ORIGINAL ARTICLE

Özgün Araştırma

Yazışma Adresi
Correspondence Address

Mehmet Enes SOZEN
Alanya Alaaddin Keykubat University, Medical Faculty, Department of Histology and Embryology, Antalya, Turkey
drenessozen@gmail.com

Geliş Tarihi : September 27, 2021
Received
Kabul Tarihi : March 22, 2022
Accepted
E Yayın Tarihi : May 01, 2023
Online published

Bu makalede yapılacak atıf
Cite this article as
Sozen ME, Akkaya O, Savas HB, Karahan O.
The Oxidative and Anti-Angiogenic Effects of Acrylamide in Chorioallantoic Membrane Model
Akd Med J 2023; 9(2): 162-168

Mehmet Enes SOZEN
Alanya Alaaddin Keykubat University, Medical Faculty, Department of Histology and Embryology, Antalya, Turkey
ORCID ID: 0000-0002-5007-6631

Ozgur AKKAYA
Alanya Alanya Training and Research Hospital, Department of Cardiovascular Surgery, Antalya, Turkey
ORCID ID: 0000-0001-6460-5066

Hasan Basri SAVAS
Mardin Artuklu University Faculty of Medicine Department of Medical Biochemistry, Mardin, Turkey
ORCID ID:0000-0001-8759-4507

Oguz KARAHAN
Medical School of Alaaddin Keykubat University, Department of Cardiovascular Surgery, Antalya, Turkey
ORCID ID: 0000-0003-0044-9476

DOI: 10.53394/akd.1015590

The Oxidative and Anti-Angiogenic Effects of Acrylamide in Chorioallantoic Membrane Model

Akrilamidin Korioallantoik Membran Modelinde Oksidatif ve Anti-Anjiyogenik Etkileri

ABSTRACT

Objective:

Acrylamide (ACR) is formed spontaneously during the preparation of carbohydrate-containing foods by exposure to high heat and can be found in large amounts in processed ready-made foods like potato crisps, biscuits, crackers, and bread. ACR is a toxic substance and increases oxidative stress. The aim of the study is to show the effects of ACR exposure at different doses on angiogenesis and oxidant-antioxidant balance in the chorioallantoic membrane (CAM) model.

Methods:

Two different concentrations of ACR were prepared (10^{-3} M and 10^{-4} M). Pellets were placed on the CAM of the embryos. Liquid samples were taken from fertilized chicken eggs before and after the experiment. Anti-angiogenic effects were evaluated through the window that was opened on the eggshell.

Results:

The 10^{-4} M ACR group caused anti-angiogenic effects (average score 0.3) which were higher than the control group, but these changes were not statistically significant. The 10^{-3} M ACR group caused moderate anti-angiogenic effects (average score 0.6). The 10^{-6} M Bevacizumab group caused powerful anti-angiogenic effects (average score 1). There is a significant increase in total oxidant capacity (TOC) and oxidative stress index (OSI) values in 10^{-3} M ACR and 10^{-4} M ACR groups, compared to the control group. Although there was a numerical increase in TOC and OSI values in 10^{-3} M ACR group compared to 10^{-4} M ACR group, this increase was not statistically significant.

Conclusion:

This study is the first to investigate the anti-angiogenic effects of ACR and is one of the first to investigate oxidative stress in the CAM model. ACR exposure increased oxidative stress in the CAM model and showed a dose-dependent anti-angiogenic effect.

Key Words:

Chorioallantoic membrane model, Acrylamide, Oxidant, Antioxidant, Angiogenesis

ÖZ

Amaç:

Akrilamid (AKR), karbonhidrat içeren gıdaların yüksek ısıya maruz bırakılarak hazırlanması sırasında oluşur ve patates cipsi, bisküvi, kraker ve ekmek gibi işlenmiş hazır gıdalarda çok miktarda bulunur. AKR toksik bir maddedir ve oksidatif stresi arttırır. Bu çalışmanın amacı, farklı dozlarda AKR maruziyetinin korioallantoik membran (KAM) modelinde anjiyogenez ve oksidan-antioksidan dengesi üzerindeki etkilerini göstermektir.

Yöntemler:

İki farklı konsantrasyonda akrilamid hazırlandı (10^{-3} M ve 10^{-4} M). Embriyoların KAM'ına peletler yerleştirildi. Deney öncesi ve sonrasında döllenmiş tavuk yumurtalarından sıvı numuneler alındı. Yumurtanın kabuğunda açılan pencereden anti-anjiogenik etki araştırıldı.

Bulgular:

10^{-4} M AKR grubu, kontrol grubuna göre daha yüksek anti-anjiyogenik etkilere (ortalama skor 0,3) neden oldu ancak bu değişiklikler istatistiksel olarak anlamlı değildi. 10^{-3} M AKR grubu orta düzeyde anti-anjiyogenik etkiye neden oldu (ortalama skor 0,6). 10^{-6} M Bevacizumab grubu güçlü anti-anjiyogenik etkiye neden oldu (ortalama skor 1). 10^{-3} M AKR ve 10^{-4} M AKR gruplarında kontrol grubuna göre total oksidan kapasite (TOK) ve oksidatif stres indeksi (OSİ) değerlerinde anlamlı artış vardı. 10^{-3} M AKR grubunda 10^{-4} M AKR grubuna göre TOK ve OSİ değerlerinde sayısal bir artış olsa da bu artış istatistiksel olarak anlamlı düzeyde değildi.

Sonuç:

Bu araştırma, KAM modelinde AKR'nin anti-anjiyogenik etkilerini ve oksidatif stresi araştıran ilk çalışmalardan biridir. AKR maruziyeti KAM modelinde oksidatif stresi artırmış ve doza bağlı olarak anti-anjiyogenik etki göstermiştir.

Anahtar Kelimeler:

Korioallantoik membran modeli, Akrilamid, Oksidan, Antioksidan, Anjiyogenez

INTRODUCTION

Acrylamide (ACR) is a toxic substance that is used widely in the field of industry such as water treatment, dyes, textile, plastics, cosmetics, and also in laboratories (1). Smoking causes exposure to ACR too (2). ACR is also formed spontaneously during the preparation of carbohydrate-containing foods by exposure to high heat and can be found in large amounts in processed ready-made foods (3,4). ACR is found in potato crisps, biscuits, crackers, and bread (5). It can cause many diseases, including cancer. Different experimental models have shown that ACR causes an increase in oxidative stress (3, 4). It also causes an elevation in the pro-inflammatory cytokines like interleukin-1β, tumor necrosis factor-alpha, and also an inducible form of nitric oxide synthase (6). Apoptosis also plays a role in ACR-induced toxicity (7).

In living organisms, there is a constant formation of free radicals under physiological conditions. These free radicals cause oxidative stress in the organism. Excessive oxidative stress causes damage to macromolecules, cells, tissues, and organs. The increase in oxidative damage is held responsible for many serious diseases. The increase in oxidative stress is constantly balanced by antioxidant mechanisms. Thus, free radicals are neutralized (8-10). There are many components of the oxidant and antioxidant mechanism. Therefore, instead of measuring and interpreting each component separately, the need for analysis, which can be a general indicator, has emerged to determine the oxidant-antioxidant balance. Total oxidant capacity (TOC), total antioxidant capacity (TAC), and oxidative stress index (OSI) measurements, as standardized colorimetric methods, show the oxidant-antioxidant balance in two ways (11-13).

The chicken chorioallantoic membrane (CAM) is an extraembryonic membrane. The mesodermal layers of the allantois and chorion fuse and then form a CAM that has very high vascularity. This high vascularity remains until day 11 and these vessels exchange gases and excrete waste products (14). CAM has two epithelial layers and a stroma between them where there is this high vascularity. CAM has a lot of advantages to study vascular functions (15). Kadawathagedara et al., (16) showed that ACR affected fetal growth and thus make us think that ACR might also affect angiogenesis.

In this study we aimed to evaluate the effects of acrylamide exposure at different doses on angiogenesis and oxidant-antioxidant balance in the CAM model.

MATERIALS and METHODS

After the design of the study, the study protocol was presented to the Animal Ethical Committee of Akdeniz University and the committee approved that this study has no need for animal research ethic (No: B.30.2.AKD.0.05.07.00/47, Date: 09.04.2021). This study was conducted in accordance with Research and Publication Ethics.

Reagents and Pellet Preparation

ACR was obtained from Sigma-Aldrich, Missouri, USA. Commercially available drug form of Bevacizumab (Avastin®, Genentech, South San Francisco, CA; Roche AG, Basle, Switzerland) was obtained from the pharmacy. Bevacizumab which is an anti-angiogenic agent was used for comparison for scoring ACR as a positive control group (n:10 embryos) as described previously (17). The negative control group (n: 10 embryos) consisted of drug-free pellets for evaluation of the normal angiogenic activity of chick embryos. The study groups were consisted of 10^{-3} M concentration ACR (n: 10 embryos) and 10^{-4} M concentration ACR (n: 10 embryos) (Table I). The concentrations were determined based on the results of previous studies.

After preparing a 1.5% solution with agarose and distilled water, the pellets were taken into the autoclave and sterilized at 121 °C and 1 atmospheric pressure. The solution was transferred to a sterile container and cooled to 37 °C. Then, the process of adding the agent to the solution was started. The initial mixtures were diluted ten-fold with agarose solution and a pellet group impregnated with two different concentrations of ACR (10^{-3}, 10^{-4} M) and a single dose (10^{-6} M) bevacizumab

was prepared according to previously described dosage (17). In order to obtain pellets of appropriate size and structure, 10 µL drops were prepared on a sterile circular stainless-steel surface with a diameter of 5 mm and solidified by rapidly dropping at room temperature. The classical molarity formula was used to find the amount of drug that would provide a drug concentration of 10^{-3} M in the final volume of 10 µL of the disc (M=m/V). In each study set, 40 discs were prepared and applied. Therefore, approximately 1 mL of agar and drug mixture was prepared for each drug (10 µLx100=1mL).

Table I: Group Creation.

Group	Number of Embryos (n)	Drug Concentrations
Control (Negative Control)	10	Drug-free agar
ACR	10	10^{-3} M
ACR	10	10^{-4} M
Bevacizumab (Positive Control)	10	10^{-6} M
Total	40	

Acrylamide (ACR)

Chicken Chorioallantoic Membrane (CAM) Model and Angiogenesis Scoring

The CAM model creation was prepared by using Ross 308 genus fertilized chickens' eggs. To sustain the natural growth process embryos were housed under conditions of 37.5°C and 80% relative humidity in a horizontal position. It was planned to have 10 embryos in each group, a total of 80 embryos were placed in the incubator to compensate for the losses during the process, and the best-developing embryos were preferred for the procedure. The eggshells were disinfected with 70% alcohol.

Five ml of albumen was removed with a syringe through the eggshell and the eggshell was removed on the opposite side of the egg in a 1x2 cm diameter on the fifth day of incubation. CAM development was examined through the aperture, normal CAM development was determined, and dead or malformed eggs were excluded. The aperture is covered with a sterile drape and embryos were incubated for 3 days more. Thereafter, when the embryo reached a diameter of approximately 2 cm, pellets containing the active substance were placed on the CAM of the embryos. Angiogenesis around the pellets (Figure 1 A) was assessed 48 hours after this procedure using a stereoscopic microscope (Nikon SMZ 745T). Eggs containing embryos that lost vital activity or showed abnormal development during the procedure were excluded from the study.

Angiogenesis Scoring

Angiogenesis was evaluated using the previously described scoring system showing the anti-angiogenic effect (18) (Table II). Ten embryos were used for each group, pellets just containing agar were used as a negative control group. A developed mean scoring system was used to evaluate the active ingredients used on the CAM. The scoring system was used according to previously published literature (17,18). The following formula was used for the determination of the average score:

Avarege score= [number of embryos (score 2) X 2+ number of embryos (score 1)]/ total number of embryos (score 0, 1, 2).

According to the average score, the obtained values were expressed as follows: Score < 0.5: Normal development or no anti-angiogenic activity, Score 0.5-1: Mild or moderate antiangiogenic property, and Score>1: Marked or powerful anti-angiogenic property.

Table II: Score values were used to evaluate the anti-angiogenic effect in the CAM.

Score	Effect	Impression/Explanation
0	No	Normal embryo formation. No change with respect to surrounding capillaries
0.5	Weak	No capillary-free areas. Decreased capillary density but not more than pellet
1	Moderate	Small capillary-free area or capillary density deceased in a specific area. Effects are not bigger than twice the pellet size
2	Strong	Capillary-free area around the pellet at least twice the pellet size

Measurement of Oxidative Parameters

Before and after the experiment, liquid samples taken from the fertilized chicken eggs feeding the fetus were centrifuged and stored in eppendorf tubes at -80 ^{O}C by the Department of Medical Biochemistry. TAC, TOC, and OSI levels were studied by the colorimetric method after liquid samples were collected. Liquid samples were brought to room temperature to thaw and stirred using a vortex stirrer (Thermo, USA) for analysis of oxidant-antioxidant capacity. The data obtained were subjected to statistical analysis, and the existence of a significant difference between the groups was checked.

Total Antioxidant Capacity, Total Oxidant Capacity, and Oxidative Stress Index Analysis

Chicken egg liquid samples' TAC, TOC, and OSI values were examined by spectrophotometry utilizing the modified Erel method (19-21). The TAC and TOC results in the serum are expressed as mmol Trolox Eq/L and mmol H_2O_2 Eq/L, respectively. OSI was determined based on the formula OSI = TOC/TAC.

Statistical Analysis

The results were presented as mean ± SEM. ANOVA test was applied to compare TAC, TOC, and OSI values between groups. Post-hoc tests, Tukey and Duncan, were applied to show the significant difference between groups. The angiogenesis scores were compared with a Kruskal Wallis followed by post hoc Mann-Whitney U test. The limit of significance was accepted as $p<0.05$.

RESULTS

The control group did not cause an anti-angiogenic effect (Figure 1 B). ACR has an anti-angiogenic effect which was dose dependent. Some ACR pellets did not show anti-angiogenic activity (Figure 1 C). 10^{-4} M ACR caused anti-angiogenic effects (average score 0.3) which were higher than the control group, but this change was not statistically significant. 10^{-3} M ACR group caused moderate anti-angiogenic property (average score 0.6). 10^{-6} M Bevacizumab caused powerful anti-angiogenic property (average score 1). (Figure 1 D, E, and F). All scores and calculated average scores for anti-angiogenic effects in each group are given (Table III).

Table III: Anti-angiogenic scoring of the ACR and Bevacizumab at different doses.

Scores / Groups	0	0,5	1	2	Average Score
Control n:10	10	0	0	0	0
ACR 10^{-3} M n:10	3	2	4	1	0.6
ACR 10^{-4} M n:10	4	3	3	0	0.3
Bevacizumab 10^{-6} M n:10	0	3	4	3	1

Acrylamide (ACR)

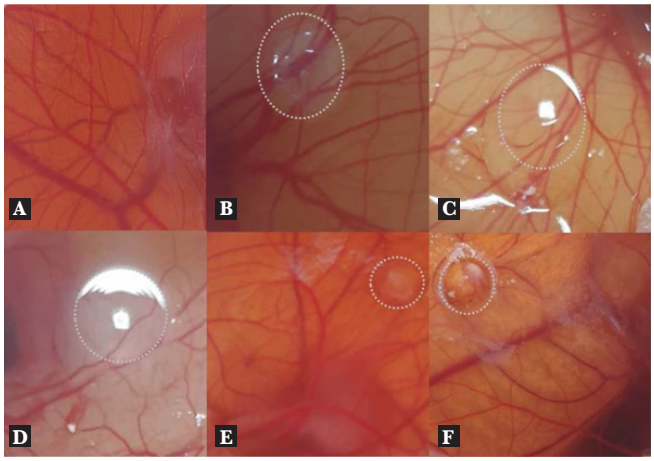

Figure 1: Figure 1 A-F: Anti-angiogenic effects of ACR in CAM model. A. Normal embryologic development of chick embryo (Well-developed vascularity). B. The development of vascular bed after free pellet implantation (white dotted circle). C. The unaffected vascular bed after pellet implantation with 10^{-4} M ACR (white dotted circle). D. Score 1 inhibition of vascular bed development after pellet implantation with 10^{-4} M ACR (white dotted circle: small capillary-free area and decreased capillary density). E. Score 2 inhibition of vascular bed development after pellet implantation with 10^{-3} M ACR (white dotted circle: Capillary-free area around the pellet). F. Score 2 inhibition of vascular bed development after pellet implantation with 10^{-6} M Bevacizumab (white dotted circle: Capillary-free area around the pellet).

According to the statistical analysis results, there is a significant increase in TOC and OSI values because of ACR exposure compared to the control group. Although the increase in dose led to a numerical increase in TOC and OSI values between the groups, that was not at a significant level. The results of TAC (Figure 2), TOC (Figure 3), and OSI (Figure 4) were given.

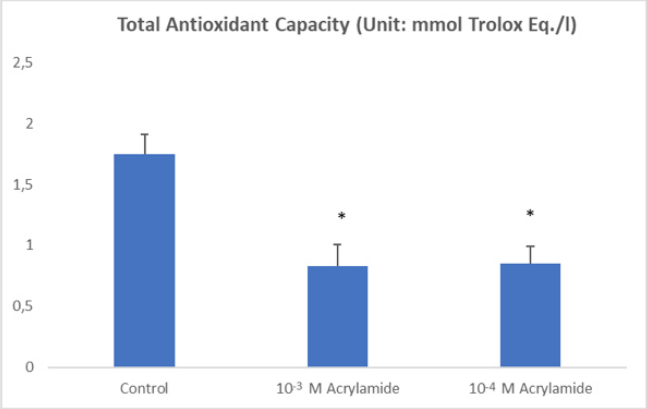

Figure 2: Comparison of total antioxidant capacity (TAC) between groups. There is a statistically significant difference in TAC values between the groups exposed to 10^{-3} M and 10^{-4} M ACR when compared to the control group ($p<0.001$). However, when the groups with 10^{-3} M and 10^{-4} M ACR exposure are compared with each other, there is no statistically significant difference ($p>0.05$). *Significant difference between control group ($p < 0.05$).

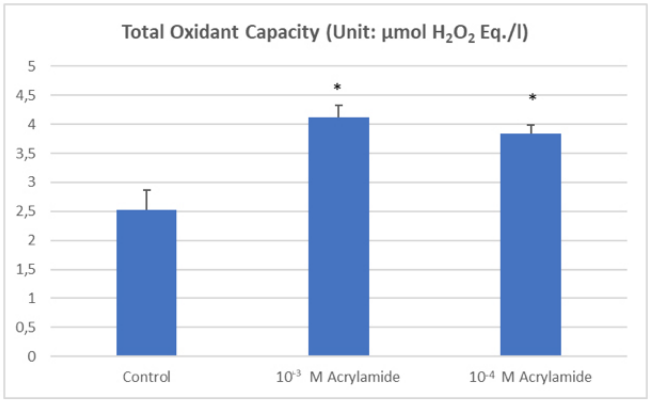

Figure 3: Comparison of total oxidant capacity (TOC) between groups. There is a statistically significant difference in TOC values between the groups exposed to 10^{-3} M and 10^{-4} M ACR when compared to the control group ($p<0.001$). However, when the groups with 10^{-3} M and 10^{-4} M ACR exposure are compared with each other, there is no statistically significant difference ($p<0.05$). *Significant difference between control group ($p < 0.05$).

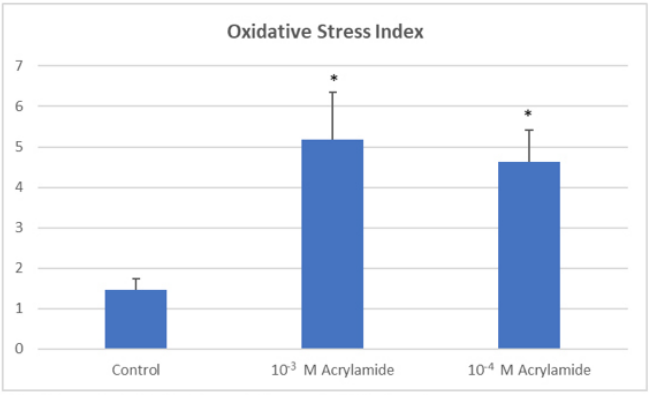

Figure 4: Comparison of oxidative stress index (OSI) between groups. There is a statistically significant difference in OSI values between the groups exposed to 10^{-3} M and 10^{-4} M ACR when compared to the control group ($p<0.001$). However, when the groups with 10^{-3} M and 10^{-4} M ACR exposure are compared with each other, there is no statistically significant difference ($p<0.05$). *Significant difference between control group ($p < 0.05$).

DISCUSSION

ACR occurs when carbohydrate-containing foods are exposed to high heat and it is found in potato crisps, french fries, biscuits and crackers, and bread (5). The risk of ACR exposure to humans is increasing. Continuous exposure to high doses of ACR is toxic and carcinogenic ACR has toxic effects on the liver, kidney, and many other organs and cells including germ cells (12, 13). This study probably is the first study that investigates the anti-angiogenic effects of ACR in the CAM model. And, our study is one of the first studies in the literature in terms of oxidant-antioxidant balance measurement in the CAM model.

The simplicity, accessibility, easy handling, quickness, and no need for animal research ethic committee approval and avoiding their regulatory rules because the embryo is considered as not a living animal until day 14 and low cost are the reasons of the usage of the chicken CAM model (14, 15). The deficit of these model is the rearrangement of the existing vessels. Angiogenic and anti-angiogenic responses affect the density of vessels around the used compounds (14).

ACR induced rat mesenteric vessels neuropathy but did not cause smooth muscle damage (22). Nurullahoğlu-Atalık et al., (23) reported that ACR treatment reduced the luminal area of rat aortas and affects vascular morphology and also contractility induced by phenylephrine and potassium chloride. ACR also caused vasorelaxation in which the acetylcholine receptor did not participate (24). Yu et al., (25) showed that ACR affects fetal blood vessels in the placental labyrinth and the formation of blood vessels and thought that this may be the reason for corruption in the development of embryos and placenta. These findings might be caused by the anti-angiogenic effects of ACR so these findings can be thought to support our findings.

ACR affects cardiac maturation by changing the N-cadherin distribution and Notch activity. ACR also caused ultrastructural changes in myofibrils and mitochondria (26). Maternal exposure to ACR causes developmental disorders, delayed growth, decreased body and brain weights (27). This might also be due to the anti-angiogenic effects of ACR.

Acrylamide-containing compounds have been investigated in tumor angiogenesis inhibition. Some derivatives have been reported to have partial effects (28,29). In the study conducted with N-Isopropyl-Acrylamide-co-Butyl Methyl Acrylate, regression in tumor growth was shown and it was suggested to investigate whether the compound could prolong the life span with further studies (28). In another study, anti-angiogenic activity was investigated for N-(pyridin-2-yl) acrylamide and it was found that this derivative inhibited angiogenesis (30). A more recent study indicated that ACR and derivatives can reduce tumorigenesis by showing concentration-dependent anti-angiogenic properties (31). However, in previous studies, the extent to which ACR abrogates angiogenesis and its dose-dependent effects have not been sufficiently clarified. The other issue concerning the effect of these materials on physiologic angiogenesis is still unknown. In addition to being the first study on this subject, our study is the only study investigating the effects of ACR on embryonic angiogenesis, rather than on tumors. Our results support that ACR may have dose-dependent anti-angiogenicc effects in physiological angiogenesis processes. Our research results showed the oxidative stress-increases effect of ACR in the CAM model. It has been previously shown in different experimental models that ACR causes an increase in oxidative stress. Our study is a pioneering and original study in that it shows the increase in oxidative stress in the CAM model. As a result of increased oxidative stress, damage up to cell death may occur because of many intracellular effects such as lipid peroxidation, protein carboxylation, and DNA damage.

The spread of this damage may cause structural and functional disorders in tissues and organs (3, 4, 8). Therefore, the increase in oxidative stress in ACR exposure emerges as an important damage mechanism. In future studies, the effects of possible antioxidant compounds that protect against oxidative damage induced by ACR can be investigated. Our research shows that role of oxidative stress in acrylamide toxicity should be supported by new scientific studies.

CONCLUSION

To the best of our knowledge, this is the first study that investigates the anti-angiogenic effects of ACR and one of the first studies that investigate oxidative stress in the CAM model. ACR has an anti-angiogenic effect and induces oxidative stress in the CAM model. To avoid these adverse effects caused by ACR, overcooking of foods and cooking with too high temperatures should be prevented.

Ethics Committee Approval:
Animal Ethical Committee of Akdeniz University Committee approved that this study has no need for animal research ethics approval (No: B.30.2.AKD.0.05.07.00/47, Date: 09.04.2021).

Authorship Contributions:
Concept – O.A., O.K.; Design – M.E.S., O.A., O.K., H.B.S.; Supervision – M.E.S., O.K., H.B.S.; Funding – M.E.S., O.A., H.B.S., O.K.; Materials – H.B.S., O.K.; Data collection &/or processing – M.E.S., H.B.S., O.K.; Analysis and/or interpretation – M.E.S., H.B.S., O.K.; Literature search - M.E.S., H.B.S; Writing – M.E.S., H.B.S, O.K.; Critical review – M.E.S, H.B.S., O.K.

Conflict of Interest:
The authors have no conflict of interest to declare.

Financial Disclosure:
The authors declared that this study has received no financial support from any institution. The necessary resources for the study were provided by the authors. There is no financial conflict between the authors.

REFERENCES

1. Semla M, Goc Z, Martiniaková M, Omelka R, Formicki G. Acrylamide: a common food toxin related to physiological functions and health. Physiol Res 2017;66(2):205-17.

2. Smith CJ, Perfetti TA, Rumple MA, Rodgman A, Doolittle DJ. "IARC group 2A Carcinogens" reported in cigarette mainstream smoke. Food Chem Toxicol 2000;38(4):371-83.

3. Hong Y, Nan B, Wu X, Yan H, Yuan Y. Allicin alleviates acrylamide-induced oxidative stress in BRL-3A cells. Life Sci 2019; 231:116550.

4. Ibrahim MA, Ibrahem MD. Acrylamide-induced hematotoxicity, oxidative stress, and DNA damage in liver, kidney, and brain of catfish (Clarias gariepinus). Environ Toxicol 2020;35(2):300-8.

5. Das AB, Srivastav PP. Acrylamide in snack foods. Toxicol Mech Methods 2012;22(3):163-9.

6. Santhanasabapathy R, Vasudevan S, Anupriya K, Pabitha R, Sudhandiran G. Farnesol quells oxidative stress, reactive gliosis and inflammation during acrylamide-induced neurotoxicity: Behavioral and biochemical evidence. Neuroscience 2015; 308:212-27.

7. Sumizawa T, Igisu H. Apoptosis induced by acrylamide in SH-SY5Y cells. Arch Toxicol 2007;81(4):279-82.

8. Gumral N, Saygin M, Asci H, Uguz AC, Celik O, Doguc DK, Savas HB, Comlekci S. The effects of electromagnetic radiation (2450 MHz wireless devices) on the heart and blood tissue: role of melatonin. Bratisl Med J 2016;117(11):665–71.

9. Savas HB, Sayar E. Oxidant Antioxidant Balance and Trace Elements In Children with Functional Dyspepsia. Turkiye Klinikleri J Med Sci 2021;41(1):70-9.

10. Savas HB, Sayar E, Kara T. Thiol Disulfide Balance Oxidative Stress and Paraoxonase 1 Activities in Children and Adolescents Aged 6-16 Years with Specific Learning Disorders. Electron J Gen Med 2021;18(3): em290.

11. Savran M, Asci H, Ozmen O, Erzurumlu Y, Savas HB, Sonmez Y, Sahin Y. Melatonin protects the heart and endothelium against high fructose corn syrup consumption- induced cardiovascular toxicity via SIRT-1 signaling. Hum Exp Toxicol 2019;38(10):1212-23.

12. Savran M, Aslankoc R, Ozmen O, Erzurumlu Y, Savas HB, Temel EN, Kosar PA, Boztepe S. Agomelatine could prevent brain and cerebellum injury against LPS- induced neuroinflammation in rats. Cytokine 2020; 127:154957.

13. Asci H, Ozmen O, Erzurumlu Y, Savas HB, Temel EN, Icten P, Hasseyid N. Ameliorative effects of pregabalin on LPS induced endothelial and cardiac toxicity. Biotech Histochem 2020; 14:1-12.

14. D. Ribatti, "The chick embryo chorioallantoic membrane (CAM) assay," Reprod. Toxicol 2017;70:97-101.

15. Nowak-Sliwinska P, Segura T, Iruela-Arispe ML. The chicken chorioallantoic membrane model in biology, medicine and bioengineering. Angiogenesis 2014;17(4):779-804.

16. Kadawathagedara M, Tong ACH, Heude B, Forhan A, Charles MA, Sirot V, Botton J, The Eden Mother-Child Cohort Study Group. Dietary acrylamide intake during pregnancy and anthropometry at birth in the French EDEN mother-child cohort study. Environ Res 2016; 149:189-96.

17. Karahan O, Yavuz C, Demirtas S, Caliskan A, Atahan E. The investigation of the antiangiogenic potential of amiodarone HCl in the chick embryo chorioallantoic membrane model. Biomedical Research 2013; 24:131-4.

18. Demirtas S, Caliskan A, Guclu O, Yazici S, Karahan O, Yavuz C, Mavitas B. Can calcium dobesilate be used safely for peripheral microvasculopathies that require neoangiogenesis? Med Sci Monit Basic Res 2013; 19:253-7.

19. Aksoy F, Aydın Bas H, A Bagcı, Savas HB. Predictive value of oxidant and antioxidant status for contrast-induced nephropathy after percutaneous coronary intervention for ST-segment elevation myocardial infarction. Rev Port Cardiol (Engl Ed) 2021;40(7):489-97.

20. Savas HB, Gultekin F, Ciris İM. Positive effects of meal frequency and calorie restriction on antioxidant systems in rats. North Clin Istanbul 2017;4(2):109–16.

21. Cankara FN, Özmen Ö, Savaş HB, Şahin Y, Günaydın C. Gastroprotective effect of tarantula cubensis extract in the indomethacin-induced peptic ulcer model in rats. Acta Med Alanya 2020;4(3):278-84.

22. Ralevic V, Aberdeen JA, Burnstock G. Acrylamide-induced autonomic neuropathy of rat mesenteric vessels: histological and pharmacological studies. J Auton Nerv Syst 1991;34(1):77-87.

23. Nurullahoğlu-Atalık E, Okudan N, Belviranlı M, Esen H, Yener Y, Oznurlu Y. Acrylamide-treatment and responses to phenylephrine and potassium in rat aorta. Acta Physiol Hung 2012;99(4):420-9.

24. Lin WD, Ou CC, Hsiao SH, Chang CH, Tsai FJ, Liao JW, Chen YT. Effects of Acrylamide-Induced Vasorelaxation and Neuromuscular Blockage: A Rodent Study Toxics 2021;9(6):117.

25. Yu D, Xie X, Qiao B, Ge W, Gong L, Luo D, Zhang D, Li Y, Yang B, Kuang H. Gestational exposure to acrylamide inhibits mouse placental development in vivo. J Hazard Mater 2019; 367:160-70.

26. Huang M, Zhu F, Jiao J, Wang J, Zhang Y. Exposure to acrylamide disrupts cardiomyocyte interactions during ventricular morphogenesis in zebrafish embryos. Sci Total Environ 2019; 656:1337-45.

27. El-Sayyad HI, El-Gammal HL, Habak LA, Abdel-Galil HM, Fernando A, Gaur RL, Ouhtit A. Structural and ultrastructural evidence of neurotoxic effects of fried potato chips on rat postnatal development. Nutrition 2011;27(10):1066-75.

28. Zhou C, Shi Q, Liu J, Huang S, Yang C, Xiong B. Effect of Inhibiting Tumor Angiogenesis After Embolization in the Treatment of HCC with Apatinib-Loaded p(N-Isopropyl-Acrylamide-co-Butyl Methyl Acrylate) Temperature-Sensitive Nanogel. J Hepatocell Carcinoma 2020;7:447-56.

29. Ahmadvand D, Rahbarizadeh F, Jafari Iri-Sofla F, Namazi G, Khaleghi S, Geramizadeh B, Pasalar P, Karimi H, Aghaee Bakhtiari SH. Inhibition of angiogenesis by recombinant VEGF receptor fragments. Lab Med 2010;41:417–22.

30. Li C, Shan Y, Sun Y, Si R, Liang L, Pan X, Wang B, Zhang J. Discovery of novel anti-angiogenesis agents. Part 7: Multitarget inhibitors of VEGFR-2, TIE-2 and EphB4. Eur J Med Chem 2017;141:506-18.

31. Zhou C, Shi Q, Liu J, Huang S, Yang C, Xiong B. Effect of Inhibiting Tumor Angiogenesis After Embolization in the Treatment of HCC with Apatinib-Loaded p(N-Isopropyl-Acrylamide-co-Butyl Methyl Acrylate) Temperature-Sensitive Nanogel. J Hepatocell Carcinoma 2020;7:447-56.