International Journal of Medical Toxicology & Legal Medicine **Volume 27, No. 4S, 2024**
e-ISSN: 0974-4614
p-ISSN: 0972-0448
https://doi.org/10.47059/ijmtlm/V27I4S/078

The protective effect of Cerium dioxide against Acrylamide Toxicity in mature rat

Mohammed A. Qathee[1], Salema Lafta Hassan[1]

[1]Department of Pathology and Poultry Diseases, College of Veterinary Medicine, University of Baghdad,Iraq.
Email: mddazz84@gmail.com
*Corresponding Author

Received: 15.09.2024 Revised: 10.10.2024 Accepted: 22.11.2024

ABSTRACT
The current experiment was designed to investigate changes in rat intestine after Acrylamide (ACR) and Cerium dioxide (Ceo2) administration to investigate the protective properties of Ceo2. In this experiment 120 mature male rat are used were divided randomly for 6 groups, 20 for each. And they were consecutively: ACR (8 mg/kg), ACR +Ceo2 (8 and 35mg/kg), ACR +Ceo2 (8 and 15mg/kg),Ceo2(35mg/kg), Ceo2(15mg/kg), and D.W. group.finally, the intestinal samples were collected for biochemical, histopathological and immunohistochemistry investigations. The result shows significant elevation in Malondialdehyde (MDA) and depletion of glutathione (GSH). administration of ceo2 lead to significant improving biochemical, histopathological and immunohistochemistry results.Conclusion, the result of this study show the ameliorative effect of ceo2 against ACR toxic effect via excellent improving of biochemical, histopathological and immunohistochemical parameters.

Keywords: Productive effect; Cerium dioxide; Acrylamide toxicity

INTRODUCTION

Acrylamide (ACR) is a nitrogenous organic chemical with the molecular formula C3H5NO. It is white, odorless, crystalline, and solid in appearance, soluble in water, alcohol, acetone, benzene, and other organic solvents (Tripathi & Singh, 2023). It has been used in the production of dyes, inks, sewage treatment chemicals, fiber treatment agents, soil conditioners, paper, and textiles (Dawood et al.2023), (Gaines, 2023). One of the sources is through processing food by frying, roasting, or baking (Das et al.2023).which formed from the Maillard reaction, driven by a combination of carbohydrates and free asparagine found mainly in plant-based foods such as potatoes and coffee. (Rifai & Saleh, 2020)Acrylamide and its metabolite glycidamide have a direct cytotoxic effect (Reshmitha & Nisha, 2021).(ACR) classified as a probable human carcinogen, and upon oral exposure, it is known to exert exceedingly harmful effects on liver and kidney structure and function (Koszucka et al.2020). Acrylamidecaninduce severe damage in the intestine, because itis penetrate the intestinal lining and distribute to systemic circulation, crossing into the body tissues and cells, due to these reasons, passive absorption may lead to local and systemic toxic effects (Idris et al.2021).The small intestine is therefore the site of potential for pathologically evaluating the negative effects of accumulated dietary acrylamide in workers who handle industrial chemicals that contain acrylamide (Pandey et al.2021). The systemic manifestation of the pathological effects of acrylamide intoxication includes symptoms such as neurological deficits and changes in body weight (Vojdani & Vojdani, 2021). The adverse health effects of acrylamide, based on the induction of oxidative stress (Zhao et al., 2022). Oxidative stress is defined as a disbalance between pro-oxidants and antioxidants; such disbalance can lead to oxidative damage to lipids, proteins, and DNA (Abbas & Jawad.2023), (Demirci-Cekic etal.2022).Malondialdehyde (MDA) is a major biomarker known to be involved in tissue damage arising from free radicals generated during oxidative stress (Abdulla & Al-Okaily, 2022), (Cordiano et al.2023).It is also frequently referred to as a major end product of lipid peroxidation caused by reactive oxygen species and reactive nitrogen species and is known to induce cellular damage (Ahmed & Mohammed,2022), (Murphy et al.2022).Cellular defense systems, such as glutathione, are severely disrupted when the host is being attacked by acrylamide (Golubkova et al.2023). Glutathione(GSH) is a naturally occurring cellular peptide that can transform the reactive oxygen and nitrogen species into nontoxic products (Vašková et al., 2023)

Cerium oxide (Ceo2) also known as ceria, is a rare earth metal oxide that has gained increased attention in recent decades because of its unique properties and wide application (Jairam et al.2023).Cerium oxide is

typically found in the form of a white to pale yellow solid with a cubic fluorite structure. Cerium oxide has excellent stability due to its high-temperature phase transitions, especially when characterized as an oxygen storage material (Sivakumar et al.2022), (Taghizadeh et al.2020). It is well known as an antioxidant that exhibits remarkable activity by providing or receiving electrons from biologically active free radicals, thereby increasing its oxidative state (Gulcin, 2020).cerium oxide catalytic ability to switch between Ce^{3+} and Ce^{4+}, CeO_2 contributes to improving the shift of the redox chemical reactions by consuming and releasing the extra oxidative radicals during its passage through the gastrointestinal tract (Zhang et al.2022).This phenomenon is likely to enhance protective mechanisms against reactive oxygen species (ROS) in a two-fold manner: direct free radical inactivation, and indirect contribution to the fat digestion process, which is crucial to decrease the amount of lipids absorbed in the gut and thereby lower the chances of new free radicals being generated (Singh, 2022).

MATERIALS AND METHODS

This experimental study utilized 120 mature, four-month-old Albino male rats, with body weights ranging from 220 to 240 grams. The duration of chronic toxicity is normally (100) days. Daily mature treatment was administered via gavage feeding. Acrylamide from CDH in India was dissolved in distilled water, in contrast cerium oxide from HC in Canada was used. This research was conducted at the University of Baghdad's College of Veterinary Medicine and received approval from the Ethical Committee, specifically via reference P.G/2197.

Experimental Design

Table 1: The experiment groups and type of treatment.

group	Type of treatment	Dose per mg/kg	Animals no.	Time of treatment
1	ACR	8	20	100 days
2	ACR +Ceo2	8+35	20	
3	ACR +Ceo2	8+15	20	
4	Ceo2	35	20	
5	Ceo2	15	20	
6	D.W.		20	

At last the animals are euthanized and through abdomen incision, intestine is removed directly for biochemical and histopathological examination (Alshumary et al. 2024)

Biochemical analysis

Intestinal samples were collected. After collection, samples were weighed and homogenized in ice-cold normal saline at a ratio of 1:9. The homogenates were centrifuged at 10,000 rpm for 10 minutes, and the supernatants were collected. Malondialdehyde (MDA) levels in tissue samples were determined using the Rat MDA ELISA kit and glutathione (GSH) was determined using the GSH ELISA Kit, all kits obtained from SUNLOG company.

Histopathological procedure

The specimens were subjected to a tap water wash, then fixation with neutral buffer formalin followed by automated processing. The process involves of incrementally increasing the alcoholic concentration from (70% to 100%) in stages to removed water from the tissues. Xylol was then used for tissue clearance. The specimens were then treated with semi-liquid paraffin wax at 58 degrees Celsius in two-step procedure. In summary, specimens were fabricated in block form using paraffin wax, and each tissue was sectioned with a 5µm uniform thickness using a. microtome. All tissue samples underwent Hematoxylin and Eosin (H&E) to all staining, and histopathological changes were examined using a light to microscope, in accordance with (Sabaawy and Al-Kaisie, 2021).

Immunohistochemistry technique

At first deparaffinization by xylene about (15) minutes and rehydration of sections through descending graded of alcohol started by absolute ethanol, 95%,70%, distilled water for (5min.) for each step. Then blocking endogenous peroxidase by using 1.5 % H_2o_2 for (10 min.) followed with immersion in distilled water.Buffer Immersions immersed of Sections in PBS (5 min) then retrieval solution (citric buffer (10 min.) at (100°C), distilled water (5 min) and PBS (5 min). followed by primary antibody blocking and secondary antibody application and finally enzyme application. (Abdel-Abbas and Hassan.2022).

Statistical analysis

Data analysis by using computer statistical program SPSS and sigma stat program. Tow way analysis variance was used $p \leq 0.05$ (wade,.2005).

RESULTS

Malondialdehyde (MDA)

Variable resultsare obtained according type of treatment and dose amount.significant differences in levelof MDA between the various groups. The first group show the highest MDA levels in intestinal tissues. The second group exhibit a marked reduction in MDA levels. While the third group it was found elevation in MDA level, but significantly reducing in this levels in both of fourth and fifth groups **table 2.**

Glutathione (GSH)

Total intensity of the glutathione (GIT) measurements reveal a significant progression among different groups. In the first (Acrylamide treated) group significant diminish in GSH level, in second group the level elevated but decline was observed in third group. While fourth and fifth (variable cerium dosing) groups show improving in GSH levels**Table 2.**

Histopathological finding

There are potential toxicological signs in first group which acrylamide treated consisting moderate inflammatory cells infiltration with goblet cell proliferation in addition of villi elongated and necrosis also appear (fig.1A). while in second group the decline in inflammatory signs. third group show mild inflammatory call infiltration with congestion. The fourth and fifth groups was without pathological finding.

Immunohistochemistry finding

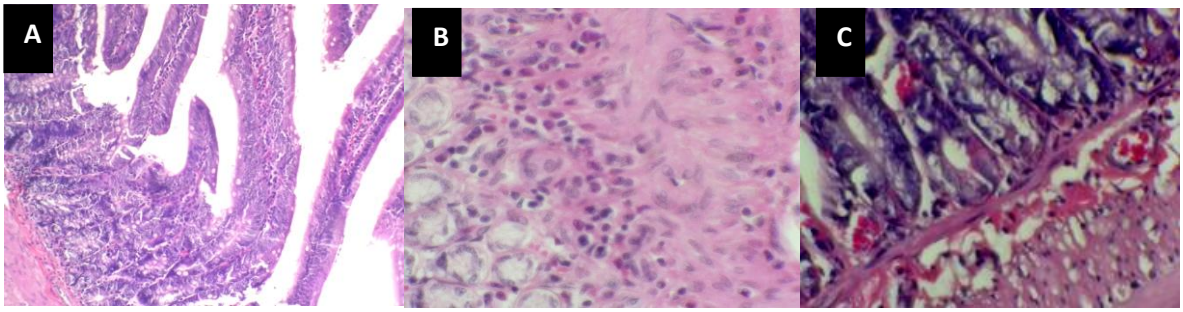
The expression significantly affected by type of treatment of each group**table 3.** First group show high expression of BAX and BCL2 protein in GIT tissue with strong reaction while in second group show significant decline in both proteins but third expression was more than second group. The expression fourth and fifth groups was non-significant when compare with control group.

Table 2: Intestinal oxidative stress levels.

	Mean±SD	
Group	**MDA**	**GSH**
Acrylamide	51.025±1.774 A	53.537±0.912 D
Acrylamide + Cerium oxide 35 mg	41.859±0.859 B	77.241±1.602 C
Acrylamide + Cerium oxide 15 mg	45.833±1.626 AB	69.556±2.214 C
Cerium oxide	25.192±1.283 C	96.037±4.608 A
Cerium oxide	29.551±2.339 C	86.225±2.486 B
Control	29.038±2.329 C	102.612±2.152 A

Table 3: Expression of BAX and BCL2 in intestine.

	Mean±SD	
Group	**BAX**	**BCL2**
Acrylamide	3.26±0.19 A/a	3.25±0.21 A/a
Acrylamide + Cerium oxide 35 mg	0.66±0.04 A/c	0.90±0.10 A/c
Acrylamide + Cerium oxide 15 mg	1.78±0.03 A/b	1.85±0.12 B/b
Cerium oxide	0.16±0.04 A/d	0.17±0.03 A/d
Cerium oxide	0.16±0.02 A/d	0.16±0.02 A/d
Control	0.16±0.04 A/d	0.15±0.03 A/d



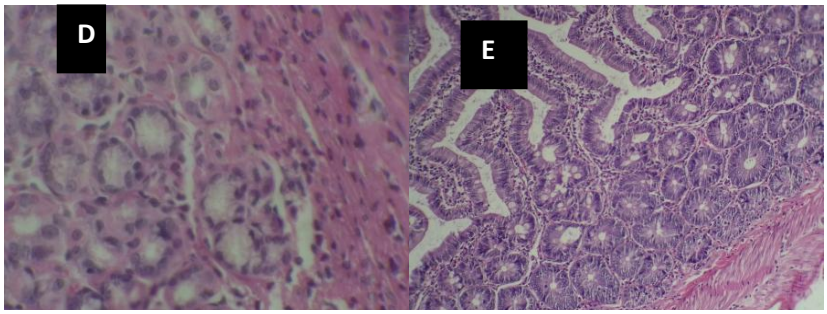


Figure1 A: Histopathological section the intestine of mature rat administration with acrylamide. (8 mg/kg B.W.) showing elongated of villi with moderate inflammatory cells infiltration in the lamina properia (H&E stain X100, B: Histopathological section in the intestine of mature rat administration with of ACR LD50 8 mg/kg B.W.) and Cerium dioxide 35mg/kg B.W.) showing moderate infiltration of mononuclear s cellular in the lamina propria and muscular layers .(H&E stain X400), C: :Histopathological section in the intestine of mature rat administration with ACR LD50(8mg/kg B.W.) and ***Cerium dioxide*** 15 mg/kg B.W.) showing congestion of blood vessels with mild inflammatory cells infiltration between mucous glands and vacculaion of muscular layers (H&E stain X400),D :no clear pathological changes,E: mild infiltration of inflammatory cells.

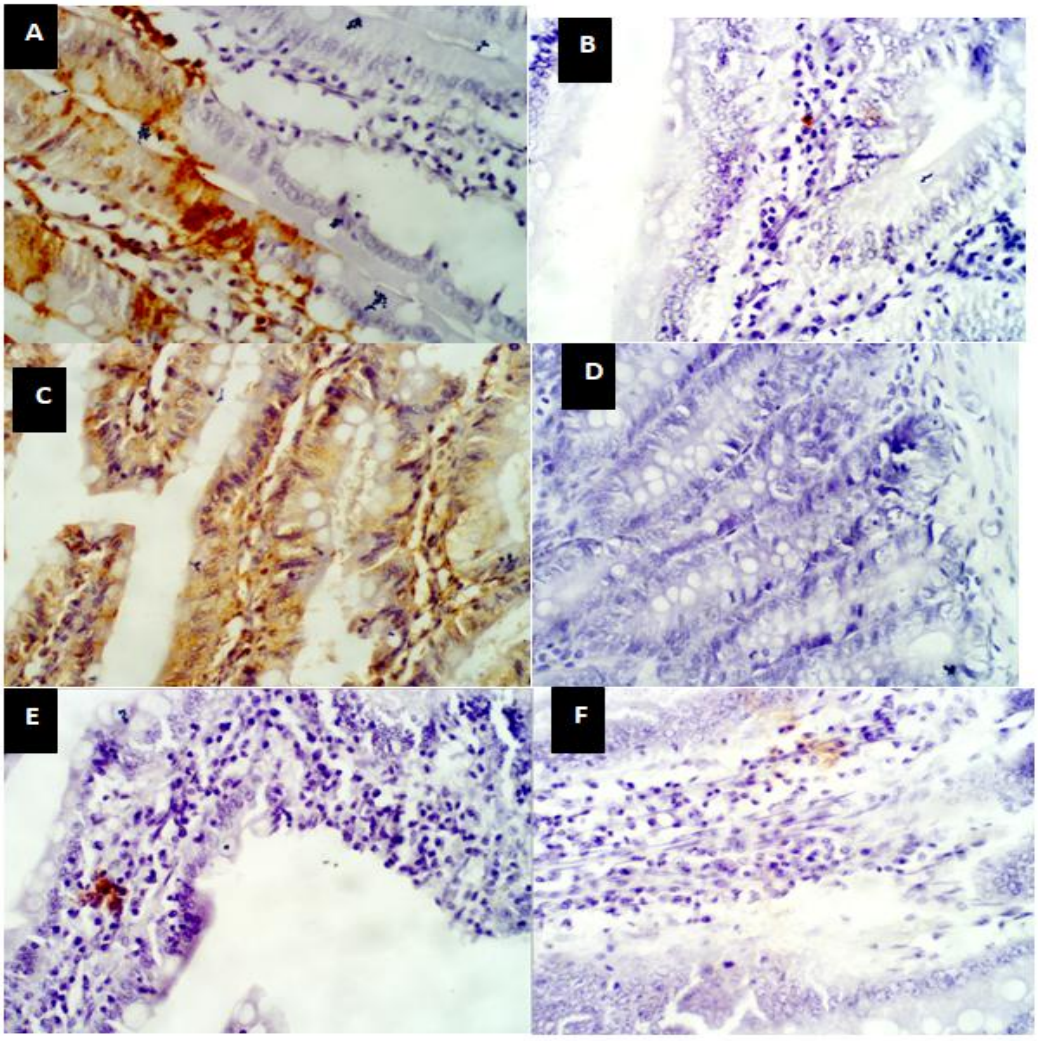


Figure 2: A: small intestine, Acrylamides group, Mature. BAX strong positive reaction which appears as cytoplasmic golden-brown patches in cells. IHC-BAX. 400x, B: small intestine, Acrylamides + antioxidant 35 mg group, Immature. BAX weak positive reaction which appears as cytoplasmic golden-brown granules. IHC-BAX. 400x.,C: small intestine: Acrylamides + antioxidant 15 mg group, Immature. BAX positive reaction which appears as cytoplasmic golden-brown granules in cells. IHC-BAX. 400x., D: small intestine, Antioxidant 35 mg group, Immature. BAX negative reaction. IHC-BAX. 400x., E: small intestine, Antioxidant 15 mg group,

Immature. BAX very weak positive reaction which appears as cytoplasmic golden-brown patch in in cells. IHC-BAX. 400x, F: small intestine, Duodenum, Control group, Mature. BAX negative reaction. IHC-BAX. 400x.

DISCUSSION

The experimental focus was on the gastrointestinal tract as one of the main targets regarding acrylamide distribution in whole animals.In addition to the harmful effects of acrylamide on intestinal tissues, it was also evaluated that it has threatening effects on the health of living organisms because it causes disturbances in the regulatory mechanisms in the cells (Albiach-Delgado et al.2022).In 1st group the main outcome show significant increasing in MDA level and decrease in GSH comparing with negative control group and this concurring with (Çelik et al., 2022) how find that (ACR) long term exposure lead to elevation of MDA. And (Firouzabadi et al.2022) who conclude the (ACR) treated group exhibit significant decline in in glutathione levels. That ensuring the pro oxidant effect of acrylamide. and oxidative stress can be enhancing with diminishing GSH level(Hasan,2019). Meanwhile 2ed and 3rd groups exhibit significant decreasing in MDA level and increasing in GSH level when comparing with 1st group. This investigation agreement with(Gallucci et al.2021). who mentioned the reducing and antioxidative ability of CeO2 NPs has been of considerable interest to be favorite solutions to minimize oxidative stress. Other paper mentioned when reduced, cerium oxide is partially reduced, giving it a capacity to both release and retain oxygen (Lord et al., 2021). this was parallel with our result in 4th and 5th groups which show elevation in GSH companied with MDA diminish level. Inflammatory response can be ignition due to (ACR) toxicity (Kopańska et al.2022) and this investigation was agreement with our pathological finding through all (ACR) exposure groups when comparing with control,4th and 5th groups. The increased number of inflammatory infiltrates indicates increased damage to cells by a toxic substance (Tschöpe et al.2021). This may be due to cytotoxic, reactive oxygen species production and disruption of cell metabolism (Koszucka et al.2020). furthermore, epidemiology research has suggested that the intestine is a complex site of chemical metabolism and signaling (Sun et al.2022). All of this investigation was agreement with our finding. In 4th and 5th groups section show with no pathological changes and this agreement with (Zhao et al.2020). Who find the prophylactic and therapeutic potentials of CeO resulted in the recovery of all deteriorations of the intestine and enabled improvement of the induced inflammatory bowel disease. The expression level of BAX to BCL2 is the main potential critical factor that leads to apoptosis (Qian et al.2022). BAX and BCL2 expression was affected by daily treatment of (ACR) (Adebayo et al.2020). and this enhance our investigation. In 2ed and 3rd groups show increase of BCL2expresion and decrease of BAX expression. This investigation was agreement with (Amiri et al.2021). Who find The treatment of cerium dioxide alone elevate expression of BCL2,but 4th and 5th groups show the ameliorative effect of cerium dioxide and was agree with (Ifijen&Omonmhenleb, 2023). Who investigate ability of cerium dioxide to mitigate apoptosis and cell damage.

CONCLUSION

Our findings revealed acrylamide's adverse impacts on gut health, leading to compromised integrity and functionality.Cerium oxide demonstrated effectiveness through its anti-inflammatory, anti-apoptotic, and antioxidant properties. Promoting cerium oxide in diets can improve gut functionality and resistance.

REFERENCE

1. Abbasa, M. I., & Jawad, Z. J. (2023). Hepatoprotective Effect of Alcoholic Extract of Ficus carica Leaves Against Cypermethrin-Induced Liver Toxicity in Male Albino Rats. The Iraqi Journal of Veterinary Medicine, 47(2), 64-72.
2. Abdel-Abbas, E., & Hassan, S. L. (2022). Immunohistochemistry Assay of Sodium Nitrate in White Mice Protected with Alpha Lipoic Acid. HIV Nursing, 22(2), 2096-2101.
3. Abdulla, J. M., & Al-Okaily, B. N. (2022). Histomorphometric and histopathological alterations of rat testis following exposure to hydrogen peroxide: Protective role of resveratrol supplement. The Iraqi Journal of Veterinary Medicine, 46(1), 17-23.
4. Aghetaa, H. F. K., Dawood, R. A., &Aladhami, A. K. (2023). Resveratrol Administration Ameliorates Hepatotoxicity in Mercuric Chloride- Induced Liver Injury in Rats. The Iraqi Journal of Veterinary Medicine, 47(2), 1-8.
5. Ahmed, R. M., & Mohammed, A. K. (2022). Role of sodium butyrate supplement on reducing hepatotoxicity induced by lead acetate in rats. The Iraqi Journal of Veterinary Medicine, 46(2), 29-35.
6. Albiach-Delgado, A., Esteve-Turrillas, F. A., Fernández, S. F., Garlito, B., & Pardo, O. (2022). Review of the state of the art of acrylamide human biomonitoring. Chemosphere, 295, 133880.
7. Al-Sabaawy, H. B., & Al-Kaisie, B. I. (2021). Histological effects of chronic sodium fluoride toxicity on some reproductive organs of male and female adult albino rats. Iraqi Journal of Veterinary Sciences, 35(4), 705-711.
8. Alshumary, H. O., Jumma, Q. S., Khorsheed, H. H., &AlKaisi, B. I. (2024). Assessment of the toxic effect of environmental pollution by 2, 3, 7, 8-Tetrachlorodibenzo-p-dioxin (TCDD) on the female reproductive

system by pathological and biochemical assay in Albino female rats. Egyptian Journal of Veterinary Sciences, 55(5), 1409-1415.
9. Amiri, G., Gholami, M., Assadollahi, V., Nemati, A., Fathi, F., Rostami, T., ... &Alasvand, M. (2021). Effect of cerium oxide nanoparticles on the expression of developmental and apoptosis genes of testicular tissue in 6-day-old NMRI mice fetuses. Biological trace element research, 1-10.
10. Çelik, R. G. G., Toprak, U. E., & Kıran, S. (2022). Neurotoxicological Effects of Environmental and Occupational Agents. Turkish Journal of Neurology.
11. Cordiano, R., Di Gioacchino, M., Mangifesta, R., Panzera, C., Gangemi, S., &Minciullo, P. L. (2023). Malondialdehyde as a potential oxidative stress marker for allergy-oriented diseases: an update. Molecules, 28(16), 5979.
12. Das, P. P., Duarah, P., & Purkait, M. K. (2023). Fundamentals of food roasting process. In High-Temperature Processing of Food Products (pp. 103-130). Woodhead Publishing.
13. Demirci-Cekic, S., Özkan, G., Avan, A. N., Uzunboy, S., Çapanoğlu, E., & Apak, R. (2022). Biomarkers of oxidative stress and antioxidant defense. Journal of pharmaceutical and biomedical analysis, 209, 114477.
14. Firouzabadi, A. M., Imani, M., Zakizadeh, F., Ghaderi, N., Zare, F., Yadegari, M., ... &Fesahat, F. (2022). Evaluating effect of acrylamide and ascorbic acid on oxidative stress and apoptosis in ovarian tissue of wistar rat. Toxicology Reports, 9, 1580-1585.
15. Gaines, L. G. T. (2023). Historical and current usage of per-and polyfluoroalkyl substances (PFAS): A literature review. American Journal of Industrial Medicine.
16. Gallucci, N., Vitiello, G., Di Girolamo, R., Imbimbo, P., Monti, D. M., Tarallo, O., ... & Paduano, L. (2021). Towards the development of antioxidant cerium oxide nanoparticles for biomedical applications: Controlling the properties by tuning synthesis conditions. Nanomaterials, 11(2), 542.
17. Golubkova, A., Leiva, T., Snyder, K., Schlegel, C., Bonvicino, S. M., Agbaga, M. P., ... & Hunter, C. J. (2023). Response of the glutathione (GSH) antioxidant defense system to oxidative injury in necrotizing enterocolitis. Antioxidants, 12(7), 1385.
18. Gulcin, İ (2020). Antioxidants and antioxidant methods: An updated overview. Archives of toxicology.
19. Hassan, S. L. (2019). Toxic pathological changes on albino mice after exposures to cypermethrin. Indian Journal of Natural Sciences, 9(52).
20. Idris, A. O., Alabi, Q. K., Ologe, M. F., Oluogun, W. A., Akanbi, M. H. J., &Iwalewa, E. O. (2021). Evaluation of acrylamide exposure in pregnant wistar rats as a risk of developing renal disease in their litters. Environmental Science and Pollution Research, 28, 39680-39691.
21. Ifijen, I., &Omonmhenleb, S. I. (2023). The Impact of Cerium Oxide Nanoparticles on Reactive Oxygen Species (ROS) Release Rate in Mice Organs. Algerian journal of Biosciences, 4(1), 026-044.
22. Jairam, L. S., Chandrashekar, A., Prabhu, T. N., Kotha, S. B., Girish, M. S., Devraj, I. M., ... & Prashantha, K. (2023). A review on biomedical and dental applications of cerium oxide Nanoparticles—Unearthing the potential of this rare earth metal. Journal of Rare Earths, 41(11), 1645-1661.
23. Kopańska, M., Łagowska, A., Kuduk, B., &Banaś-Ząbczyk, A. (2022). Acrylamide neurotoxicity as a possible factor responsible for inflammation in the cholinergic nervous system. International Journal of Molecular Sciences, 23(4), 2030.
24. Koszucka, A., Nowak, A., Nowak, I., & Motyl, I. (2020). Acrylamide in human diet, its metabolism, toxicity, inactivation and the associated European Union legal regulations in food industry. Critical reviews in food science and nutrition, 60(10), 1677-1692.
25. Lord, M. S., Berret, J. F., Singh, S., Vinu, A., &Karakoti, A. S. (2021). Redox active cerium oxide nanoparticles: current status and burning issues. Small, 17(51), 2102342.
26. Murphy, M. P., Bayir, H., Belousov, V., Chang, C. J., Davies, K. J., Davies, M. J., ... & Halliwell, B. (2022). Guidelines for measuring reactive oxygen species and oxidative damage in cells and in vivo. Nature metabolism, 4(6), 651-662.
27. Pandey, M., Choudhury, H., D/O Segar Singh, S. K., Chetty Annan, N., Bhattamisra, S. K., Gorain, B., & Mohd Amin, M. C. I. (2021). Budesonide-loaded pectin/polyacrylamide hydrogel for sustained delivery: Fabrication, characterization and in vitro release kinetics. Molecules, 26(9), 2704.
28. Qian, S., Wei, Z., Yang, W., Huang, J., Yang, Y., & Wang, J. (2022). The role of BCL-2 family proteins in regulating apoptosis and cancer therapy. Frontiers in oncology, 12, 985363.
29. Reshmitha, T. R. & Nisha, P. (2021). Lycopene mitigates acrylamide and glycidamide induced cellular toxicity via oxidative stress modulation in HepG2 cells. Journal of Functional Foods.
30. Rifai, L. & Saleh, F. A. (2020). A review on acrylamide in food: Occurrence, toxicity, and mitigation strategies. International journal of toxicology.
31. Singh, D. (2022). Juggling with reactive oxygen species and antioxidant defense system–A coping mechanism under salt stress. Plant Stress.

32. Sivakumar, A., Ramya, S., Dhas, S. S. J., Almansour, A. I., Kumar, R. S., Arumugam, N., ... &Dhas, S. M. B. (2022). Assessment of crystallographic and electronic phase stability of shock wave loaded cubic cerium oxide nanoparticles. Ceramics International, 48(2), 1963-1968.
33. Sun, J., Fang, R., Wang, H., Xu, D. X., Yang, J., Huang, X., ... & Huang, Y. (2022). A review of environmental metabolism disrupting chemicals and effect biomarkers associating disease risks: Where exposomics meets metabolomics. Environment International, 158, 106941. sciencedirect.com
34. Taghizadeh, A., Taghizadeh, M., Azimi, M., Alsagri, A. S., Alrobaian, A. A., & Afrand, M. (2020). Influence of cerium oxide nanoparticles on thermal conductivity of antifreeze: preparation and stability of nanofluid using surfactant. Journal of Thermal Analysis and Calorimetry, 139(1), 225-236.
35. Tripathi, I. & Singh, S. K. (2023). Safety Aspects of Fried Foods with Reference to Acrylamide and Other Polar Compounds. Frying Technology.
36. Tschöpe, C., Ammirati, E., Bozkurt, B., Caforio, A. L., Cooper, L. T., Felix, S. B., ... & Van Linthout, S. (2021). Myocarditis and inflammatory cardiomyopathy: current evidence and future directions. Nature Reviews Cardiology, 18(3), 169-193.
37. Vašková, J., Kočan, L., Vaško, L., &Perjési, P. (2023). Glutathione-related enzymes and proteins: a review. Molecules, 28(3), 1447.
38. Vojdani, A. & Vojdani, E. (2021). The role of exposomes in the pathophysiology of autoimmune diseases I: toxic chemicals and food. Pathophysiology.
39. Wade AL. A handbook of statistical analyses using SPSS. Boca Raton: Wiley; 2005. 36-77 p.
40. Zhang, Y., Zhao, S., Feng, J., Song, S., Shi, W., Wang, D., & Zhang, H. (2021). Unraveling the physical chemistry and materials science of CeO2-based nanostructures. Chem, 7(8), 2022-2059.
41. Zhao, M., Zhang, B., & Deng, L. (2022). The mechanism of acrylamide-induced neurotoxicity: current status and future perspectives. Frontiers in Nutrition.
42. Zhao, S., Li, Y., Liu, Q., Li, S., Cheng, Y., Cheng, C., ... & Wei, H. (2020). An orally administered CeO2@ montmorillonite nanozyme targets inflammation for inflammatory bowel disease therapy. Advanced Functional Materials, 30(45), 2004692.