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Research Article/Özgün Araştırma

Impact of black carrot juice on acrylamide-induced structural alterations in rats' testicles

Sıçanlarda akrilamid ile testiste oluşan yapısal değişiklikler üzerine siyah havuç suyu'nun etkisi

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Abstract

Aim: The purpose of this study was to investigate the deleterious effects of acrylamide on rat testicular tissue and to determine how these effects might vary in response to black carrot juice.

Materials and Methods: Four groups of adult male Wistar albino rats were formed: Control, Acrylamide, Black carrot juice and Acrylamide + Black carrot juice. For 30 days, 20 mg/kg acrylamide dose was administered intraperitoneally and 4 mg/kg black carrot juice dose was administered orally every second day.

Results: Malondialdehyde and glutathione S-transferase levels rose in the acrylamide group relative to the control group, whereas the levels of the enzymes glutathione and carboxylesterase dropped. Malondialdehyde and glutathione S-transferase levels were lower in the acrylamide+black carrot juice group than in the acrylamide group, whereas glutathione and carboxylesterase enzyme activity levels were higher.

Conclusion: Lipid peroxidation was discovered as a result of acrylamide's detrimental effects on the antioxidant enzyme system. It was observed that black carrot juice had positive effects.

Keywords: Acrylamide; Testis; Black carrot; Oxidative stress; Histopathology.

Öz

Amaç: Bu çalışmanın amacı akrilamidin sıçan testis dokusu üzerindeki zararlı etkilerini araştırmak ve bu etkilerin siyah havuç suyuna yanıt olarak nasıl değişebileceğini belirlemekti.

Gereç ve Yöntem: Yetişkin erkek Wistar albino sıçanlar dört gruba ayrıldı: Kontrol, Akrilamid, Siyah havuç suyu ve akrilamid + siyah havuç suyu. 30 gün boyunca, 20 mg/kg akrilamid dozu intraperitoneal olarak uygulandı ve 4 mg/kg siyah havuç suyu dozu her iki günde bir oral olarak uygulandı.

Bulgular: Malondialdehit ve glutatyon S-transferaz seviyeleri akrilamid grubunda kontrol grubuna göre artarken, glutatyon ve karboksilesteraz enzimlerinin seviyeleri düştü. Malondialdehit ve glutatyon S-transferaz seviyeleri akrilamid + siyah havuç suyu grubunda akrilamid grubuna göre daha düşüktü, buna karşın glutatyon ve karboksilesteraz enzim aktivite seviyeleri daha yüksekti.

Sonuç: Akrilamid'in antioksidan enzim sistemi üzerindeki zararlı etkilerinin bir sonucu olarak lipid peroksidasyonu keşfedildi. Siyah havuç suyu'nun pozitif etkileri olduğu görüldü.

Anahtar Kelimeler: Akrilamid; Testis; Siyah havuç; Oksidatif stres; Histopatoloji.

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Introduction

Acrylamide (ACR) was declared a carcinogenic agent in 1994 by the International Agency for Research on Cancer. ACR is a very widely used substance that negatively affects all the systems in organisms.[1-3] The level of ACR in food depends on processes such as frying or baking, and the temperature and time applied. It has been determined that it is found in excessive amounts in some starchy foods cooked at high temperatures. Daily exposure is relatively high in those who consume fried potato products, toasted bread, potato chips, some breakfast cereals, and roasted coffee. ACR is used in printing to increase the durability of paper, oil well processes, water treatment, and cosmetics.[3-5] It has been shown that ACR causes oxidative stress and histological alterations in the tissues of the testes.[6] While ACR exposure increased lipid peroxidation levels in these tissues, a decrease was observed in antioxidant enzyme systems.[7-10] Consequently, oxidative stress plays crucial roles in ACR-induced toxicity due to over-production of reactive oxygen species (ROS). ROS lead to apoptosis, decreased motility, chromatin damage, and impaired fertilisation ability.[4,11]

Vitamin-rich black carrot (*Daucus carota L.*) is widely consumed. Carrots contain many substances such as kaempferol, quercetin, luteolin, myricetin and kaempferol which are flavonoid derivatives.[12,13] The amount of flavonoids found in black carrot carrots is much higher compared to other types of carrots.[14] The chemical component black carrot includes important pigments such as carotenoids, anthocyanins, polyacetylenes and falcarindiol. These component found in black carrots have many biochemical effects such as antioxidant, antitumor, anti-inflammatory, antimicrobial, anti-allergic, and anti-atherosclerotic activities. [15-18]

Free oxygen radicals and antioxidants are produced under control in tissues. However, when free oxygen radicals are overproduced, tissue damage known as oxidative stress occurs. Lipids in cell membranes are oxidized by free oxygen radicals, thus forming toxic products such as MDA.[19] When free oxygen radicals are overproduced, antioxidant defense systems counteract the harmful effects of free oxygen radicals for tissue integrity and normal functions. Antioxidant defense systems show their effects by blocking radical production and eliminating the harmful effects of formed radicals. GSH and GST are essential antioxidants. The GST enzyme has many functions in cells. It has an antioxidant effect against compounds such as hydroxyalkenals, propanals, and hydroperoxides formed in the cell. GST enzymes require the presence of the GSH molecule for its activity.[20,21] Many drugs are metabolized by the Ces. Ces has been detected in the liver, testis, and kidney tissues of mammals and is a member of esterases that catalyze the hydrolysis of esters, amides, and thioesters and convert esters into carboxylic acid and hydroxylated products. For this reason, changing activities in tissues are clinically very important.[22,23]

It is important to discover new substances or natural products to correct the negative effects of toxic substances on human health. In the literature review, it was observed that the effects of BCJ on testicular tissues were not studied against the oxidative stress caused by ACR in rats. Therefore, in this study, the effects of BCJ on MDA, GSH, GST, Ces, and histopathological parameters in rat testicular tissues exposed to ACR were investigated.

Materials and Methods

Animals

The Experimental Animal Production Implementation and Research Center of Adıyaman University in Turkey provided 32 male Wistar Albino rats, weighing between 200 and 250 g, which were used for the study. The rats were aged between 10 and 12 weeks. Throughout the study, the rats were provided with unlimited access to food and water, and they were kept in a room temperature of 22 ± 20 C with a 12-hour light and 12-hour dark cycle. The study was approved by Adıyaman University Animal Experiments Local Ethics Committee with the number 2022/88.

Experimental procedure

The rats were randomly divided into four groups as the Control (C), ACR, Black carrot juice (BCJ), and ACR+ BCJ (n=8 each group).

The vehicle solutions administered to the rats in the C group consisted solely of distilled water. The rats were administered 20 mg/kg intraperitoneally (IP) of dissolved ACR in distilled water.[19] The animals were given an orally administered dose of 4 ml/kg of BCJ. [20]ACR and BCJ were administered every other day for 30 days; ACR between 08:00 am and 09:00 am, and BCJ between 04:00 pm and 05:00 pm was given every other day for 30 days.

At the end of the 30-day experimental period, the combination of ketamine/xylazine HCl was administered to rats intramuscularly, and the blood was taken intracardiacally under anesthesia. Then, the testis and epididymis were removed, and the adipose tissue was cleaned and weighed. For biochemical analyses, it was stored at -80^{o} C. Testicular tissue samples were fixed with 10% formaldehyde for histopathological examination. When we look at the content of black carrot juice in the literature, total phenolics are 7.98–291.48 mg/100 g, anthocyanins are 837 mg/100 g, favo noids are 3.00–111.70 mg/100 g, falcarinol is 1.55 mg/100 g and favonols were reported as 51.6 mg/100 g.[24]

Biochemical analyses

Preparation of tissue homogenates

Testicular tissue samples were homogenized using a homogenizer (Heidolph RZ 2021, Germany) in a cooled potassium phosphate buffer (0.1 M, pH 7.4; 0.15 M KCl, 1 mM EDTA, and 1 mM DTT). For MDA analysis, five hundred microliters of homogenate were divided. The remaining homogenates were centrifuged (Hettich 460 R) at 16,000 x g for 20 minutes at 4 °C. The supernatants (S16) were then transferred into Eppendorf tubes to assess biomarkers other than MDA.

Determination of testis malondialdehyde (MDA) level and reduced glutathione (GSH) activity

MDA levels were measured using the Placer et al. technique in testicular tissue samples.21 MDA generates a pink-colored molecule when it interacts with thiobarbituric acid. The absorbancies of the resulting samples were measured at 532 nm using spectrophotometry (ThermoTM Varioskan Flash, Finland). The expression for the MDA level was nmol/mg protein.

The Moron et al.[25] method was used to calculate the quantity of GSH. Its response to 5,5'-dithiobis-2-nitrobenzoic acid was used to measure it. Using a spectrophotometer set at 412 nm, the absorbancies of the samples were measured (ThermoTM Varioskan Flash, Finland). The GSH concentration was given as nmol/mg of protein.

Determination of testis glutathione S-transferase (GST) activity

For the GST activity, 10 µL of supernatant, 100 µL of phosphate buffer (0.1 M, pH 6.5), and 100 µL of GSH mixture were produced. Subsequently, a substrate solution of 20 mM 1-Chloro-2,4 dinitrobenzene (CDNB) was produced in 96% ethanol and added to microplate wells. After the microplates were put in the microplate reader, the absorbance at 344 nm changed in less than two minutes at 25 °C. The formula for calculating specific GST activity was nmol/min/mg protein.

Determination of testis carboxylesterases (Ces)

26 mM p-nitrophenyl acetate (PNPA) was produced in 96% ethanol and utilized as a substrate in the Ces activity analysis. The reaction mixture, including 250 µL of 50 mM trizma buffer (pH, 7.4) and 5 µL of sample, was incubated at 25 °C for three minutes. 5 µL of substrate was added to start the reaction, which was then seen for two minutes at 25 °C at 405 nm. The protein activity was reported as nmol/min/mg.

Determination of total protein

The amount of protein was measured according to the Bradford[26] assay, using a bovine serum albumin (0–1.4 mg BSA / mL) standard.

Histological and immunohistochemical evaluation

Haematoxylin-eosin staining procedure

Following standard light microscopy methods, testicular tissue samples were

embedded in paraffin and preserved with 10% formaldehyde. After that, portions of these blocks, 4-6 µm thick, were removed and stained with hematoxylin-eosin (HxE). Under a light microscope (Leica DM500 connected Leica DFC295 Digital Image Analyze System), the preparations were inspected and photographed.

Immunohistochemistry for caspase 3

The technique employed was the streptavidin-biotin-peroxidase combination. By using this technique, slices of the blocked tissues, 4-6 µm thick, were removed and deparaffinized. Using a Thermo Scientific TM TP-015-HA commercial kit, the primary antibody Caspase-3 (Rabbit polyclonal IgG, Abcam, ab2302, London, UK) was diluted at a ratio of 1/200. Both the positive and negative controls were operated in accordance with the manufacturer's guidelines. The samples were stained with Mayer Hematoxylin, taken with a Leica DFC295 Digital Image Analyze System attached to a Leica DM500, and examined under a light microscope after applying AEC Chromogen.

The prevalence (0.1:<25%, 0.4:26-50%, 0.6:51-75%, 0.9:76-100%) and severity (0: none, +0.5: very mild, +1: mild, +2: moderate,+3: severe) of immunoreactivity in staining were used to produce the histoscore. (Severity x prevalence= Histoscore)

Spermatological examinations

Epididymal spermatozoa density

In a petri plate with 1 ml of physiological saline (0.9% NaCl), the epididymis was minced and allowed to incubate for four hours at room temperature. The supernatant containing spermatozoa up to 0.5 lines of red blood cell pipette was diluted at 1: 200, by drawing up to 101 lines of eosin solution (5 g sodium bicarbonate, 1 ml formalin, 25 mg eosin, and 100 ml distilled water). After this procedure, the supernatant was placed on the Neubauer slide (0.1 mm depth, 0.0025 mm^2 area, LABART, Munich, Germany) and was counted and calculated at 200 magnification under a light microscope.[27]

Spermatozoa motility

After placing the sample on the warming table of a slide microscope, its temperature reached 37 °C. A 200 µl Tris buffer solution was applied to the slide, which contained 3.63 g of Tris (hydroxymethyl) aminomethane, 0.50 g of glucose, 1.99 g of citric acid, and 100 ml of distilled water.

Next epididymis was sectioned, the 5–10 µl spermatozoa suspension was poured over the Tris buffer solution, and the entire mixture was mixed together. The motility percentage was computed using a 400 magnification light microscope.

Johnsen scoring

For light microscopic evaluations, modified Johnsen scoring was used to evaluate spermatogenesis in seminiferous tubules at 10× magnification in 30 randomly selected seminiferous tubules per section. Spermatogenic cells were examined using a Leica DM500 microscope and were evaluated according to maturation and density using a scoring table that gives scores ranging from 1 to 10. The Johnson scoring is shown in Table 1.[26]

Table 1. Modified Johnsen scoring.

10	Complete spermatogenesis with mature sperm cells
9	There are few sperm cells with disorganized germinal epithelium
8	There are less than 10 sperm cells (less than 5-10)
7	There are no sperm cells, there are spermatids
6	No sperm cells, less than 10 spermatids (less than 5-10)
5	There are no sperm cells and spermatids, there are spermatocytes
4	No sperm cells and spermatids, less than 5 spermatocytes
3	There are only spermatogonia as germ cells
2	There are no germ cells, only Sertoli cells
1	There are no cells in the seminiferous tubule

Statistical analysis

All of the computations were performed using the statistical program SPSS 22.0. For the results, the mean ± SEM was tabulated. The Tukey-HSD test was used to identify the significant groups after the groupings were statistically assessed using One-way analysis of variance (ANOVA).

Ethics committee approval

The study was approved by the Adıyaman University Experimental Animals Ethics Committee at the meeting dated 10.05.2018 with the decision numbered 2018/006 and received permission. All experimental procedures were carried out in accordance with the ethical guidelines for the care and use of laboratory animals.

Results

MDA, reduced GSH, GST, and Ces levels in the testis

The MDA levels of the rats exposed to ACR were higher than those of the C group with statistical significance ($p<0.001$). Between the BCJ and C groups, there was no statistically significant difference ($p>0.05$). In comparison to the C group, the MDA level of the ACR + BCJ group was shown to have increased ($p<0.001$), whereas it decreased ($p<0.01$) when compared to the ACR group. In contrast to the other groups, the GSH level in the ACR group dropped ($p<0.001$; $p<0.05$).

Other groups than the C group showed higher levels of GST enzyme activity ($p<0.001$). The GST enzyme activity levels in the BCJ and ACR + BCJ groups were found to have dropped ($p<0.001$) in contrast to the ACR group. The BCJ and ACR + BCJ groups' levels of Ces enzyme activity were found to be higher than those of the ACR group ($p<0.05$), but the ACR group's level was found to be lower than that of the C group ($p<0.001$). Moreover, it was shown that the levels of Ces enzyme activity in the ACR and ACR + BCJ groups had decreased relative to the C group ($p<0.05$). The biochemical parameter levels of the testicular tissue are listed in Table 2.

Table 2. Biochemical parameters in C, ACR, BCJ And ACR + BCJ treated groups (n=8).

Parameters	C	ACR	BCJ	ACR+BCJ
MDA (nmol/mg protein)	25.7±2.8	43.2±1.6c	29.5±3.5z	35.6±1.2cy
GSH (nmol/mg protein)	57.5±5.7	31.3±1.4c	45.3±2.7x	49.3±1.8x
GST (nmol/min/mg protein)	98.48±1.10	151.32±1.22c	111.03±1.15cz	123.24±1.99cz
Ces (nmol/min/mg protein)	0.72±0.04	0.42±0.02c	0.58±0.03ax	0.57±0.03ax

ANOVA
Values are expressed as means ± SE; n=8 for each treatment group.
Comparison with group C. a: $p<0.05$, b: $p<0.01$, c: $p<0.001$
Comparison with group ACR. x: $p<0.05$, y: $p<0.01$, z: $p<0.001$

Histologic analysis of testis tissue

Examining sections of the testicular tissues of the C and BCJ groups stained with Hematoxylin-Eosin (H.E.), it was discovered that the cells of the spermatogenic series and seminiferous tubules were normal (Figure 1A, 1B). Degeneration (arrow) in the seminiferous tubules and a decrease in the cells of the spermatogenic series (star) were observed when the sections of the rat testicular tissues of the ACR-applied group stained with H.E. were analyzed (Figure 1C). When the sections of the rat testicular tissues of the ACR and BCJ group were examined, a decrease in degeneration (arrow) and an increase in the cells of the spermatogenic series were observed (star) (Figure 1).

1A: The C group seen under a microscope. Testicular tissues in normal condition. 1B: A close-up of the BCJ-treated group. View of normal testicular tissue. 1C: ACR group seen under a microscope. Significant seminiferous degeneration (arrow) and a drop in spermatogenic series cells (star) were seen in the tubules. 1D: Under a microscope, tubules from ACR+BCJ showed a marked reduction in degeneration (arrow) and an increase in spermatogenic series cells (star). The scale bars represent 100 μm.

Caspases-3 immunohistochemical staining. The presence of caspase-3 in spermatogenic cells. Groups 2A–C: Testicular positivity is minimal or absent. 2B-BCJ: The testicles appear normally. The scale bars show 25 μm. 2C-ACR group: Severe positivity in the testis;

2D-ACR+ BCJ group: Diminished positivity in the testis.

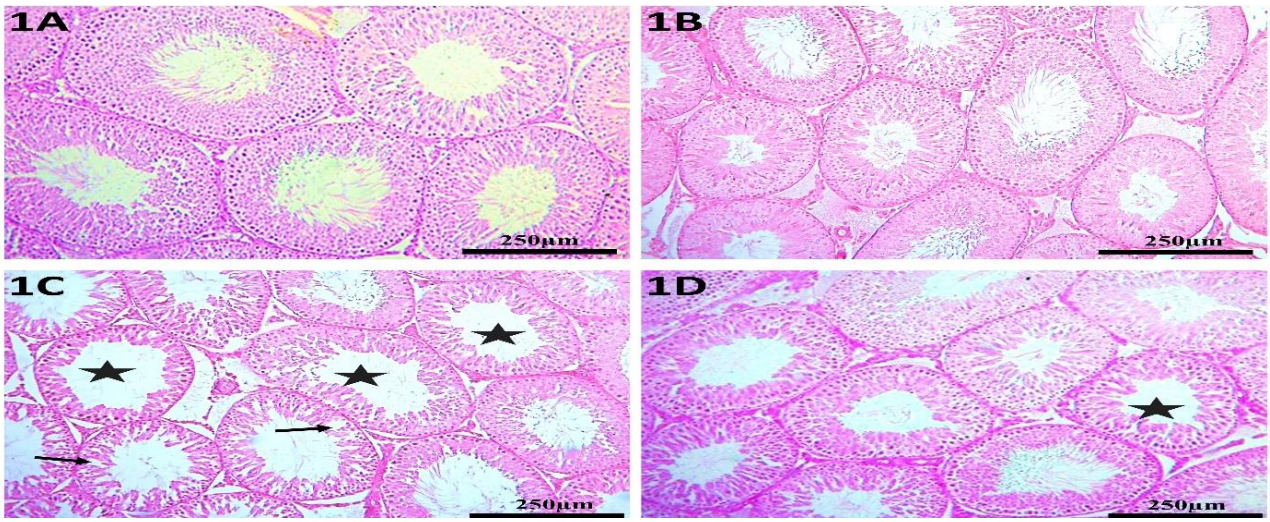

Figure 1. Histopathological effects of black carrot juice on testicular tissue of rats against damage caused by acrylamide are given in the figure. Accordingly: In the control and black carrot juice groups, testicular tissue was observed to have a normal histological structure. However, in the ACR group, to which only acrylamide was applied, degeneration in seminiferous tubules and spermatogenesis decreased and histopathological damage increased compared to the control group. However, in the treatment group, the ACR+ black carrot juice group, when compared to the ACR group, degeneration in seminiferous tubules decreased, spermatogenesis increased and therefore histopathological damage decreased. (H&E staining black arrow: seminiferous tubule degeneration, black star: decreased spermatogenesis. Scala bar 250 µm)

Immunohistochemical analysis of testis tissue

Caspase-3 immunoreactivity was detected in the testicular tissue of seminiferous tubules as a consequence of the immunohistochemical staining evaluation under light microscopy (red arrow). Caspase-3 immunoreactivities in testicular tissues were similar in the C (Figure 1 2A) and BCJ (Figure 1 2B) groups. Caspase-3 immunoreactivity was found to be statistically significantly increased in the ACR group (Figure 1 2C) compared to the C group (p<0.001). The Caspase-3 immunoreactivity in the ACR + BCJ group was found to be statistically lower than in the ACR group (Figure 1 2D) (p<0.001). Caspase-3 immunoreactivity parameter levels are given in Table 3.

Table 3. Caspase-3 activation in C, ACR, BCJ and ACR + BCJ treated groups (n=8)

Parameters	C	ACR	BCJ	ACR+BCJ
Histoscore	0.14±0.17	1.22±0.36c	0.15±0.18z	0.92±0.37cz

ANOVA
Values are expressed as means ± SE; n=8 for each treatment group.
Comparison with group C. a: p<0.05, b: p<0.01, c: p<0.001
Comparison with group ACR. x: p<0.05, y: p<0.01, z: p<0.001

According to the results of the modified Johnsen scoring system used to evaluate spermatogenesis in the seminiferous tubules, the control and BJC groups were similar (p=0.750). Spermatogenesis in the ACR group was statistically decreased compared to the control group (p<0.05). The Johnsen score of the ACR+BJC group, which was the treatment group, was statistically increased compared to the ACR group (p<0.05). (Figure 2D)

It was shown that there was no difference in testis weight, epididymal spermatozoa numbers, and motility between the C group and the BCJ group (p>0.05). Rats who received ACR application showed statistically lower testicular weight, seminal vesicle weight, and epididymal spermatozoa counts and motility (p<0.001) in comparison to the C group. The ACR + BCJ group showed a statistically significant increase (p<0.001). Spermatological parameter levels are given in Table 4.

Discussion

Many studies have indicated that negative effects on organism systems caused by ACR, which is commonly used nowadays.[7-9]

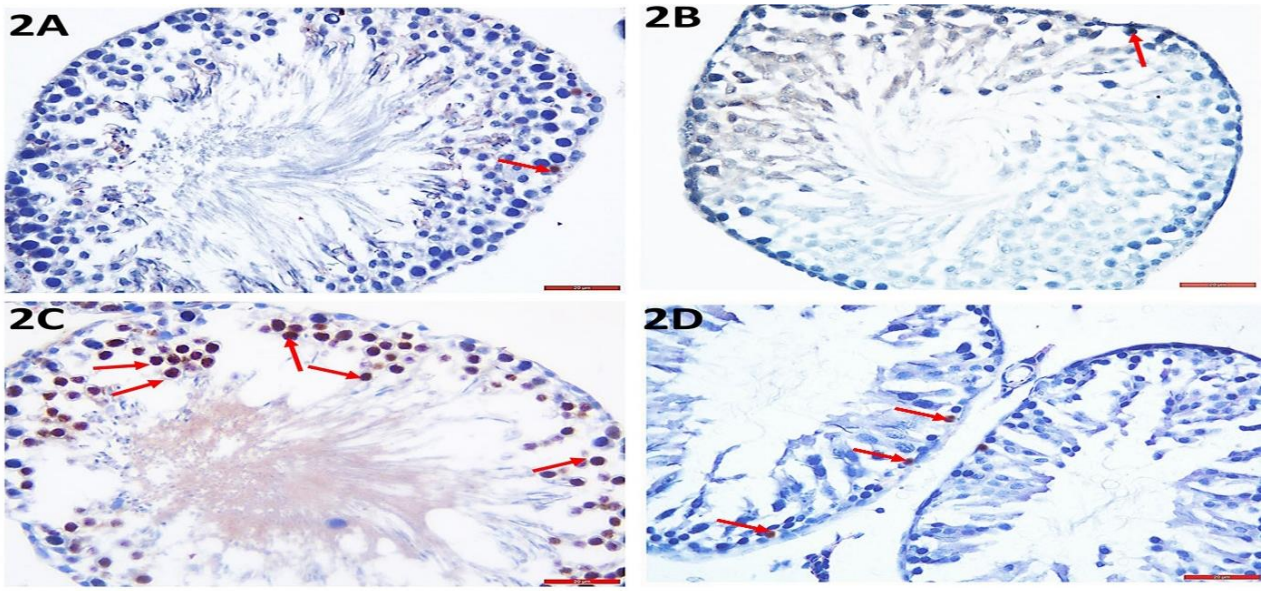

Figure 2. Caspase-3 immunoreactivity (red arrow) in rat testicular tissue of experimental groups is shown. No statistically significant difference was observed in caspase-3 immunoreactivity in control and black carrot groups. However, caspase-3 immunoreactivity was statistically increased compared to control and black carrot groups due to the damage caused by acrylamide. Only in the ACR group given acrylamide, caspase-3 immunoreactivity was statistically increased compared to the treatment group ACR+black carrot juice group. (A: control B: BCJ, C: ACR, D: ACR+BJR immunohistochemical staining AEC chromogen scala bar:20 µm)

Table 4. Spermatological parameters in C, ACR, BCJ, and ACR + BCJ treated groups (n=8).

Parameters	**C**	**ACR**	**BCJ**	**ACR+BCJ**
Testicular weight (g)	1.29±0.23	0.98±0.24c	1.34±0.14z	1.19±0.11bz
Epididymis weight (g)	0.39±0.07	0.214±0.11c	0.40±0.01z	0.30±0.10cz
Seminal gland weight (g)	0.78±0.09	0.41±0.11c	0.87±0.08cz	0.68±0.10cz
Spermatozoon count (million / cauda epididymis)	83.75±1.82	27.50±2.50c	85.62±1.45z	75.00±2.67az
Spermatozoon motility (%)	87.50±0,90	40.12±1.64c	88.12±0.83z	74.37±1.20cz

ANOVA
Values are expressed as means ± SE; n=8 for each treatment group.
Comparison with group C. a: $p<0.05$, b: $p<0.01$, c: $p<0.001$
Comparison with group ACR. x: $p<0.05$, y: $p<0.01$, z: $p<0.001$

ACR causes oxidative stress, and consequently, free radical levels increase which induces lipid peroxidation. As a result, the MDA, which is a lipid peroxidation product, increases.[14] In previous studies conducted on ACR applications, According to previous studies, testicular tissue contains higher MDA levels, which results in oxidative stress.[6,8,15,27,28] The results of these previous studies are similar to ours. Additionally, when compared to the C group, we discovered that rats exposed to ACR had statistically significant higher amounts of MDA (a consequence of lipid peroxidation) in their testicular tissue. Our analysis revealed that the MDA level was lower in the ACR + BCJ group compared to the ACR group. We believe that the antioxidant content of BCJ is the cause of the MDA level reduction in the ACR + BCJ group. Black carrot juice's flavonoid, phenolic, and antioxidant components have been shown to have strong antioxidant activity.

In contrast to the C group, we found a statistically significant drop in GSH levels in the rats that had ACR application in our investigation. Numerous investigations have revealed that the application of ACR in rats and mice results in a reduction in GSH levels in the testicular tissues. When comparing the ACR group to the C group in our study, there was a rise in GST levels. Also, Yildizbayrak et al[28] found similar results to our study. There

are studies that showed a decrease in GST levels with ACR application.[10,29] The GST enzyme has many functions in the cell. It has an antioxidant effect against compounds such as hydroxyalkenals, propanals, and hydroperoxides formed in the cell. GST enzymes require the presence of the GSH molecule for its activity.[20] In our study, we believe that the decrease of the GSH molecule and increase of the GST enzyme in the ACR-applied rat testicular tissues is because the GST enzyme excessively consumes the GSH molecule, which is necessary for activation.

Many drugs and toxins are metabolized by the Ces enzymes. Ces has been detected in the liver, testis, and kidney tissues of mammals, and is a member of esterases that catalyze the hydrolysis of esters, amides, and thioesters and convert esters into carboxylic acid and hydroxylated products. For this reason, activity changes in tissues are clinically very important.[22,23] Our study revealed that the Ces enzyme activities of the other groups were lower than those of the control group. According to the Ces enzyme activity of the ACR group, an increase was detected in the BCJ and ACR + BCJ groups. We think that ACR has the same negative effects on the Ces enzyme as it has on the antioxidant system. There are many biomolecules in black carrot content. It can be said that polyphenolic compounds, especially, react with molecules in Ces enzymes and change the activities of the structure.[30] However, we see the increase of Ces enzyme activities in BCJ groups as a positive effect compared to the ACR group.

In our study, light microscopic examination of the testicular tissue of rats exposed to ACR showed a degeneration in the seminiferous tubular epithelium and a decrease in cells of the spermatogenic series.[6] It has been reported in earlier research that ACR causes the seminiferous tubular epithelium and germ cells to degrade, a decrease in spermatogenic cells, and a decrease in mature sperm and spermatogonia.[15,29,31,32] Our research supports the findings of the previously mentioned studies. Similar to our study, there are studies reporting that the Caspase-3 immunoreactivity increases and apoptosis occur with ACR treatment.[7,28]

In a study on rats, Lebda et al determined that the seminiferous tubules lacked spermatitis and spermatozoa due to the application of ACR.[6,33] Detected changes in testicular weights and seminiferous tubules in rats exposed to ACR. In our study, rats given ACR had lower testicular weights, seminal vesicle weights, epididymal sperm counts, and sperm motility than the C group. In the ACR + BCJ group, the numerical increases in these parameters were observed to be statistically significant.

Limitations

This study has some limitations. Since the experimental period was only 30 days, this period may not be sufficient to understand the long-term effects of acrylamide and black carrot juice.

The study was conducted only on Wistar albino rats. This may limit its generalizability to other species and humans.

Conclusion

Our study detected a decrease in testicular function of the rats exposed to ACR, and this decrease could be prevented with the antioxidant properties of BCJ. Chemicals that harm the organism cause oxidative stress and thus damage the cells and tissues. Antioxidants are thought to be an effective treatment method in the prevention of tissue damage caused by oxidative stress. It is important to discover new substances or natural products in order to ameliorate the negative effects of harmful chemicals. As a result, We think that BCJ may be able to lessen the harmful effects of ACR-induced oxidative stress and toxic consequences.

Ethics Committee Approval

The Adıyaman University Animal Experiments Local Ethics Committee provided ethical approval (2018/006). All experimental procedures were carried out in accordance with the ethical guidelines for the care and use of laboratory animals.

Author Contributions

Conceptualization: HP; Design: GE, SÇ, EMBY; Auditing: SÇ, AY, AT; Resources: HP, AK, MFA; Data collection: AK, AT, AY;

Data analysis and interpretation: EMBY, AT; Literature review: SÇ, AK, MFA; Writers: GE, AY; The final version of this article was read and approved by all authors.

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None

Conflict of Interest

The authors declare that there is no conflict of interest for this article.

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Statements

These data have not been presented or published anywhere previously.

Peer-review

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