Ankara Sağlık Bilimleri Dergisi (ASBD)
Journal of Ankara Health Sciences (JAHS)
ISSN:2146-328X (print)
e-ISSN: 2618-5989 (online)
DOI: https://www.doi.org/10.46971/ausbid.747684

Özgün Araştırma/Research Article

Siyah havuç suyu: Sıçanlarda akrilamide bağlı hepatotoksisiteyi tedavi etmede yeni bir yaklaşım

Alper YALÇIN [1]
Hıdır PEKMEZ [2]

Black Carrot Juice: A New Approach to Cure Acrylamide-Induced Hepatotoxicity in Rats

Özet

Amaç: Karaciğer, oksidatif stres yoluyla hepatotoksisiteye neden olan birçok zararlı maddeye maruz kalmaktadır. Akrilamid kanserojen bir ajandır ve karaciğer üzerinde toksik etkileri vardır. Bu çalışmada siyah havuç suyunun, sıçan karaciğerini, akrilamid toksisitesine karşı koruyup koruyamayacağını araştırmayı amaçladık. **Yöntem:** Otuz iki erkek Wistar albino sıçan; kontrol, akrilamid, siyah havuç suyu ve akrilamid + siyah havuç suyu olmak üzere dört eşit gruba ayrıldı. Tüm uygulamalar 30 gün boyunca her gün yapıldı. Karaciğer dokuları rutin histopatoloji ve apoptoz açısından analiz edildi, serum örnekleri ise oksidasyon seviyesi açısından değerlendirildi. **Bulgular:** Akrilamid grubunda, hepatosit dejenerasyonu, sinüzoidal dilatasyon ve pasif hiperemi gibi ciddi histopatolojik hasar ile beraber, kaspaz-3 immunreaktivitesinde önemli bir artış gözlendi. Biyokimyasal olarak serum toplam antioksidan seviyesi önemli ölçüde azaldı ve serum toplam oksidan seviyesi önemli ölçüde arttı. Histopatolojik inceleme, akrilamid + siyah havuç suyu grubunda, akrilamid grubuna göre doku hasarında anlamlı bir düzelme olduğunu gösterdi. Ayrıca, toplam oksidan seviyesi düzeyi önemli ölçüde azalırken, toplam antioksidan düzeyi önemli ölçüde arttı. Kaspaz-3 immünoreaktivitesi ise önemsiz derecede azaldı. **Sonuç:** Siyah havuç suyu, antioksidan özelliklerinden dolayı, akrilamide bağlı hepatotoksisiteye karşı terapötik etkiler sergilemektedir.

Anahtar Kelimeler: akrilamid, kara havuç suyu, kaspaz-3, hepatotoksisite, total antiosidan seviye, total oksidan seviye

Alındığı tarih/Received Date:
03.06.2020
Kabul tarihi/Accepted Date:
14.06.2020

Sorumlu yazar:
Alper YALÇIN
e-mail:
alperyalcin0171@hotmail.com

[1] Kahramanmaraş Sütçü İmam Üniversitesi, Tıp Fakültesi, Histoloji ve Embriyoloji Ana Bilim Dalı, Türkiye.

[2] Adıyaman Üniversitesi, Tıp Fakültesi, Anatomi Ana Bilim Dalı, Türkiye.

Abstract

Objective: The liver is exposed to many harmful agents, which cause hepatotoxicity due to oxidative stress. Acrylamide is a carcinogenic agent and has toxic effects on the liver. In this study we aimed to examine whether black carrot juice might protect rat liver from toxicities of acrylamide. **Method:** Thirty-two male Wistar albino rats were divided into four equal groups as follows: control, acrylamide, black carrot juice and acrylamide + black carrot juice All the treatments were administered every other day for 30 days. Liver tissues were analysed for routine histopathology and apoptosis whereas serum samples were evaluated for oxidation state. **Results**: In the acrylamide group, severe histopathological damage, including hepatocyte degeneration, sinusoidal dilatation, and passive hyperemia, along with a significant increase in caspase-3 immunoreactivity was observed. Biochemically the serum total antioxidant status decreased significantly, and the serum total oxidant status increased significantly. The histopathological examination revealed significant amelioration of tissue damage in the acrylamide + black carrot juice group as compared with that in the acrylamide group. Moreover, total oxidant status decreased significantly, whereas the total antioxidant status increased significantly. Caspase-3 immunoreactivity also decreased insignificantly. **Conclusion:** Black carrot juice appears to exhibit therapeutic effects against acrylamide-induced hepatotoxicity due to its antioxidant properties.

Key words: acrylamide, black carrot juice, caspase-3, hepatotoxicity, total antioxidant status, total oxidant status

INTRODUCTION

Acrylamide (ACR) is a strongly reactive organic compound, that is widely used in many industries (Semla et al. 2017) including cosmetic and dye (LoPachin et al. 2002) fibers, glue, pharmaceutical, paper, textile, and polyacrylamide gel electrophoresis (Kuklenyik et al. 2005). In addition previous studies have reported that smoking is an important source of ACR in humans (Huang et al. 2007; Vesper et al. 2007). ACR also forms when foods containing high amounts of protein and carbohydrate (e.g., fried foods, potato products, bread, crackers, and cereals) are heated at high temperatures (Erdreich & Friedman 2004; Jin-Heon et al. 2016). ACR emanates as a result of the Maillard browning reaction in which amino acids, mostly asparagine, interact chemically with reducing sugars at temperatures above 120 (Parzefall 2008). ACR that forms as a by-product during the frying/cooking process is defined as a carcinogenic substance (Alturfan et al. 2012). Acrylamide is categorized as probably carcinogenic to humans (IARC 1994). ACR exposure was reported as dangerous agent for public health (Powers et al., 2013).The Food and Agriculture Organization, United States Food and Drug Administration, and World Health Organization reported that ACR that forms in food is a threat to human health (Rosen 2002). Therefore, many studies have investigated the toxicity of ACR and its possible effects on human health, with some research concluding that it has neurotoxic, genotoxic, and carcinogenic effects (Acaroz et al. 2018; Friedman et al. 2008). Oxidative stress has been documented in many chemical-induced cellular damages (Wang et al. 2005). It leads increased production of reactive oxygen species (ROS) and reducing of antioxidant defense in the tissues (Zhu et al. 2008). ACR-induced oxidative stress causes to cell damage by effecting critical molecules, such as DNA and proteins, and death (Mannaa et al. 2006). Antioxidants can be taken in account as an alternative way to combat ACR-induced toxicity (Lakshmi et al. 2012). In a previous research, natural antioxidants have been notified to reduce ACR-induced toxicity (Hamdy et al. 2017). It has been reported that phytochemicals can strengthen endogenous cellular defense mechanisms and have potential to prevent and treat diseases (Houghton 2019). Phenolic agents present in foods have been reported to have significant role in defense against oxidative stress caused by ROS (Tsao 2010). Anthocyanins increase physiological activity and strengthen the health-promoting aspects of foods because of their strong antioxidant properties (Netzel et al. 2007). Black carrot (BC) (Daucus carota L.) include anthocyanins, phenolic acids and carotenoids as basic phytochemicals. Anthocyanins in BC are phytochemicals with high antioxidant capacity and play a preventive role against several diseases (Akhtar et al. 2017). Due to the high yield potential of BC it is admitted one of the most important vegetable among the top 10 vegetable crops in the world (Dawid et al. 2015). As it has high dietary value it plays a major role in human nutrition (Garba et al. 2015). BC is rich in anthocyanin as compared with the content in other common fruits and vegetables (Espinosa-Acosta et al. 2018) and contains relatively higher phenolic content with high antioxidant potential (Kaur & Kapoor 2002). Anthocyanins have been shown to have antioxidant and anti-proliferative characteristics against different cancer cell lines (Wang & Stoner 2008). Akhtar et al. (2017) also reported that the phytochemicals in BC exhibit preventive effects against variety of diseases including many types of cancer, diabetes mellitus, cardiovascular diseases and oxidative stress. There has not been any report in the literature that investigated the therapeutic effects of BCJ against ACR induced liver cytoxicity. Therefore we aim to examine the possible ameliorative effects of BCJ on ACR induced hepatotoxicity in a rat model.

MATERIALS AND METHODS

Source and preperation of black carrot extracts

BC was purchased from a local grocer in Adiyaman, Turkey (April 2019). After washing with tap water, carrots were divided into small pieces, passed through a domestic mixer, and filtered, and administered to the animals by oral gavage.

Chemicals

ACR was purchased as a commercial preparation (Acrylamide, Cas no: 79-06-1, Acros Organıcs, New-Jersey US)

Animals, diets and experimental designs

Thirty-two male Wistar albino rats aged 10–12 wk and weighing 200–250 g were divided into four groups, with eight animals in each group. Prior to the commencement of the experiment, the rats were kept in cages for 7 d without any treatments for adaptation. During the experimental period, the rats were housed in cages at room

temperature of 22 ± 2° C under 12/12 h light/dark conditions, with free access to food (standard rodent diet) and water.

The treatment groups were as follows: control group; 1 ml of physiological serum was administered by oral gavage; Black carrot juice (BCJ) group: 1 ml of BCJ was administered by oral gavage; ACR group: 20 mg/kg of ACR was administered via intraperitoneal injection (i.p.); ACR + BCJ group: 20 mg/kg ACR was administered by i.p. injection, followed by 1 ml of BCJ. All the treatments were administered every other day for 30 d.

The experiment was performed in accordance with ethical guidelines for the care and maintenance of laboratory animals, and the study was approved by the animal experiments local ethics committee of Adiyaman University (protocol no.: 2019/30).

At the end of the experiment, the animals were anesthetized using a combination of ketamine (75 mg/kg) and xylazineHCl (10 mg/kg). Blood samples were collected by intracardiac puncture. The sera from all the groups were stored at -20° C until the biochemical evaluation. Liver tissues were fixed with 10% formalin for histopathological and immunohistochemical examinations.

Histopathological examination

The liver tissues were processed in accordance with routine histological procedures. Sections 4–6 μm thick were cut from paraffin blocks and stained with hematoxylin-eosin and then blindly analyzed using a light microscope (Leica DM500, Germany) and digital image analysis system (Leica DFC295, Germany).

Biochemical analysis

Serum samples were collected for analysis of total antioxidant status (TAS), and total oxidant status (TOS).

Measurements of TAS and TOS levels

To determine the degree of oxidant damage, TAS and TOS levels were evaluated using TAS and TOS (Rel Assay DiagnosticR, Gaziantep, Turkey) assay test kits in accordance with the method of Erel (2004, 2005). Prior to the analysis, the serum samples were allowed to reach room temperature. The TAS and TOS levels were analyzed by colorimetric analysis using an enzyme-linked immunosorbent assay (Anthos Zenyth 200rt) in the biochemistry laboratory of Adiyaman University Faculty of Pharmacy.

Immunohistochemistry for Caspase-3

Tissue sections 4–6 μm thick taken from paraffin blocks were transferred to polylysine-coated slides. Deparaffinized and rehydrated sections were boiled in a microwave oven for antigen retrieval. The sections were then left at room temperature for about 20 min for cooling. Subsequently, hydrogen peroxide was applied for 5 min to block endogenous peroxidase activity. To remove dye from the tissue sections, they were washed with phosphate-buffered saline for 3 × 5 min, followed by the application of Ultra V Block solution for 5 min. Next, caspase primary antibody (ratio of 1/200) (Caspase 3, rabbit polyclonal IgG; ab2302; Abcam, London, UK) was applied for 60 min in a humidity chamber. The sections were then treated with anti-mouse/rabbit IgG secondary antibody for 30 min at room temperature. Finally, the sections were washed again with phosphate-buffered saline and incubated with streptavidin peroxidase. Microscopic images were taken by using a solution of 3-amino-9-ethylcarbazole substrate and of 3-amino-9-ethylcarbazole chromogen tissues stained with Mayer's hematoxylin and covered with aqueous medium.

Histopathological scores for immunoreactivity were based on the prevalence interval, as follows: < 25%, 0.1; 26–50%, 0.4; 51–75%, 0.6; and 76–100%, 0.9. Staining intensity was classified as absent (0), very low (+0.5), low (+1), moderate (+2), and severe (+3). The histopathological score was determined as the prevalence ×staining intensity.

Statistical Analysis

The SPSS 15.0 program was used for statistical analysis. For data with a normal distribution (TAS, TOS, and immunoreactivity variables), the Kolmogorov–Smirnov test was applied. For between-group comparisons of TAS, TOS, and immune variables, a one-way analysis of variance (ANOVA) was used. Levene's test was performed for testing the homogeneity of variances. Tukey's multiple comparison test was used to reveal differences between groups of significant variables. The results are presented as mean ± standard deviation (SD). The level of statistical significance was considered as $p < 0.05$.

RESULTS

Histopathological results

In the histopathological evaluation of liver tissues (Figure 1), normal histological structures were observed in the control and BCJ groups. In the ACR group, hepatocyte degeneration, sinusoidal dilatation and passive hyperemia were observed. As compared with the ACR group, hepatocyte degeneration, sinusoidal dilatation, and passive hyperemia decreased significantly in the ACR + BCJ group.

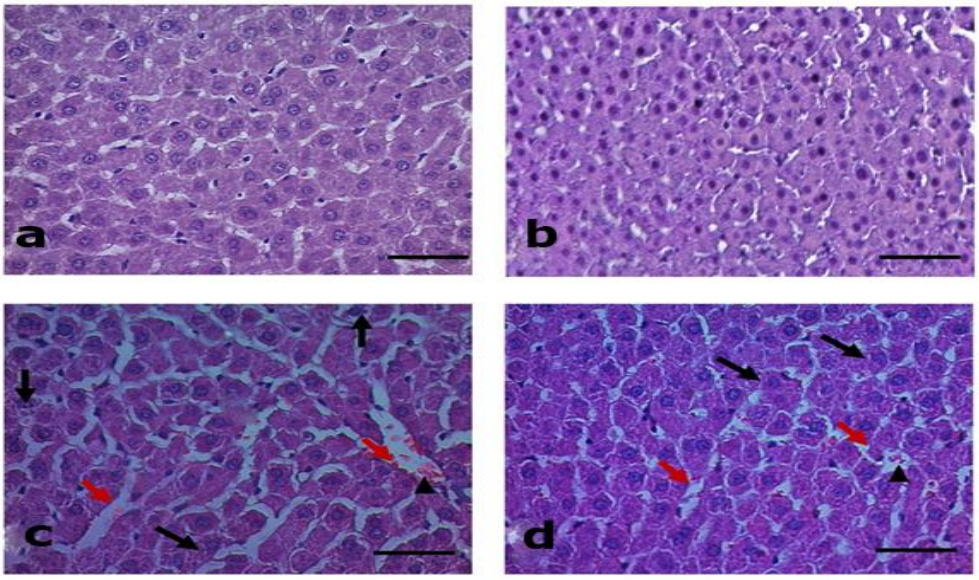

Figure 1: H-E stained liver tissues. The scale bars represent 200 µm. (a) Normal histological structure of liver tissue in the control group. (b) Normal histological structure liver tissue in the BCJ group (c) Degenerated hepatocytes (black arrow), sinusoidal dilatation (red arrow) and passive hyperemia (arrow head) in the ACR group. (d) Significantly decreased degenerated hepatocytes (black arrow), sinusoidal dilatation (red arrow head) and passive hyperemia (arrow head) in the ACR + BCJ group.

Effects of ACR and BCJ on TAS and TOS levels

In the biochemical analysis, serum TAS and TOS (Table 1) levels in the control and BCJ groups were similar. As compared with the control group, TAS levels decreased significantly and those of TOS levels increased significantly in the ACR group ($p < 0.05$). In the ACR + BCJ group, TAS levels were significantly increased, where as TOS levels were significantly decreased as compared with those in the ACR group ($p < 0.05$).

Table 1: Serum levels of TAS and TOS

GROUPS	TAS	TOS
CONTROL(n=8)	$2.592^{a}\pm1.88$	$15.86^{c}\pm2.63$
BCJ (n=8)	$2.486^{a}\pm1.31$	$15.85^{c}\pm1.97$
ACR (n=8)	$2.104^{b}\pm1.65$	$25.04^{a}\pm2.35$
ACR+BCJ (n=8)	$2.420^{a}\pm2.46$	$21.27^{b}\pm2.21$
p* values	<0.001	<0.001

[abc]Means within the same column with differing superscripts are significantly different (p <0.05). *One Way Anova

Immunohistochemial results

The immunohistochemical staining of caspase in the liver (Figure 2) revealed that similar caspase-3 immunoreactivity was observed in the control group and BCJ group ($p > 0.05$). As compared with the control group, caspase-3 immunoreactivity increased significantly in the ACR group ($p < 0.05$). As can be seen in Table 2, caspase-3 immunoreactivity decreased in the ACR + BCJ group as compared with that in the ACR group, although the decrease was statistically insignificant ($p > 0.05$).

Table 2. Caspase-3 immunoreactivity

GROUPS	Caspase-3
CONTROL (n=8)	$0.83^{b}\pm0.17$
BCJ (n=8)	$0.88^{b}\pm0.2$
ACR (n=8)	$1.99^{a}\pm0.64$
ACR+BCJ (n=8)	$1.41^{ab}\pm0.64$
p* values	<0.001

[ab]Means within the same column with differing superscripts are significantly different (p <0.05). *One Way Anova

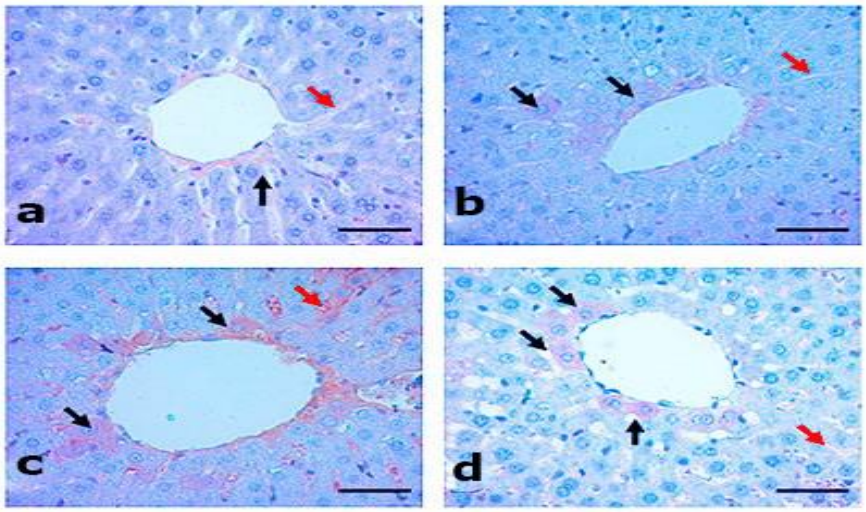

Figure 2: Livers stained with Streptavidin biotin peroxidase complex method with Mayer's Hematoxylin counterstain for caspase-3 immunoreactivity. The scale bars represent 200 µm. Caspase-3 immunoreactivity in hepatocytes (black arrows) and sinusoidal cells (red arrows) of liver tissue. (a) Control group. (b) BCJ group. (c) ACR group and (d) ACR + BCJ group.

DISCUSSION

ACR has been reported to have neurotoxic, genotoxic, hepatotoxic, and teratogenic effects (Lai et al. 2017) due to its rapid absorption and biodistribution ability (Al-Serwi & Ghoneim 2015). It causes damage to many parenchymal organs including the liver by inducing free radical production (Dahiru et al. 2010). Injury of the liver is an important public health issue as it has significant role in detoxifying and depositing both endogenous and exogenous compounds (Zhang et al. 2012). Toxic effects of ACR have been shown in many organs such as the brain, heart, lung, liver, kidney, and testis (Acaroz et al. 2018). ACR cytotoxicity is attributed to oxidative stress (Rodríguez-Ramiro et al. 2011). Liver is a target organ for ACR toxicity, but promising restorative results were obtained with some natural compounds (Acaroz et al. 2018; Altinoz et al. 2015; Alturfan et al. 2012; Gedik et al. 2017; Hamdy et al. 2017) in experimental ACR-induced liver injury.

Polyphenols are the main class of phenols found in fruits and vegetables and have many bioactive properties. (Ignat et al. 2011). These phenolic compounds have anti-aging, anti-inflammatory and antiproliferative properties (Stan et al. 2008) and protect cells against oxidative stress caused by excess reactive oxygen species with their antioxidant capacities (Tsao 2010). BC includes relatively higher phenolic content (Kaur & Kapoor 2002). Phytochemicals in carrots have been reported to have significant effects on human health due to their, antioxidant, anti-inflammatory, antibacterial, plasma lipid modification, anticancer and serotogenic potential (Ahmad et al. 2019). In previous studies BC have been reported to be effective on modifying cholesterol absorption, bile acid excretion, increases antioxidant capacity (Nicolle et al. 2003) and act as a defense system to combat against oxidative stress (Akhtar et al. 2017).

The course of oxidative stress depends on the rate of oxidant formation and all of the antioxidant molecules (Davies 2000). Under normal circumstances, a physiological equilibrium exists between pro-oxidant species and antioxidant molecules. On the other hand deterioration of this homeostasis leads oxidative stress resulting pathological conditions (Zafar et al. 2019). It is possible to measure activity of different oxidant species and antioxidants in laboratories separately in serum, but this might be time-consuming, labor-intensive and costly process also require different and complicated techniques. (Tarpey et al. 2004). Therefore evaluating total oxidant status (TOS) and total antioxidant status (TAS) may count cumulative and synergistic effects of all oxidant and antioxidant compounds and is a simple way to exhibit oxidant/antioxidant status (Erel 2004, 2005; Horoz et al. 2006). In the current study TOS was evaluated as an oxidant marker, and TAS, as an antioxidant marker. Similar to previous studies (Erdemli et al. 2019; Gedik et al. 2017; Yerlikaya et al. 2013) we observed decreased TAS levels and increased TOS levels in serum samples of the ACR treated rats which indicates ACR

administration led oxidative stress in the treatment groups. BCJ treatment decreased TOS levels and increased TAS levels in a significant manner in the ACR + BCJ group compared to the ACR group.

However BCJ administration caused significant decrease in these enzyme levels in ACR+BCJ group compared to ACR group. These biochemical ameliorating effects of BCJ could be related to its antioxidant (Ahmad et al. 2019) and scavenging free-radical generation properties (Zhang & Hamauzu 2004).

Previous studies conducted with ACR in rats, significantly damaged hepatocytes (Altinoz et al. 2015), cell necrosis, cytoplasmic vacuolation, disarrangement and mild degeneration of hepatocytes (Ansar et al. 2016), and chronic periportal inflammatory cell infiltration with central vein blood deposition were reported (Singh et al. 2015). In addition ACR has been reported to cause hemorrhage, necrosis and intracytoplasmic vacuolization in hepatocytes, and inflammatory cell infiltration (Hamdy et al. 2017). Acaröz et al. (2018) demonstrated that ACR-treated rats showed sinusoidal dilatation, degenerated hepatocytes and kuppfer cell activation in the liver tissue. All these histopathological results showed that exposure to ACR treatment caused to hepatotoxicity. As in previous reports, the current study clearly demonstrated that ACR treatment caused severe histopathologic changes such as degenerated hepatocytes, sinusoidal dilatation and passive hyperemia. On the other hand BCJ treatment exhibited a decrease in all these histopathological results when compared to ACR group because it has protective and therapeutic (Akhtar et al. 2017) also antioxidant effects (Tanriseven et al. 2020; Yen et al. 2008) against different diseases as determined in the current study.

The balance that exists between pro-apoptotic and anti-apoptotic proteins has an important role for cell survival. The increase in apoptotic proteins is related to cell death (Zheng et al. 2016), and caspases, one of the factors affecting apoptosis, have significant roles in this process called programmed cell death (Cohen 1997). Most important markers of apoptosis are partially or fully involved in a number of structural and regulatory protein cleavages (Peña-Llopis et al. 2003). Caspase-3 has been reported to be the principal effective caspase that cleaves the majority of the proteins in apoptotic cells (Porter & Janicke1999), and has an important role in apoptosis (Boeddeker et al. 2015). The course of apoptosis was examined through caspase-3 in this study, and an upregulated caspase-3 immunoreactivity was determined to be a result of ACR induced toxicity in accordance with the literature in cerebrum, cerebellum, hippocampus, spinal cord and sciatic nerve (Elblehi et al. 2020) stomach (El-Mehi & El-Sherif 2015) retina (Albalawi et al. 2018), ovary (Hułas-Stasiak et al. 2013) and leydig cells (Li et al. 2017). Apoptotic effect of ACR could be attributed to its inductive effect on cell death (Kacar et al. 2018). An insignificant deprivation of apoptotic activity in the ACR + BCJ group was observed compared to the ACR group.

CONCLUSION

In conclusion, BCJ exhibited ameliorative effects by normalizing hepatic architecture and regulating TOS and TAS levels significantly, which also slightly decreased caspase-3 immunoreactivity. The results of the present study suggest that BCJ can reduce oxidative stress in liver tissue. Thus, BCJ consumption is recommended as it may have a potential in delaying the progression of hepatic damage in ACR-related hepatotoxicity and preventing associated complications.

KAYNAKLAR

Acaroz, U., Ince, S., Arslan-Acaroz, D., Gurler, Z., Kucukkurt, I., Demirel, H. H., Arslan, HO., Varol, N.,Zhu, K. 2018. The ameliorative effects of boron against acrylamide-induced oxidative stress, inflammatory response, and metabolic changes in rats. Food Chem Toxicol; 118: 745-752.

Ahmad, T., Cawood, M., Iqbal, Q., Ariño, A., Batool, A., Tarıq, RMS., Azam, M., Akhtar, S. 2019. Phytochemicals in Daucus carota and Their Health Benefits. Foods; 8(9): 424.

Akhtar, S., Rauf, A., Imran, M., Qamar, M,. Riaz, M, Mubarek, MS. 2017. Black carrot (Daucus carota L.), dietary and health promoting perspectives of its polyphenols: A review. Trends Food Sci Technol; 66: 36-47.

Albalawi, A., Alhasani, RHA., Biswas, L., Reilly, J., Akhtar, S., Shu, X. 2018. Carnosic acid attenuates acrylamide-induced retinal toxicity in zebrafish embryos. Exp. Eye Res; 175: 103-114.

Al-Serwi, RH and Ghoneim, FM. 2015. The impact of vitamin E against acrylamide induced toxicity on skeletal muscles of adult male albino rat tongue: Light and electron microscopic study. J Microsc Ultrastruct; 3: 137-147.

Altinoz, E., Turkoz, Y., Vardi, N. 2015. The protective effect of N-acetylcysteine against acrylamide toxicity in liver and small and large intestine tissues. Bratisl Med J ;116(4): 252-258.

Alturfan, AA., Tozan-Beceren, A., Şehirli, AÖ., Demiralp, E., Şener, G., Omurtag, GZ. 2012. Resveratrol ameliorates oxidative DNA damage and protects against acrylamide-induced oxidative stress in rats. Mol Biol Rep; 39(4): 4589-4596.

Alturfan, EI., Beceren, .A, Şehirli, AO., Demiralp, ZE., Şener, G., Omurtag, GZ. 2012. Protective effect of N-acetyl-L-cysteine against acrylamide-induced oxidative stress in rats. Turk J Vet Anim Sci; 36(4): 438-445.

Ansar, S., Siddiqi, N. J., Zargar, S., Ganaie, M. A., & Abudawood, M. 2016. Hepatoprotective effect of Quercetin supplementation against Acrylamide-induced DNA damage in wistar rats. BMC Complem Altern M; 16(1): 327.

Boeddeker, SJ and Alexandra PH. 2015. The role of apoptosis in human embryo implantation. J Reprod Immunol; 108: 114-122

Cohen GM. 1997. Caspases: the executioners of apoptosis. Biochem J; 326(1): 1-16.

Dahiru, D., Mamman, D. N., Wakawa, H. Y.2010. Ziziphus mauritiana fruit extract inhibits carbon tetrachloride-induced hepatotoxicity in male rats. Pakistan J Nutr; 9(10): 990-993.

Davies KJ. 2000. Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. IUBMB Life; 50(4-5): 279-289.

Dawid, C., Dunemann, F., Schwab, W., Nothnagel, T., and Hofmann, T. . 2015. Bioactive C17-polyacetylenes in carrots (Daucus carota L.): Current knowledge and future perspectives. J Agric Food Chem; 63(42): 9211-9222.

El-Mehi, AE and El-Sherif, NM. 2015. Influence of acrylamide on the gastric mucosa of adult albino rats and the possible protective role of rosemary. Tissue Cell; 47(3): 273-283.

Elblehi, SS., El Euony, OI., El-Sayed, YS. 2020. Apoptosis and astrogliosis perturbations and expression of regulatory inflammatory factors and neurotransmitters in acrylamide-induced neurotoxicity under ω3 fatty acids protection in rats. Neurotoxicology; 76: 44-57.

Erdemli, ME., Aksungur, Z., Gul, M., Yigitcan, B., Bag, HG., Altinoz, E., Turkoz, Y.. 2019. The effects of acrylamide and vitamin E on kidneys in pregnancy: an experimental study. J Matern-Fetal Neo M; 32(22): 3747-3756.

Erdreich, LS and Friedman MA. 2004. Epidemiologic evidence for assessing the carcinogenicity of acrylamide. Regul Toxicol Pharm; 39(2): 150-157.

Erel O. 2004. A novel automated direct measurement method for total antioxidant capacity using a new generation. more stable ABTS radical cation. Clin Biochem; 37(4): 277-85.

Erel O. 2005. A new automated colorimetric method for measuring total oxidant status. Clin Biochem; 38(12): 1103-1111.

Espinosa-Acosta, G., Ramos-Jacques, AL., Molina, GA., Maya-Cornejo, J., Esparza, R., Hernandez-Martinez, AR, Sanchez-Gonzales I., Estevez M. 2018. Stability Analysis of Anthocyanins Using Alcoholic Extracts from Black Carrot (Daucus Carota ssp. Sativus Var. Atrorubens Alef.). Molecules; 23(11): 2744.

Friedman, MA., Zeiger, E., Marroni, DE., Sickles, DW. 2008. Inhibition of Rat Testicular Nuclear Kinesins (krp 2; KIFC5A) by Acrylamide as a Basis for Establishing a Genotoxicity Threshold. J Agric Food Chem; 56(15): 6024-6030.

Garba, U., Kaur, S., Gurumayum, S., and Rasane, P. 2015. Effect of hot water blanching time and drying temperature on the thin layer drying kinetics of and anthocyanin degradation in black carrot (Daucus carota L.) shreds. Food Technol Biotechi 53(3): 324-330.

Hamdy, SM., Shabaan, AM., Latif, AKMA., Abdel-Aziz, AM., Amin, AM. 2017. Protective effect of Hesperidin and Tiger nut against Acrylamide toxicity in female rats. Exp Toxicol Pathol, 69(8), 580-588.

Houghton CA. 2019. Sulforaphane: Its "Coming of Age" as a Clinically Relevant Nutraceutical in the Prevention and Treatment of Chronic Disease. Oxid Med Cell Longev; https://doi.org/10.1155/2019/2716870

Horoz, M., Bolukbas, C., Bolukbas, FF., Aslan, M., Koylu, AO., Selek, S., Erel, O. 2006. Oxidative stress in hepatitis C infected end-stage renal disease subjects. BMC Infect Dis; 6(1): 114. doi:10.1186/1471-2334-6-114

Huang, CCJ., Li, CM., Wu, CF., Jao, SP., Wu, KY. 2007. Analysis of urinary N-acetyl-S-(propionamide)-cysteine as a biomarker for the assessment of acrylamide exposure in smokers. Environ Res; 104(3) 346-351.

Hułas-Stasiak, M., Dobrowolski, P., Tomaszewska, E., Kostro, K. 2013. Maternal acrylamide treatment reduces ovarian follicle number in newborn guinea pig offspring. Reprod Toxicol; 42: 125-131.

Jın-Heon L, Kee-Jae L & Hee-Sook K. 2016. Estimation of the daily human intake of acrylamide (AA) based on urinary N-acetyl-S-(2-carbamoylethyl)-cysteine (AAMA) and the contribution of dietary habits in South Korean adults. 한국환경보건학회지; 42(4): 235-245.

Kacar, S., Vejselova, D., Kutlu, HM., Sahinturk, V.. 2018. Acrylamide-derived cytotoxic, anti-proliferative, and apoptotic effects on A549 cells. Hum Exp Toxicol; 37: 468-474.

Kaur C and Kapoor HC. 2002. Anti-oxidant activity and total phenolic content of some Asian vegetables. Int J Food Sci Technol; 37: 153-161

Kuklenyik Z, Needham LL & Calafat AM. 2005. Measurement of 18 perfluorinated organic acids and amides in human serum using on-line solid-phase extraction. Anal Chem; 77(18): 6085-6091.

Lai, SM., Gu, ZT., Zhao, MM., Li, XX., Ma, YX., Luo, L., Liu, J. 2017.Toxic effect of acrylamide on the development of hippocampal neurons of weaning rats. Neural Regen Res; 12(10): 1648-1654.

Lakshmi, D., Gopinath, K., Jayanthy, G., Anjum, S., Prakash, D., Sudhandiran, G. 2012. Ameliorating effect of fish oil on acrylamide induced oxidative stress and neuronal apoptosis in cerebral cortex. Neurochem Res; 37(9): 1859-1867.

Li, M., Sun, J., Zou, F., Bai, S., Jiang, X., Jiao, R., Ou, S., Zhang, H., Su, Z., Huang, Y., Bai, W.2017. Glycidamide inhibits progesterone production through reactive oxygen species-induced apoptosis in R2C Rat Leydig Cells. Food Chem Toxicol; 108: 563-570.

LoPachin, RM., Ross, JF., Reid, ML., Das, S., Mansukhani, S., Lehning, EJ 2002. Neurological evaluation of toxic

axonopathies in rats: Acrylamide and 2, 5-hexanedione. Neurotoxicology; 23(1): 95-110.

Mannaa, F., Abdel-Wahhab, MA., Ahmed, HH., Park, MH. 2006. Protective role of Panax ginseng extract standardized with ginsenoside Rg3 against acrylamide-induced neurotoxicity in rats. J Appl Toxicol; 26(3): 198-206.

International Agency for Research on Cancer (IARC). 1994. Acrylamide. In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans; Some Industrial Chemicals, vol. 60. IARC, Lyon, France.

Netzel, M., Netzel, G., Kammerer, DR., Schieber, A., Carle, R., Simons, L., Bitsch I, Bitsch R., Konczak. I 2007. Cancer cell antiproliferation activity and metabolism of black carrot anthocyanins. Innov Food Sci Emerg Technol; 8(3): 365-372.

Nicolle C et al. 2003. Effect of carrot intake on cholesterol metabolism and on antioxidant status in cholesterol-fed rat. Eur J Nutr ;42(5): 254-261.

Parzefall W. 2008. Minireview on the toxicity of dietary acrylamide. Food Chem Toxicol; 46(4): 1360-1364.

Peña-Llopis, S., Ferrando, MD., Peña, JB. 2003. Fish tolerance to organophosphate-induced oxidative stress is dependent on the glutathione metabolism and enhanced by N-acetylcysteine. Aquat Toxicol; 65(4): 337-360.

Porter AG and Janicke RU. 1999. Emerging roles of caspase-3 in apoptosis. Cell Death Differ; 6: 99–104.

Powers, SJ., Mottram, DS., Curtis, A., Halford, NG. . 2013. Acrylamide concentrations in potato crisps in Europe from 2002 to 2011. Food Addit Contam; 30(9): 1493-1500.

Rodríguez-Ramiro, I., Martín, MÁ., Ramos, S., Bravo, L., Goya, L. 2011. Olive oil hydroxytyrosol reduces toxicity evoked by acrylamide in human Caco-2 cells by preventing oxidative stress. Toxicology; 288(1-3): 43-48.

Rosen JD. 2002. Acrylamide in food: is it a real threat to public health. American Council on Science and Health. New York, pp. 1-17

Semla, M., Goc, Z., Martiniaková, M., Omelka, R., Formicki, G. 2017. Acrylamide: a common food toxin related to physiological functions and health. Physiol Res; 66(2): 205-217

Singh, MP., Jakhar, R., Kang, SC. 2015. Morin hydrate attenuates the acrylamide-induced imbalance in antioxidant enzymes in a murine model. Int J Mol Med; 36(4): 992-1000.

Stan, SD., Kar, S., Stoner, GD., Singh,SV. 2008. Bioactive food components and cancer risk reduction. J Cell Biochem; 104(1): 339-356.

Tanriseven, D., Kadiroglu, P., Selli, S., Kelebek, H. 2020. LC-DAD-ESI-MS/MS-assisted elucidation of the phenolic compounds in shalgams: Comparison of traditional and direct methods. Food chem; 305: 125505. https://doi.org/10.1016/j.foodchem.2019.125505

Tarpey MM, Wink DA & Grisham MB. 2004. Methods for detection of reactive metabolites of oxygen and nitrogen: in vitro and in vivo considerations. Am J Physiol-Reg I 286(3): R431-R444.

Tsao R. 2010. Chemistry and biochemistry of dietary polyphenols. Nutrients; 2(12): 1231-1246.

Ignat, I., Volf, I., & Popa, VI. 2011. A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. Food Chem; 126(4): 1821-1835.

Vesper, HW., Bernert, JT., Ospina, M., Meyers, T., Ingham, L., Smith, A., Myers, GL. 2007. Assessment of the relation between biomarkers for smoking and biomarkers for acrylamide exposure in humans. Cancer Epidem Biomar; 16(11): 2471-2478.

Wang LS & Stoner GD 2008. Anthocyanins and their role in cancer prevention. Cancer Lett; 269(2): 281-290.

Wang, QS., Xie, KQ., Zhang, CL., Zhu, YJ., Zhang, LP., Guo, X., Yu, SF. 2005. Allyl chloride-induced time dependent changes of lipid peroxidation in rat nerve

tissue. Neurochem Res; 30(11): 1387-1395.

Yen YH, Shih CH & Chang CH. 2008. Effect of adding ascorbic acid and glucose on the antioxidative properties during storage of dried carrot. Food Chem; 107(1): 265-272.

Yerlikaya FH, Toker A., Yener Y. 2013. Effects of acrylamide treatment on oxidant and antioxidant levels in rats. Kafkas Univ Vet Fak Derg; 19(4): 607-612.

Zafar, MS., Quarta, A., Marradi, M., Ragusa, A. 2019. Recent Developments in the Reduction of Oxidative Stress through Antioxidant Polymeric Formulations. Pharmaceutics; 11(10): 505.

Zhang DL & Hamauzu Y. 2004. Phenolic compounds and their antioxidant properties in different tissues of carrots (Daucus carota L.). J Food Agric Environ; 2(1): 95-100.

Zheng, JH., Viacava Follis, A., Kriwacki, RW., Moldoveanu, T . 2016. Discoveries and controversies in BCL2 protein mediated apoptosis. The FEBS J; 283(14): 2690-2700.

Zhu, YJ., Zeng, T., Zhu, YB., Yu, SF., Wang, QS., Zhang, LP.,Guo. X., Xie, KQ. 2008. Effects of acrylamide on the nervous tissue antioxidant system and sciatic nerve electrophysiology in the rat. Neurochem Res; 33(11): 2310-2317.