonewald L, Poser J, Mundy GR. Oxygen-derived free radicals stimulate osteoclastic bone resorption in rodent bone in vitro and in vivo. *J Clin Invest* 1990;85:632–9. [CrossRef]
- Basu S, Michae K, Olofsson H, Johansson S, Melhus H. Association between oxidative stress and bone mineral density. *Biochem Biophys Res Commun* 2001;288:275–9. [CrossRef]
- Tawonsawatruk T, Hamilton DE, Simpson AH. Validation of the use of radiographic fracture-healing scores in a small animal model. *J Orthop Res* 2014;32:1117–9. [CrossRef]
- Huo MH, Troiano NW, Pelker RR, Gundberg CM, Friedlaender GE. The influence of ibuprofen on fracture repair: Biomechanical, biochemical, histologic, and histomorphometric parameters in rats. *J Orthop Res* 1991;9:383–90. [CrossRef]
- Sahoo S, Chakraborti CK, Behera PK, Mishra SC. FTIR and raman spectroscopic investigations of a norfloxacin/carbopol934 polymeric suspension. *J Young Pharm* 2012;4:138–45. [CrossRef]
- Muchtaridi M, Levita J, Rahayu D, Rahmi H. Influence of using coconut,

- palm, and corn oils as frying medium on concentration of acrylamide in fried tempe. *Food Public Health* 2012;2:16–20. [CrossRef]
21. Friedman M. Chemistry, biochemistry, and safety of acrylamide. A review. *J Agric Food Chem* 2003;51:4504–26. [CrossRef]
 22. Lambert M, Inthavong C, Hommet F, Leblanc JC, Hulin M, Guérin T. Levels of acrylamide in foods included in the first French total diet study on infants and toddlers. *Food Chem* 2018;240:997–1004. [CrossRef]
 23. Sarocka A, Babosova R, Kovacova V, Omelka R, Semla M. Acrylamide-induced changes in femoral bone microstructure of mice. *Physiol Res* 2017;8408:1067–71. [CrossRef]
 24. Benetou V, Orfanos P, Feskanich D, Michaëlsson K, Pettersson-Kymmer U, Byberg L, et al. Mediterranean diet and hip fracture incidence among older adults: The CHANCES project. *Osteoporos Int* 2018;29:1591–9.
 25. Sarocka A, Kovacova V, Omelka R, Grosskopf B, Kapusta E, Goc Z, et al. Single and simultaneous effects of acrylamide and ethanol on bone microstructure of mice after one remodeling cycle. *BMC Pharmacol Toxicol* 2019;20:1–9. [CrossRef]
 26. Yener Y. Effects of long term low dose acrylamide exposure on rat bone marrow polychromatic erythrocytes. *Biotech Histochem* 2013;88:356–60. [CrossRef]
 27. World Health Organization. Health Implications of Acrylamide in Food. Report of a Joint FAO/WHO Consultation. Geneva: World Health Organization; 2002. p. 1–35.
 28. Svensson K, Abramsson L, Becker W, Glynn A, Hellenäs KE, Lind Y, et al. Dietary intake of acrylamide in Sweden. *Food Chem Toxicol* 2003;41:1581–6. [CrossRef]
 29. LoPachin RM, Ross JF, Reid ML, Das S, Mansukhani S, Lehning EJ. Neurological evaluation of toxic axonopathies in rats: Acrylamide and 2, 5-hexanedione. *Neurotoxicology* 2002;23:95–110. [CrossRef]
 30. Riboldi BP, Vinhas AM, Moreira JD. Risks of dietary acrylamide exposure: A systematic review. *Food Chem* 2014;157:310–22. [CrossRef]
 31. Brisson B, Ayotte P, Normandin L, Gaudreau É, Bienvenu JF, Fennell TR, et al. Relation between dietary acrylamide exposure and biomarkers of internal dose in Canadian teenagers. *J Expo Sci Environ Epidemiol* 2014;24:215–21. [CrossRef]
 32. Baum M, Loepky RN, Thielen S, Eisenbrand G. Genotoxicity of glycidamide in comparison to 3-N-nitroso-oxazolidin-2-one. *J Agric Food Chem* 2008;56:5989–93. [CrossRef]
 33. Ghorbel I, Elwej A, Chaabene M, Boudawara O, Jamoussi K, Boudawara TS, et al. Effects of acrylamide graded doses on metallothioneins I and II induction and DNA fragmentation: Biochemical and histomorphological changes in the liver of adult rats. *Toxicol Ind Health* 2017;33:611–22. [CrossRef]
 34. Tarskikh MM. Damage to erythrocyte membranes as the mechanism for acrylate toxicity. *Bull Exp Biol Med* 2006;142:690–2. [CrossRef]
 35. Benziane AB, Bouras AD, Belhadri A, Benali M. Effect of oral exposure to acrylamide on biochemical and hematologic parameters in Wistar rats. *Drug Chem Toxicol* 2019;42:157–66. [CrossRef]
 36. Lineback DR, Coughlin JR, Stadler RH. Acrylamide in foods: A review of the science and future considerations. *Annu Rev Food Sci Technol* 2012;3:15–35. [CrossRef]
 37. Yousef MI, El-Demerdash FM. Acrylamide-induced oxidative stress and biochemical perturbations in rats. *Toxicology* 2006;219:133–41. [CrossRef]
 38. Wigner NA, Luderer HF, Cox MK, Sooy K, Gerstenfeld LC, Demay MB. Acute phosphate restriction leads to impaired fracture healing and resistance to BMP-2. *J Bone Miner Res* 2010;25:724–33. [CrossRef]
 39. Ali S, Singh A, Mahdi A, Srivastava R, Shantanu K. Does serum albumin level affect the healing outcomes of simple diaphyseal tibial fractures? *Saudi J Med Med Sci* 2016;4:93. [CrossRef]

DENEYSSEL ÇALIŞMA - ÖZ

Akrilamidin sıçanlarda kırık iyileşmesi üzerindeki etkisinin araştırılması

Dr. Özhan Pazarcı,¹ Dr. Erkan Gumus,² Dr. Ayca Taş,³ Dr. Neşe Keklikioğlu Çakmak,⁴ Dr. Seyran Kılıncı,¹ Dr. Şeyma Nur Yıldız,⁵ Dr. Okay Bulut¹

¹Sivas Cumhuriyet Üniversitesi Tıp Fakültesi, Ortopedi ve Travmatoloji Anabilim Dalı, Sivas

²Aydın Adnan Menderes Üniversitesi Tıp Fakültesi, Histoloji ve Embriyoloji Anabilim Dalı, Aydın

³Sivas Cumhuriyet Üniversitesi Sağlık Bilimleri Fakültesi, Beslenme ve Diyet Etik Bölümü, Sivas

⁴Sivas Cumhuriyet Üniversitesi Mühendislik Fakültesi, Kimya Mühendisliği Bölümü, Sivas

⁵Sivas Cumhuriyet Üniversitesi Tıp Fakültesi, Biyokimya Anabilim Dalı, Sivas

AMAÇ: Bu çalışmanın amacı, bir sıçan femur kırığı modelinde akrilamidin (AA) histolojik, biyokimyasal ve radyolojik olarak kırık iyileşmesi üzerindeki etkilerini araştırmaktır.

GEREÇ VE YÖNTEM: Akrilamid karakterizasyonu için taramalı elektron mikroskobu (SEM) görüntüleme ve Fourier dönüşüm kızılötesi spektroskopisi (FTIR) ve UV (ultraviyole) -Vis (görünür) spektrofotometre incelemesi yapıldı. Bu çalışmada, femur kırığı modeli oluşturulduktan sonra gruplar, her grupta (G) sekiz sıçan olacak şekilde oluşturuldu; G1: 15. gün kontrol, G2: 15. gün AA, G3: 30. gün kontrol, G4: 30. gün AA. G2 ve G4'te 5 mg/kg akrilamid, mide gavajı ile haftada üç kez uygulandı. Kırık, Lane-Sandhu skorlamasına göre radyolojik olarak ve Huo skorlamasına göre histolojik olarak değerlendirildi. Sıçanların ağırlık değişiklikleri kaydedildi. Kan örneklerinde; albümin, total protein, kolesterol, HDL, LDL, trigliserit, ALP, LDH, vitamin D, PTH, Ca, P, WBC, Hb, Plt değerleri incelendi.

BULGULAR: Akrilamidin karakterizasyon özellikleri doğrulandı. Çalışma sırasında sıçanlarda önemli bir ağırlık değişikliği gözlenmedi. Kan değerleri karşılaştırıldığında albümin, total protein, fosfor, WBC ve hemoglobin grupları arasında istatistiksel olarak anlamlı bir fark tespit edildi (sırasıyla, p=0.41, p=0.00, p= 0.003, p=0.019 ve p=0.017). Histolojik skor karşılaştırmalarına göre G3, G1, G2 ve G4'ten anlamlı olarak farklıydı (p<0.05) ve G4, G1 ve G2'den anlamlı olarak farklıydı (p<0.05). Lane-Sandhu skorlamasına göre sırasıyla G2 ile G3 ve G4 (p=0.0, p=0.034), G1 ve G3 (p=0.001) arasında anlamlı fark vardı.

TARTIŞMA: Akrilamidin çalışmamızda olduğu gibi düşük dozlarda bile kırık iyileşmesini olumsuz etkilemektedir. Bu çalışmanın sonuçlarına göre, yazarlar, kırık tedavisi sırasında AA bakımından fakir bir diyet önermektedir. AA'nın kemik iyileşmesi ve vücut üzerindeki karmaşık etkisini bulmak için daha fazla insan çalışmasına ihtiyaç vardır.

Anahtar sözcükler: Akrilamid; kemik; kırık; kırık iyileşmesi, sıçan.

Ulus Travma Acil Cerrahi Derg 2022;28(5):570-578 doi: 10.14744/tjtes.2021.94848