Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.

Therapeutic role of methanolic extract of pomegranate flower on tissues of albino rats dosed with cadmium sulphide nanoparticles

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Article info

Received 04.08.2025
Received in revised form 02.09.2025
Accepted 30.09.2025

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Al-Obaidi, F. J., Rashied, R. M., Jasim, R. A., AlRawi, M. S., Ramizy, A., & Almehemdi, A. F. (2025). Therapeutic role of methanolic extract of pomegranate flower on tissues of albino rats dosed with cadmium sulphide nanoparticles. Regulatory Mechanisms in Biosystems, 16(3), e25160. doi:10.15421/0225160

Cadmium (Cd) is a hazardous toxin to humans and a major source of pollution in industrial and environmental fields. Compared with cadmium sulfide (CdS), cadmium is more toxic. Pomegranate plants are known for their antioxidant properties and potential medicinal uses due to their high levels of phenols and other compounds. This study was conducted to confirm the therapeutic activity of pomegranate flowers against cadmium sulfide poisoning. White rats were used as experimental subjects by analyzing tissue sections from multiple organs of animals after treatment with metabolite extracts of pomegranate flowers and organs of untreated animals. Pomegranate flowers were collected from several locations, and the methanolic preparations were analyzed using gas chromatography with electron impact mass spectrometry (GC-MS). Electron microscopy was used to evaluate the physical properties of the laboratory-generated nano-cadmium sulfide solution. Five groups of white rats were assigned to the experiment. These groups were given 0.00, 0.025, 0.05 and 0.10 mg extract/kg + 0.1 μg CdS/mL, and just 0.1 μg CdS/mL. These treatments were applied.intraperitoneally Nano-cadmium sulfide doses were administered to the rats regularly. Rats were given concentrated extract of pomegranate flower after 30 days. Three days later, the rats were dissected, and the organs – liver, kidney, spleen and heart – were taken out for laboratory analysis. The study concluded that methanol extracts of pomegranate flowers reduced tissue toxicity, especially at high doses of 0.05 and 0.1 mg extract/kg. The heart responded better to this treatment than other studied organ tissues. Therefore, nanotoxicity of nano-cadmium sulfide was significantly reduced in albino rats by methanolic extract of pomegranate flowers.

Keywords: albino rat; cadmium sulfide; histology; nanoparticle; pomegranate.

Introduction

Nanoparticles (NPs) are increasingly used by medical researchers due to their special properties, small sizes, large surface areas, and, most importantly, their ability to transport substances (Altammar et al., 2023). Nanoparticles have made single-cell diagnosis easier and have completed difficult tasks such as cancer diagnosis and blood vessel imaging (Rahim et al., 2023). The abovementioned researcher claimed that such cells could be used to treat neurological disorders and cancer due to their ability to transfer genes. As a result, recent advancements in NP synthesis technology over the last ten years have increased the use of NPs, not only opening up new therapeutic paths (Varmazyari et al., 2020), but also serving as potential game-changers in this field. Despite those possible advancements, a thorough investigation on their toxicity has not yet been completed. Due to its inherent abundance in the Earth's crust, the heavy metal cadmium (Cd) has been recognized as one of the major drivers of industrial and environmental contamination (Jadaa & Mohammed, 2023). This component is found in many dietary products, which has led to extensive research on its toxicity in both human and animal models (Alalwan et al., 2020). Cadmium sulphide (CdS), an impurity found in zinc ore that contains cadmium, is the element's inorganic state. Pure Cd metal is more hazardous than CdS, according to Regmi et al. (2023). CdS is a common colorant used in many industrial items. Its application has also produced unique light-emitting semiconductor nanocrystals. The effectiveness of CdS NPs as a drug delivery mechanism was examined by researchers (Ghasempour et al., 2023). The toxicity of CdS particles in a biological system is directly correlated with their size, according to (Xuan et al., 2023), indicating that expanding an NP's functional area may in fact increase the risk of toxicity (Al-Obaidi et al., 2023).

This poses a significant problem to our field of study. In the current era of nanoparticle synthesis, the increasing need for a reliable, nontoxic, clean, and environmentally acceptable experimental methods is overwhelming. One synthesis technique that does fit the aforementioned criteria is the use of natural processes, such as fermentation.

Pomegranates (*Punica granatum*) have been utilized for a very long time. Pomegranate flowers are used as astringents or antidiarrheal medications in traditional Chinese medicine (Wei et al., 2022). Pomegranate blossoms contain a variety of secondary metabolites, mostly polyphenols with strong antioxidant properties such as gallic acid, ellagic acid, and ethyl brevifolincarboxylate (Ökmen et al., 2023). Furthermore, pomegranate juice contains higher levels of vitamin E, β-carotene, and ascorbic acid than green tea, and it is an old fruit that is grown in many areas. Pomegranates have strong health benefits and can help avoid a number of conditions, such as cancer, inflammatory illnesses, arthritis, Alzheimer's disease, hyperglycaemia, hypertension, and hyperlipidaemia. Additionally, pomegranate extract promotes wound healing, has antibacterial, antimalarial, and dental antiplaque properties, and works well for severe acute respiratory syndrome coronavirus-2 infections (Aziz et al., 2020).

The fruit can be eaten raw or as a salad dressing, jam, or juice, and it is helpful in treating hyperglycaemia and dyslipidaemia (Wang et al., 2020). Due to its high phytochemical content, pomegranates are used as valuable ingredients in cooking, cosmetics, and health goods. If 0.50% of dried pomegranate peel extracts were added to other liquids, like orange or tomato juice, the quantity of antioxidants present would be increased (Karwasra et al., 2011). Furthermore, pomegranate fruit is one of the essential components used to make pigment and ink (Kulkarni et al., 2019). Pomegranate peel can be used to dye cotton textiles naturally (Sudha et al., 2021). Pomegranate fruit has been used as a reducing agent in the production of NPs. There are no negative environmental impacts associated with NP synthesis from pomegranates or other low-capacity plants. Therefore, "green synthesis" refers to the production of NPs from plant extracts (Farhan et al., 2022). Pomegranate has been used to remove metals, trash, and hazardous materials from natural ecosystems (Celiksoy et al., 2020). In several varieties, including those from Israel, Turkey, Tunisia, and Iran, the peel of the pomegranate fruit contains more polyphenol phytochemicals than the arils, seeds, juice, leaves, and flowers (Tito et al., 2021). The identification of metallothionein (MT) as a cadmium protein from horse kidney, which came about as a result of the quest for a biological function for Cd (II), started a new line of inquiry into the potential role of MT in the detoxification of certain metal ions. Cadmium MT is discussed here for another reason: Cd (II) increases the stability of the protein when it is introduced during the heterologous synthesis of recombinant MT proteins or when it is used to induce MT in animals. Exposure to Cd significantly boosted hepatic MT (by 3.9 times), but it had no effect on interstitial cell MT translation.

Cadmium-containing nanomaterials have been created recently for use in consumer goods, engineering, biology, and medicine. Toxicologists now face additional difficulties as a result of the development and commercial application of these cadmium-containing nanoparticles (NPs). When nanoparticles enter the human body, they may be carried to secondary main sites of interaction by a variety of mechanisms. A comparison of the toxicity of nanoparticles and their micro size counterparts demonstrates that nanoparticles have a higher potential for toxicity (Awashra & Młynarz, 2023). Thereby, the aim of this study is to determine whether pomegranate flower extract influences the histology of albino rats that have been injected with nano-CdS in any way.

Materials and methods

A fresh sample of *Punica granatum* L. var Rawah flower was collected at the flowering stage on July 15, 2023, from a garden at the University of Anbar in Anbar, Iraq (33°24′24.10″ N, 43°15′44.89″ E, and 56 m above sea level, Fig. 1). The plants were removed completely, placed in polyethylene bags, and sent directly to the laboratory. Prof. Dr Mohammed Othman Mosa of the University of Anbar's Centre of Desert Studies subsequently classified them. The plantlist website was used to compare this specimen (http://ipni.org/urn:lsid:ipni.org:names:554129-1).

The phytochemical profiles of the methanolic pomegranate extract were investigated using gas chromatography in conjunction with electron impact mass spectrometry (GC-MS). An autoinjector and a 5-millisecond capillary column of 30 by 0.25 mm, holding film with a thickness of 0.25 μm, were features of a Shimadzu GC-MS-QP2010 plus device (Kyoto, Japan). The helium gas was used as the carrier gas, and its flow rate was 1.15 mL/min. The mass spectroscopic analysis was conducted using an ionizing instrument set to 70 eV. The temperature was gradually increased to 280 °C over the course of five minutes, at a rate of 10 °C per minute, after being maintained at 80 °C for two minutes. The sample was injected in split mode at 250 °C. Retention lengths and mass spectrum data were used to identify the recovered bioactive compounds (Hooks et al., 2021).

It was possible to obtain commercial nano-CdS powder with a diameter of less than 100 nm. Using an ESM microscope, the material's nanoscale characteristics were investigated. To avoid molecule aggregation and maintain the material's nature following the application of additional organic solvents, the powder was dissolved in distilled deionized water using an ultrasonic probe (Ghasempour et al., 2023).

Twenty-five mature male Sprague-Dawley rats (*Rattus norvagegicus*; male albino rats) were used in this study. The rats weighed 200–250 g and were 12–14 weeks old. A specialist physician inspected them after they were raised at the animal house unit of the Department of Biological Sciences, College of Education for Pure Sciences, University of Anbar. They were then put in plastic cages measuring 64 x 31 x 19 cm and covered with metal. The experimental animals were exposed to the proper laboratory settings, which included 11-hour light/13-hour dark cycles and a temperature of 225 °C. We regularly cleaned and sterilized the cages, keeping them in an area with good ventilation. The sawdust was changed every day, and the trial rats had fed and watered twice a day. Those rats were guaranteed to be free of any illnesses by giving them two weeks to get used to the new surroundings.

Fig. 1. Pomegranate used in the study

The proportion of the feed components was as follows; wheat – 43.4%, yellow maize – 35.0%, soybeans – 20.0%; 10% was made up of concentrated protein; 1% was made up of dry milk powder, which also contained trace levels of preservatives and antifungal substances. Throughout our 30-day research project, we continuously supplied sufficient water and feed without disruptions or shortages.

The experimental animals.were randomly assigned to five groups. The second group was treated with CdS of 0.1 g/mL (4cc), whereas the control group was given a normal diet and 0.5 mL/kg of 0.9% normal saline solution via tube feeding. A two-week dosage of CdS (4 cc) and a second two-week dosage of a 0.025 mg/L pomegranate alcoholic extract (5 cc) were administered to the third group. After receiving a two-week dose of CdS(4 cc), the fourth group received a two-week dose of the alcoholic extract at a concentration of 0.05 mg/L (5 cc). The fifth group received doses of the CdS (4 cc) and 0.1mg/L alcoholic extract (5 cc) for two weeks.

The rats were not given any treatment for two days following the conclusion of the allotted time for each group. They were weighed and euthanized by chloroform anaesthesia on day three. Following their dissection, tissues from the liver, kidney, spleen, and heart were gathered and stored in a 10% formalin solution. The tissue sections were prepared in accordance with the methods described by Bancroft & Gambl (2008).

SPSS version 25.0 (IBM Corp., USA) was used for all statistical tests. Any P-value with two tails less than 0.05 was deemed statistically significant. The Mann-Whitney U-test was used to evaluate non-parametric variables, and the independent t-test was used to compare parametric variables. The Chi-square test was used to assess categorical data. Through binary logistic regression, odds ratios (ORs) and 95% confidence intervals (CIs) were determined. PTPRC's diagnostic performance was assessed through the use of receiver operating characteristic (ROC) curve analysis.

Results

While the liver tissue of the rats in two groups displayed alterations, the liver tissue of the control rats in group A and the rats given 0.05% alcoholic extract displayed normal morphology (Fig. 2). Group B exhibited considerable changes. The hepatocyte in the perivascular cuffing showed acute cell swelling (black arrow), while the tissue showed lipid changes (red arrow) and inflammatory cell infiltration (blue arrow). Histological imaging of the E group showed histological alterations, such as a pyknotic nucleus (black arrow).

The histological appearance of the spleen tissue sections in groups A, D, and E was normal (Fig. 4). Compared to group A, groups B and C displayed hyperplasia of lymphoid follicles (black arrow). The group exhibited hemosiderin-laden cells (blue arrow), red pulp blood sinusoids engorged with blood cells (red arrow), and white pulp depletion (black arrows).

Figure 6 reveals the morphology of the lab-prepared cadmium sulphide investigated using a silicon substrate-based field emission scanning electron microscope at a high magnification. The pictures showed diffusion throughout the entire base and good homogeneity among the samples. We used ImageJ to determine the regular Gaussian distribution of semi spherical NPs, which showed a particle diameter of 40–60 nm. The thin-film morphology is crucial as a catalyst for electrochemical processes in biological applications.

Atomic force microscopy can provide a complete description of the surface topography of the thin films that are formed (Fig. 7a). The three-dimensional photographs of the surface topography were used to specify the dimensions, as shown in Figure 7. The result showed how important is the surface roughness factor (24.16 nm), which represented surface quality and grain growth. It also acts as a cell-wall snatcher, which allows nanotechnologies to affect living cells. It could be determined that 17.97 nm was the ideal outcome after computing the maximum height, which represents the pace of nanoparticle formation. The horizontal dimensions of the surface were calculated using the three-dimensional photos. With 2θ values of 28.3610°, 26.9620°, and 44.1840°, respectively, Figure 7b shows three main peaks that represent crystal planes (002), (100), and (103). These were all linked to the composition of CdS films based on the numbered card (JCPDS No. 41-1049).

Phytochemical analysis of the pomegranate fruit extract of the 'Rawah' variety using gas chromatography-mass spectrometry (GC-MS) revealed the presence of a diverse range of compounds. Ricinolic acid was the most abundant compound, recording the highest surface area of 44.55%, indicating that it is the major component of the extract. 5-Hydroxymethylfurfural was also identified in significant quantities at 17.08%. Among the other detected compounds, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one was the third most abundant at 7.23%. In addition, compounds belonging to various chemical groups were found, including fatty acid esters such as methyl palmitate, methyl oleate, and methyl stearate, as well as compounds from the aldehyde, amine, alcohol, lactone, and other families. The analysis also indicated the presence of one compound for which no match was found within the database used.

Discussion

In the present study, the action of CdS in the liver may be responsible for this observation, although other mechanisms were identified for the caused hepatotoxicity. Importantly, exposure of essential organelles such as mitochondria, microsomes, and peroxisomes increased lipid peroxidation and free radical production. Cadmium, a nonredox metal, indirectly harms the liver through oxidative stress because it reduces cellular antioxidant levels, especially those of glutathione and protein-bound sulfhydryl groups, which encourage the production of reactive oxygen species (ROS) like superoxide ions, hydroxyl radicals, and hydrogen peroxide (Ren et al., 2003; Rana et al., 2018). The antioxidant properties of methanolic extract of pomegranate flowers, which lessened tissue damage from exposure to cadmium sulfide NP and free radical-induced oxidative stress, was what caused the ameliorative effect (Smaoui et al., 2019; Campos et al., 2022) as Groups C and D showed normal histology.

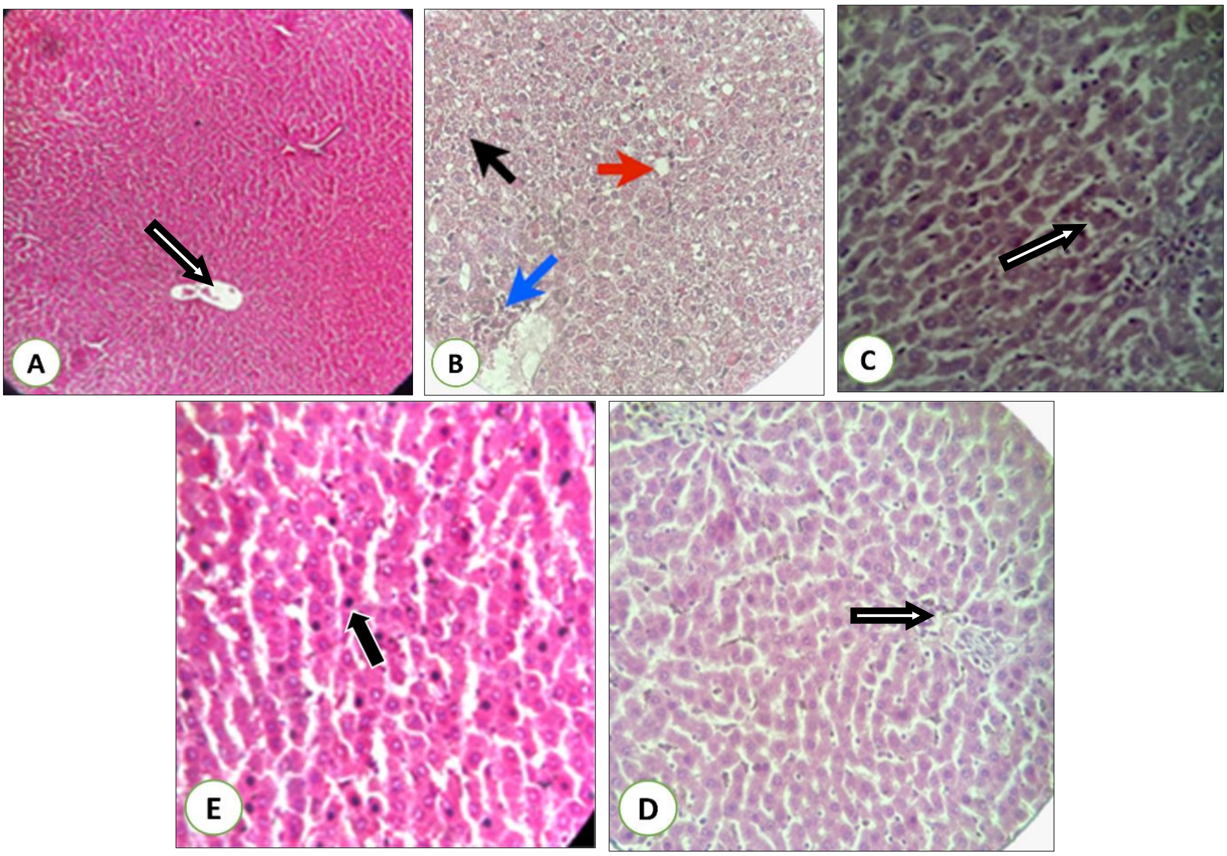

Fig. 2. Section of liver tissue of control group rat showing normal histological appearance (*A*, hematoxylin and eosin $^{X}400$); section of liver tissue of rat exposed to CdS (*B*), showing hepatocyte with acute cell swelling (black arrow), fatty changes (red arrow) and inflammatory cells infiltration (blue arrow, hematoxylin and eosin $^{X}400$); section of liver tissue of rat exposed to 0.025 alcoholic extract, showing normal histological appearance (*C*, hematoxylin and eosin $^{X}400$); section of liver tissue of rat exposed to 0.05 alcoholic extract showing normal histological appearance (*D*, hematoxylin and eosin $^{X}400$); section of liver tissue of rat exposed to 0. 1 alcoholic extract showing mild histological changes including pyknotic nucleus (black arrow) (*E*, hematoxylin and eosin $^{X}400$)

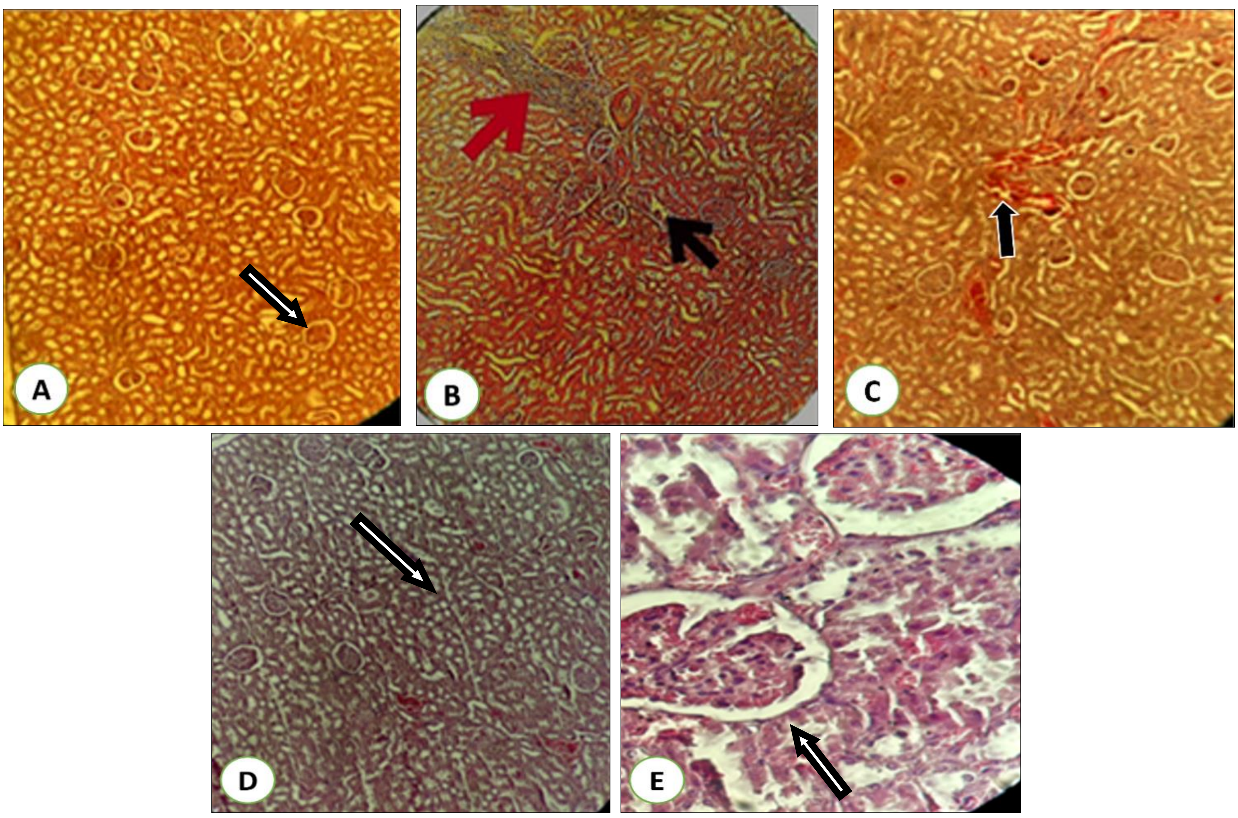

Fig. 3. Kidney of control group rat showing normal histological appearance (*A*, hematoxylin and eosin ×400); kidney of T group rat showing blood vessels congestion (black arrow) and severe inflammatory cells infiltration (red arrow) (*B*, hematoxylin and eosin ×400); kidney of rat exposed to 0.025 alcoholic extract showing blood vessels congestion (black arrow) (*C*, hematoxylin and eosin ×400); kidney of rat exposed to 0.05 alcoholic extract showing normal histological appearance (*D*, hematoxylin and eosin ×400); kidney of rat exposed to 0.1 alcoholic extract showing normal histological appearance (*E*, hematoxylin and eosin ×400)

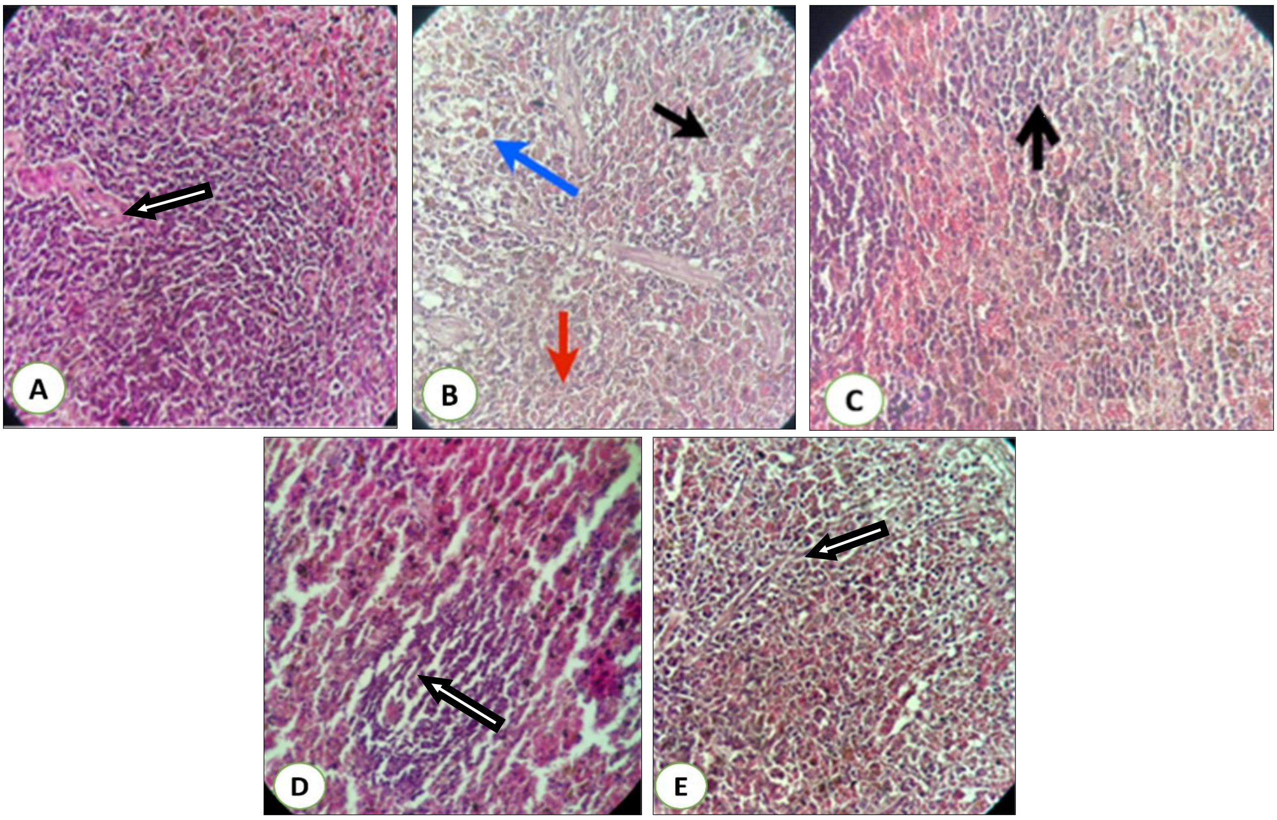

Fig. 4. Spleen of control group rat showing normal histological appearance (*A*, hematoxylin and eosin ×400) ; spleen of T group rat showing white pulp depletion (black arrows) and red pulp blood sinusoids engorged with blood cells (red arrow) and presence of hemosiderin laden cell (blue arrow) (*B*, hematoxylin and eosin ×400); spleen of rat exposed to 0.025 alcoholic extract showing lymphoid follicles hyperplasia (black arrow) (*C*, hematoxylin and eosin ×400); spleen of rat exposed to 0.05 alcoholic extract showing normal histology (*D*, hematoxylin and eosin ×400); spleen, of rat exposed to 0.1alcoholic extract showing no pathological changes (*E*, hematoxylin and eosin ×400)

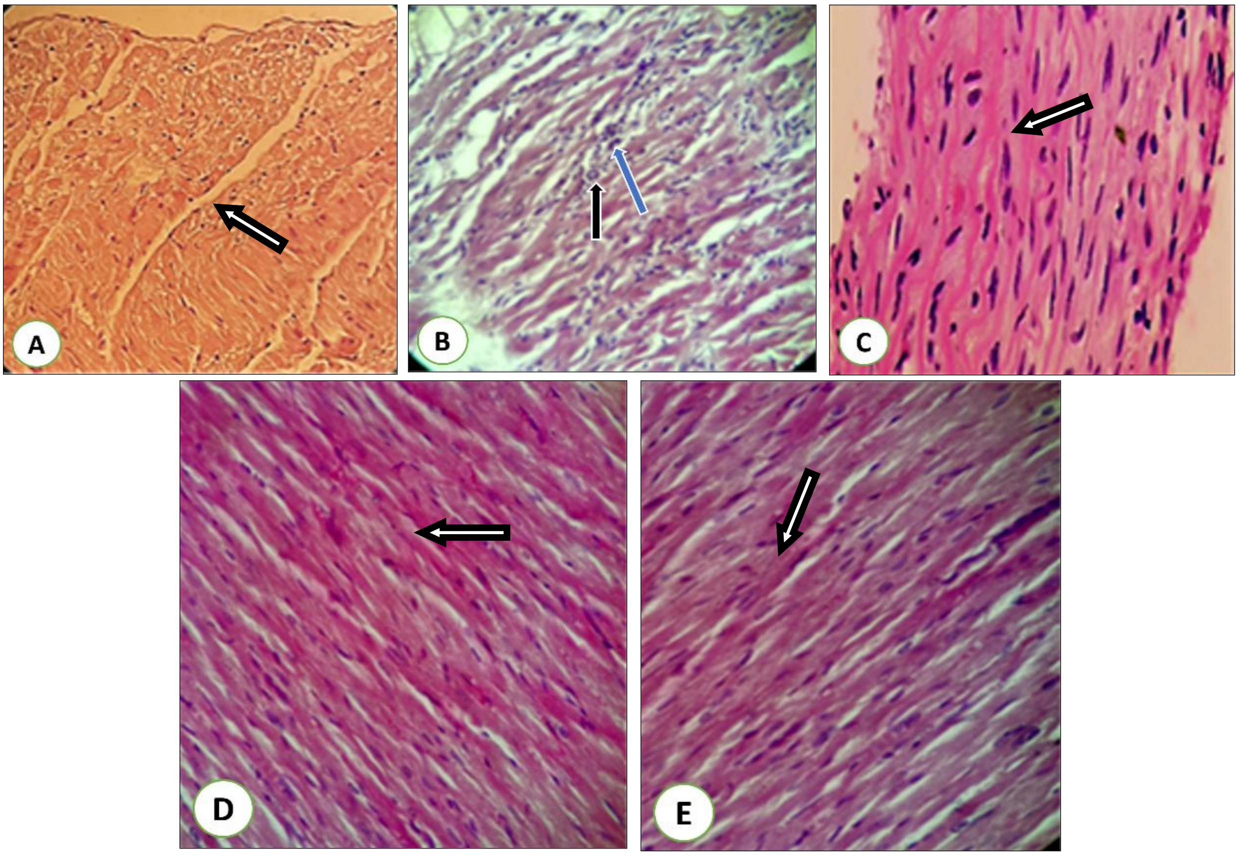

Fig. 5. Heart of control group rat showing normal histological appearance (*A*, hematoxylin and eosin ×400); heart of CdS group rat showing myocardial cells necrosis (black arrow) with acute inflammatory cells infiltration (blue arrow) (*B*, hematoxylin and eosin ×400); heart of rat exposed to 0.025 alcoholic extract showing normal histological appearance (*C*, hematoxylin and eosin ×400); heart of rat exposed to 0.05 alcoholic extract showing normal histological appearance (*D*, hematoxylin and eosin ×400); heart of rat exposed to 0.1 alcoholic extract showing normal histological appeariance (*E*, hematoxylin and eosin ×400)

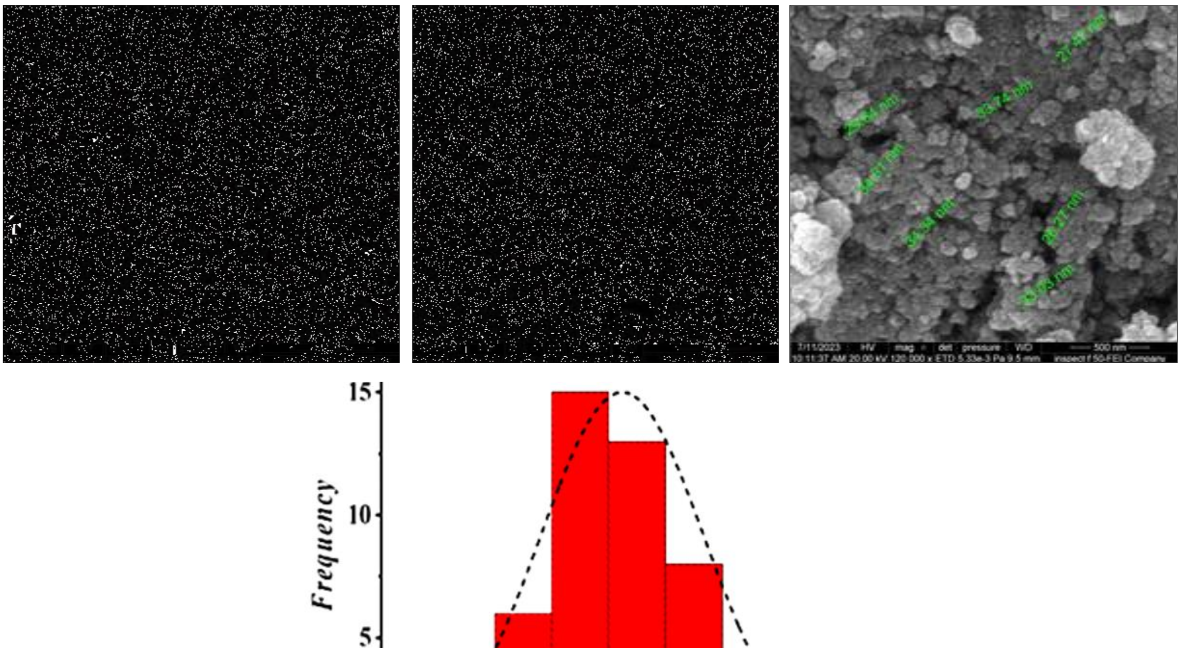

Fig. 6. Shows field emission scanning electron microscopy (FESEM) pictures showing the topographic surface features of the produced nanoparticles at different magnifications with histogram of the particle size distribution together

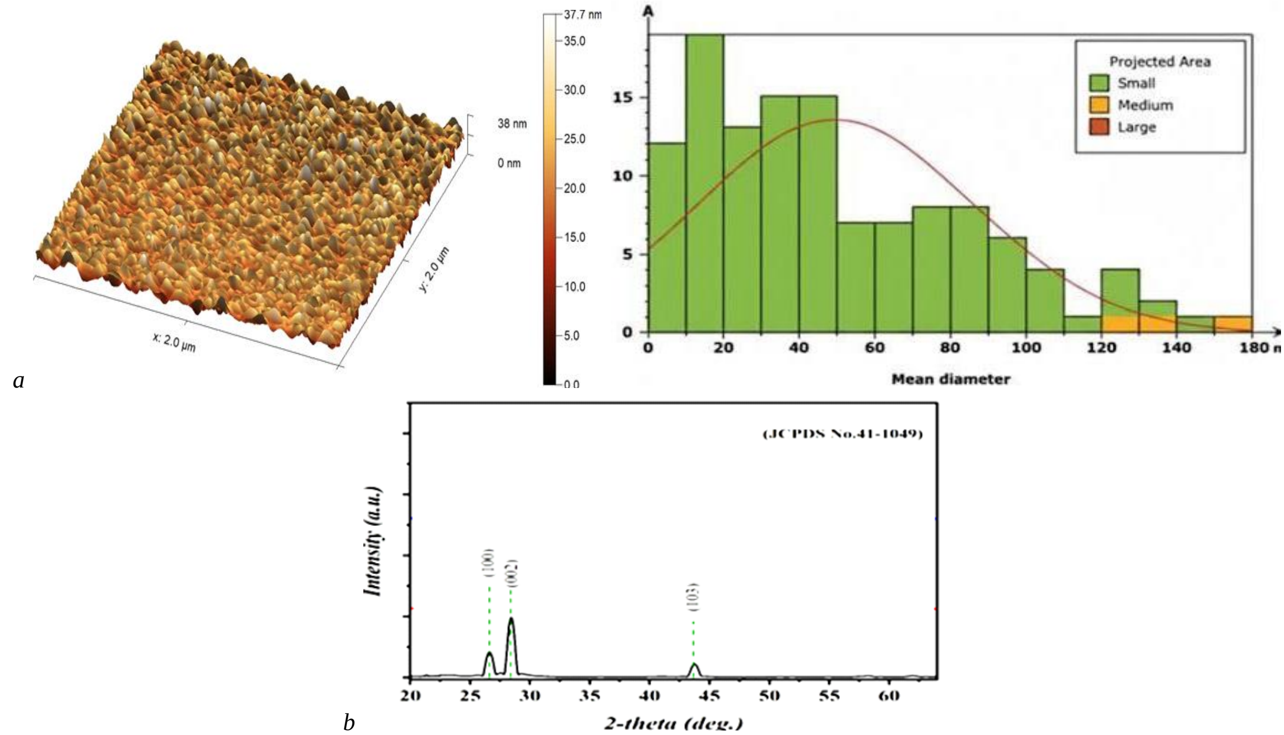

Fig. 7. Atomic force microscopy (AFM) image of cadmium sulfide (CdS) nanoparticles (*a*) displaying their particle size and surface distribution and the synthetic CdS nanoparticles' (*b*) crystalline structure is confirmed by the X-ray diffraction (XRD) pattern

Table 1
Phytochemical analysis of pomegranate (*Punica granatum* L.)

Pk#	RT	Area	Identity	Group
1	4.236	2.92	Y-butyrolactone	lactone
2	4.972	2.05	5-methylfurfural	furan
3	5.266	1.35	methyl glyoxylate	methyl ester
4	6.054	1.03	2,5-di-tert-butylhydroquinone	hydroquinone
5	6.348	1.45	4-heptanol	alcohol
6	6.988	3.24	N-cyclohexyl-3,4-methylenedioxyamphetamine	amine
7	7.534	1.19	N-ethylpropionamide	amide
8	8.140	7.23	2,3-dihydro-3,5-dihydroxy-6-methyl-4h-pyran-4-one	pyrone
9	8.780	1.15	pyrrolidine-2-thione	pyrroline
10	9.360	1.22	succinaldehyde	aldehyde
11	9.923	17.08	5-hydroxymethylfurfural	furan
12	11.117	0.84	no match found	–
13	11.342	0.86	triacetin	triglyceride
14	14.371	0.87	carbonyldiamide	amide
15	19.893	2.57	methyl palmitate	fatty acid ester
16	20.395	1.36	heptyl vinyl sulfide	alkane
17	21.997	7.11	methyl oleate	fatty acid ester
18	22.317	1.02	methyl stearate	fatty acid ester
19	24.230	44.55	ricinoleic acid	hydroxy fatty acid
20	26.662	0.91	cis-aconitic anhydride	carboxylic acid

CdS promotes reactive oxygen species (ROS), such as hydrogen peroxide, superoxide ions, and hydroxyl radicals, by smashing glutathione and protein-bound sulfhydryl groups. Because of that, CdS is an especially dangerous environmental pollutant (Ghasempour et al., 2023). It appears that pomegranate therapy can boost antioxidant enzymes, which can reduce oxidative stress and preserve kidney tissues (Polat et al., 2024).

In spleen tissue, it is possible that the proliferation of lymphoid follicles encouraged the death of cells exposed to CdS. Infarction, inflammation, or damage can all cause fibrosis inside the spleen (Sharma et al., 2014). Pomegranates can decrease blood and tissue levels of cadmium by blocking the absorption of lead from the gastrointestinal tract and lowering the amount of cadmium that is retained in metabolism because they are high in polyphenolic components, such as tannins, flavonoids, alkaloids, and organic acids (Smaoui et al., 2019).

In cardiac histology, the increased production of reactive oxygen species could be contributing factors in exposure to CdS (Veličkov et al., 2013). Since pomegranate extracts are rich in potent antioxidants, the groups that received them showed improvement. Pomegranate can be considered an antioxidant quality; pomegranate fruit is one of the primary medicinal plants used to cure a wide range of illnesses; pomegranate antioxidants are made up of a wide range of polyphenols, they have a broader range of activity against various types of free radicals than vitamin E, β-carotene, and ascorbic acid. These fruits contain phenolic compounds that can function as hydrogen donors and bind transition metal pro-oxidants like iron and copper. Low-density lipoprotein oxidation can be inhibited by phenolic compounds (Gil et al., 2000; Singh et al., 2002; Lazeeza, 2021).

Overall, the data indicate that an unusual hydrogen bonding network may exist, either involving the OH group from Ser residues, which may contribute to steric hindrance of SH groups due to their near proximity, or involving the SH group directly (Mahmood & Jabar, 2023; Siddiqu et al., 2024). Despite its decreased susceptibility to alkylation, literature data shows that thionine is easily oxidized, including DTNB, which is utilized to quantify thiol/thiolate group content (Tumbarski et al., 2025). Metallothionein has two domains (α/β-domains) that help capture heavy metals like cadmium (Yang et al., 2024).

Conclusion

The high quantities of pomegranate xtract dramatically decreased the nanotoxicity of cadmium sulphide in albino rats. Compared to other examined organs, the heart responded more favourably to these extracts. The findings of the GC-MAS analysis, which are aforementioned, demonstrated the existence of antioxidant chemicals that effectively reduce the severe damage brought on by exposure to the nano-cadmium utilized in this investigation. Some phenolic compound that is found in methanolic extract of pomegranate could be such a preventor of cadmium toxicity as 2,5-di-tert-butylhydroquinone and cis-aconitic anhydride.

References

Alalwan, H. A., Kadhom, M. A., & Alminshid, A. H. (2020). Removal of heavy metals from wastewater using agricultural byproducts. Journal of Water Supply: Research and Technology-Aqua, 69(2), 99–112.

Al-Obaidi, F. J., AlRawi, M. S., Ramizy, A., Almehemdi, A. F., & Thaker, A. A. (2024). Phytochemical, molecular docking and expressing the *ALAD* gene protected via *Moringa* extract against nano lead in rat blood. In: Obaid, A. J., Al-Heety, E. A., Radwan, N., & Polkowski, Z. (Eds.). Advanced studies on environmental sustainability. Springer Nature Switzerland, Cham. Pp. 205–218.

Altammar, K. A. (2023). A review on nanoparticles: Characteristics, synthesis, applications, and challenges. Frontiers in Microbiology, 14, 1155622.

Awashra, M., & Młynarz, P. (2023). The toxicity of nanoparticles and their interaction with cells: An *in vitro* metabolomic perspective. Nanoscale Advances, 5(10), 2674–2723.

Aziz, Z., Huin, W. K., Hisham, M. D. B., & Ng, J. X. (2020). Effects of pomegranate on lipid profiles: A systematic review of randomised controlled trials. Complementary Therapies in Medicine, 48, 102236.

Bancroft, J. D., & Gamble, M. (Eds.). (2008). Theory and practice of histological techniques. Churchill Livingstone.

Campos, L., Seixas, L., Henriques, M. H. F., Peres, A. M., & Veloso, A. C. A. (2022). Pomegranate peels and seeds as a source of phenolic compounds: Effect of cultivar, by-product, and extraction solvent. International Journal of Food Science, 2022, 9189575.

Celiksoy, V., Moses, R. L., Sloan, A. J., Moseley, R., & Heard, C. M. (2020). Evaluation of the *in vitro* oral wound healing effects of pomegranate (*Punica granatum*) rind extract and punicalagin, in combination with Zn (II). Biomolecules, 10(9), 1234.

Farhan, M., Rizvi, A., Ali, F., Ahmad, A., Aatif, M., Malik, A., Alam, M. W., Muteeb, G., Ahmad, S., Noor, A., & Siddiqui, F. A. (2022). Pomegranate juice anthocyanidins induce cell death in human cancer cells by mobilizing intracellular copper ions and producing reactive oxygen species. Frontiers in Oncology, 12, 998346.

Ghasempour, A., Dehghan, H., Ataee, M., Chen, B., Zhao, Z., Sedighi, M., Guo, X., & Shahbazi, M.-A. (2023). Cadmium sulfide nanoparticles: Preparation, characterization, and biomedical applications. Molecules, 28(9), 3857.

Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., Holcroft, D. M., & Kader, A. A. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. Journal of Agricultural and Food Chemistry, 48(10), 4581–4589.

Hooks, T., Niu, G., Masabni, J., Sun, Y., & Ganjegunte, G. (2021). Performance and phytochemical content of 22 pomegranate (*Punica granatum*) varieties. HortScience, 56(2), 217–225.

Jadaa, W., & Mohammed, H. (2023). Heavy metals – definition, natural and anthropogenic sources of releasing into ecosystems, toxicity, and removal methods – an overview study. Journal of Ecological Engineering, 24(6), 249–271.

Karwasra, R., Singh, S., Sharma, D., Sharma, S., Sharma, N., & Khanna, K. (2019). Pomegranate supplementation attenuates inflammation, joint dysfunction via inhibition of NF-κB signaling pathway in experimental models of rheumatoid arthritis. Journal of Food Biochemistry, 43(8), e12959.

Kulkarni, S. S., Gokhale, A. V., Bodake, U. M., & Pathade, G. R. (2011). Cotton dyeing with natural dye extracted from pomegranate (*Punica granatum*) peel. Universal Journal of Environmental Research and Technology, 1(2), 135–139.

Lazeeza, S. O. (2021). Antioxidant activity of pomegranate. Iraqi Journal of Agricultural Sciences, 52(1), 196–203.

Mahmood, A., & Jabar, H. (2023). Characterization of biochemical compounds in different accessions of pomegranate (*Punica granatum* L.) peels in Iraq. Passer Journal of Basic and Applied Sciences, 5(2), 382–390.

Ökmen, G., Giannetto, D., Fazio, F., & Arslan, K. (2023). Investigation of pomegranate (*Punica granatum* L.) flowers' antioxidant properties and antibacterial activities against different *Staphylococcus* species associated with bovine mastitis. Veterinary Sciences, 10(6), 394.

Polat, Y., Çelik, F., & Kafkas, N. E. (2024). Evaluation possibilities of different parts of pomegranate, a historical fruit and its effects on health. BIO Web of Conferences 85, 01030.

Raheem, M. A., Rahim, M. A., Gul, I., Zhong, X., Xiao, C., Zhang, H., Wei, J., He, Q., Hassan, M., Zhang, C. Y., Yu, D., Pandey, V., Du, K., Wang, R., Han, S., Han, Y., & Qin, P. (2023). Advances in nanoparticles-based approaches in cancer theranostics. OpenNano, 12, 100152.

Rana, K., Verma, Y., Rani, V., & Rana, S. V. S. (2018). Renal toxicity of nanoparticles of cadmium sulphide in rat. Chemosphere, 193, 142–150.

Regmi, A., Basnet, Y., Bhattarai, S., & Gautam, S. K. (2023). Cadmium sulfide nanoparticles: Synthesis, characterization, and antimicrobial study. Journal of Nanomaterials, 2023, 8187000.

Ren, X.-Y. (2003). Expression of metallothionein gene at different time in testicular interstitial cells and liver of rats treated with cadmium. World Journal of Gastroenterology, 9(7), 1554.

Sharma, B., Singh, S., & Siddiqi, N. J. (2014). Biomedical implications of heavy metals induced imbalances in redox systems. BioMed Research International, 2014, 640754.

Siddiqui, S. A., Singh, S., & Nayik, G. A. (2024). Bioactive compounds from pomegranate peels – biological properties, structure–function relationships, health benefits and food applications – a comprehensive review. Journal of Functional Foods, 116, 106132.

Singh, R. P., Chidambara Murthy, K. N., & Jayaprakasha, G. K. (2001). Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using *in vitro* models. Journal of Agricultural and Food Chemistry, 50(1), 81–86.

Smaoui, S., Hlima, H. B., Mtibaa, A. C., Fourati, M., Sellem, I., Elhadef, K., Ennouri, K., & Mellouli, L. (2019). Pomegranate peel as phenolic compounds source: Advanced analytical strategies and practical use in meat products. Meat Science, 158, 107914.

Smaoui, S., Hlima, H. B., Mtibaa, A. C., Fourati, M., Sellem, I., Elhadef, K., Ennouri, K., & Mellouli, L. (2019). Pomegranate peel as phenolic compounds source: Advanced analytical strategies and practical use in meat products. Meat Science, 158, 107914.

Sudha, T., Mousa, D. S., El-Far, A. H., & Mousa, S. A. (2020). Pomegranate (*Punica granatum*) fruit extract suppresses cancer progression and tumor angiogenesis of pancreatic and colon cancer in chick chorioallantoic membrane model. Nutrition and Cancer, 73(8), 1350–1356.

Tito, A., Colantuono, A., Pirone, L., Pedone, E., Intartaglia, D., Giamundo, G., Conte, I., Vitaglione, P., & Apone, F. (2021). Pomegranate peel extract as an inhibitor of SARS-CoV-2 spike binding to human ACE2 receptor (*in vitro*): A promising source of novel antiviral drugs. Frontiers in Chemistry, 9, 638187.

Tumbarski, Y., Ivanov, I., Vrancheva, R., Mazova, N., & Nikolova, K. (2025). Pomegranate peels: A promising source of biologically active compounds with potential application in cosmetic products. Cosmetics, 12(4), 169.

Varmazyari, A., Taghizadehghalehjoughi, A., Sevim, C., Baris, O., Eser, G., Yildirim, S., Hacimuftuoglu, A., Buha, A., Wallace, D. R., Tsatsakis, A., Aschner, M., & Mezhuev, Y. (2020). Cadmium sulfide-induced toxicity in the cortex and cerebellum: *In vitro* and *in vivo* studies. Toxicology Reports, 7, 637–648.

Veličkov, A., Jančić, N., Đinđić, N., Rančić, I., Bojanić, N., & Krstić, M. (2013). Histological and histochemical characteristics of rat myocardium in cadmium toxicosis. Acta Medica Medianae, 52(2), 15–22.

Wang, P., Zhang, Q., Hou, H., Liu, Z., Wang, L., Rasekhmagham, R., Kord-Varkaneh, H., Santos, H. O., & Yao, G. (2020). The effects of pomegranate supplementation on biomarkers of inflammation and endothelial dysfunction: A meta-analysis and systematic review. Complementary Therapies in Medicine, 49, 102358.

Wei, Y., Khalaf, A. T., Ye, P., Fan, W., Su, J., Chen, W., Hu, H., Menhas, R., Wang, L., & Oglah, Z. (2022). Therapeutic benefits of pomegranate flower extract: A novel effect that reduces oxidative stress and significantly improves diastolic relaxation in hyperglycemic *in vitro* in rats. Evidence-Based Complementary and Alternative Medicine, 2022, 4158762.

Xuan, L., Ju, Z., Skonieczna, M., Zhou, P., & Huang, R. (2023). Nanoparticles-induced potential toxicity on human health: Applications, toxicity mechanisms, and evaluation models. MedComm, 4(4), e327.

Yang, R., Roshani, D., Gao, B., Li, P., & Shang, N. (2024). Metallothionein: A comprehensive review of its classification, structure, biological functions, and applications. Antioxidants, 13(7), 825.