BIU Journal of Basic and Applied Sciences 11(1): 86 – 95, 2026.

ISSN: 2563-6424

INVESTIGATION OF THE EFFECTS OF 6-GINGEROL ON OXIDATIVE STRESS AND INFLAMMATION IN RATS MODEL OF CADMIUM-INDUCED SPLENOTOXICITY

***MADUAKO, I. C.[1] AND OSAWE, S. O.[1,2]**
[1]Chemoprevention and Toxicology Laboratory, Department of Medical Biochemistry, College of Medical Sciences, Benson Idahosa University, Benin City, Nigeria
[2]Biochemistry Option, Department of Biological Sciences, Faculty of Science, Benson Idahosa University, Benin City, Nigeria.
*Corresponding author: imaduako@biu.edu.ng

ABSTRACT
Cadmium (Cd) is a toxic heavy metal and a significant environmental contaminant that causes splenotoxicity through mechanisms involving oxidative stress and inflammation. 6-Gingerol (6GR), a naturally occurring phenolic compound, exhibits potent antioxidant and anti-inflammatory activities. This study aimed to evaluate the protective effects of 6GR against Cd-induced spleen injury in rats. Sixty adult male Wistar rats were randomly allocated into six groups (n = 10 per group): Control, negative control (corn oil), 6GR 100, Cd, 6GR 50 + Cd, and 6GR 100 + Cd. Cadmium and/or 6GR were administered orally for 7 consecutive days. Biochemical markers of oxidative stress (SOD, GSH, CAT, and MDA) and pro-inflammatory indices (NO and MPO) were measured in spleen tissue homogenates. Cadmium exposure significantly decreased splenic antioxidant enzyme activities (SOD, GSH, and CAT) and increased MDA, NO levels, and MPO activity. 6GR co-administration dose-dependently ameliorated these pathological alterations, restoring oxidative balance and suppressing inflammation. These findings suggest that 6GR may be a promising splenoprotective agent in conditions of heavy-metal-induced splenic injury.

KEYWORDS: *6-Gingerol, Cadmium, Splenotoxicity, Oxidative stress, Inflammation, Antioxidant*

INTRODUCTION

Among the most hazardous non-essential heavy metals, cadmium (Cd) has emerged as a major global environmental and public health concern due to its persistence, low rate of excretion, and remarkable tendency for prolonged biological retention and preferential accumulation in soft tissues such as the liver, kidneys, and spleen (Davidova *et al*., 2024; Charkiewicz *et al*., 2023). Food and tobacco smoke are the main routes of entry into humans, while occupational exposure is highest among workers in manufacturing, construction, electroplating, and battery recycling industries due to Cd widespread use in batteries, pigments, metal coatings, and plastics (Charkiewicz *et al*., 2023; Davidova *et al*., 2024).

Cadmium disrupts cellular redox homeostasis by suppressing key

antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), while simultaneously promoting the accumulation of reactive oxygen species (ROS) and lipid peroxidation, as indicated by elevated malondialdehyde (MDA) concentrations in target tissues (Peana *et al*., 2023; Qu and Zheng, 2024). This oxidative environment is further exacerbated by a concurrent inflammatory response, in which Cd stimulates excessive nitric oxide (NO) production and markedly increases myeloperoxidase (MPO) activity a hallmark of neutrophil activation and tissue-level inflammatory injury (Charkiewicz *et al*., 2023; Davidova *et al*., 2024). The convergence of these oxidative and inflammatory mechanisms underlies the broad organotoxic potential of Cd across multiple biological systems.

The spleen, as a pivotal secondary lymphoid organ responsible for immune surveillance, erythrocyte clearance, and antibody production, represents a biologically significant yet relatively understudied target of Cd toxicity. While most studies on Cd toxicity have focused on organ and tissue damage, the immunotoxicity of Cd has recently drawn increasing attention, with evidence showing that Cd accumulates in immune cells, modulates immune system function, triggers immunological responses, and leads to diverse health problems (Xia *et al*., 2023). At the molecular level, Cd exposure induces spleen necroptosis through reactive oxygen species (ROS)-mediated activation of the signal transducer and activator of transcription 1 (STAT1) and receptor-interacting protein kinase 3 (RIPK3) signaling pathway, while cadmium-mediated cellular damage exposes nuclear factor kappa B (NF-κB) binding sites, leading to its nuclear translocation and the subsequent synthesis of pro-inflammatory proteins such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) (Li *et al*., 2025). Understanding Cd-induced splenotoxicity is therefore of considerable clinical and toxicological significance.

Natural polyphenols derived from dietary plants have emerged as a promising frontier for mitigating heavy-metal-induced cytotoxicity, with 6-gingerol (6GR), the primary pungent phenolic and main bioactive compound of fresh ginger (*Zingiber officinale)* demonstrating well-established antioxidant, anti-inflammatory, anticancer, and neuroprotective properties (Yu *et al*., 2024). At the molecular level, 6-gingerol exerts its cytoprotective effects by downregulating Janus kinase/signal transducer and activator of transcription 3 (JAK/STAT3), mitogen-activated protein kinase (MAPK), and inhibitor of kappa B alpha (IκBα) signaling pathways, and notably demonstrates favourable safety at therapeutically relevant concentrations without inducing cytotoxicity or apoptosis (Kang *et al*., 2026). These mechanistic attributes, including free radical scavenging, upregulation of antioxidant enzymes, and suppression of pro-inflammatory transcription factors, collectively justify investigating 6GR as a splenoprotective agent against cadmium-induced immunotoxicity.

Although the hepatoprotective and nephroprotective effects of 6GR have been explored in various models of chemical and heavy-metal toxicity, its potential to mitigate cadmium-induced splenotoxicity remains poorly characterized. As the spleen is a critical immunological organ, elucidating how 6GR modulates Cd-induced oxidative

stress and inflammatory responses may have important implications for both experimental toxicology and clinical medicine. Accordingly, this study systematically investigated the protective effects of 6GR at two dose levels (50 and 100 mg/kg) against cadmium-induced splenotoxicity in Wistar rats, focusing on oxidative stress biomarkers (SOD, GSH, CAT, and MDA) and pro-inflammatory mediators (NO and MPO).

MATERIALS AND METHODS

Animals and Ethical Approval

Sixty adult male Wistar rats weighing 180–220g were obtained from the Veterinary Department, University of Benin animal facility. All animals were housed under standard laboratory conditions (temperature: 22 ± 2°C; relative humidity: 55 ± 5%; 12-hour light/dark cycle) with free access to standard rat chow and water ad libitum. Animals were acclimatized for one week before the experiment began. All experimental procedures were performed in accordance with internationally accepted principles for the care and use of laboratory animals, and approval was obtained from the Institutional Animal Ethics Committee (Number LS21046).

Chemicals and Reagents

Cadmium chloride ($CdCl_2$; purity > 99%) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and reagents used in this study were of analytical grade.

Isolation of 6-Gingerol

In the current study, 6-gingerol (6GR) with a confirmed purity of > 91% was obtained by phytochemical extraction from the rhizome of *Zingiber officinale* Roscoe using established isolation protocols (Ajayi et al., 2015). Fresh ginger plants (*Zingiber officinale*) were purchased from Bodija Market, Ibadan, Nigeria, and authenticated at the University of Ibadan Herbarium (Voucher No. UIH-22390). The rhizomes were subsequently pulverized, extracted with appropriate solvent, concentrated, and purified through column chromatography and HPLC, followed by purity determination of the isolated 6-gingerol.

Experimental Design and Treatment Protocol

Following acclimatization, the sixty rats were randomly divided into six groups of ten animals each, as follows:

Group I (Control) was orally gavaged with 2 mL/kg of distilled water for 7 days.
Group II (negative control) was orally gavaged with 2 mL/kg corn oil for 7 days.
Group III (6GR) was orally gavaged with 100 mg/kg 6GR for 7 days.
Group IV (Cd) was orally gavaged with 5 mg/kg cadmium chloride for only 7 days.
Group V (6GR 50 + Cd) was orally gavaged with 50 mg/kg + 5 mg/kg cadmium chloride for 7 days.
Group VI (6GR 100 + Cd) was orally gavaged with 100 mg/kg + 5 mg/kg cadmium chloride for 7 days.

6GR was dissolved in corn oil and administered by oral gavage 30 minutes prior to cadmium chloride administration. The cadmium chloride was dissolved in distilled water and administered orally at 5 mg/kg body weight once daily. All treatments were given once daily for seven consecutive days.

Sample Collection and Tissue Preparation

Twenty-four hours after the final administration, all animals were anesthetized using ketamine/xylazine (80/10 mg/kg, i.p.) and sacrificed by cervical dislocation. The spleens were excised rapidly, and rinsed with ice-cold normal saline. A portion of each spleen

was homogenized in ice-cold phosphate-buffered saline (PBS, pH 7.4) at a ratio of 1:10 (w/v) using a tissue homogenizer. The homogenate was centrifuged at 3,000 × g for 15 minutes at 4°C, and the resulting supernatant was collected and stored at −80°C until biochemical analysis.

Biochemical Assays

Oxidative Stress Markers

Superoxide dismutase (SOD) activity was determined according to the method of Misra and Fridovich (1972), based on the inhibition of epinephrine autoxidation. Catalase (CAT) activity was measured by the method of Aebi (1984), based on the rate of H_2O_2 decomposition at 240 nm. Reduced glutathione (GSH) content was estimated by Ellman's reagent method (Ellman, 1959), which involves the reaction of the sulfhydryl group with 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB). Malondialdehyde (MDA) levels, as an index of lipid peroxidation, were quantified using the thiobarbituric acid reactive substances (TBARS) assay (Ohkawa *et al*., 1979). Protein content in tissue homogenates was determined by the Bradford method as modified by Kruger (2009) using bovine serum albumin (BSA) as a standard.

Inflammatory Markers

Nitric oxide (NO) levels were estimated colorimetrically using the Griess reagent method (Green et al., 1982), which measures the stable end products of NO metabolism (nitrite/nitrate). Myeloperoxidase (MPO) activity, as a marker of neutrophil infiltration and inflammatory activation, was assayed spectrophotometrically using a method based on the oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) in the presence of H_2O_2 (Bradley *et al*., 1982).

Histopathological Examination

Additional spleen samples were fixed in 10% neutral buffered formalin, processed through a graded ethanol series, embedded in paraffin wax, and sectioned at 5 µm thickness. Sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope for histopathological changes including lymphoid depletion, congestion, inflammatory cell infiltration, and morphological disruption of splenic architecture.

Data Analysis

All data are expressed as mean ± standard deviation (SD). Statistical comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS software version 19 (IBM Corp., Armonk, NY, USA).

RESULTS AND DISCUSSION

6GR Abated Splenic Oxidative Stress Associated with Cd Exposure

Table 1 illustrates the modulatory effects of 6GR on splenic antioxidant status and oxidative stress indices in Cd-intoxicated rats. Cadmium administration significantly ($p < 0.05$) reduced SOD, CAT, and GSH levels while markedly elevating lipid peroxidation (LPO) levels relative to controls, consistent with severe oxidative stress. Co-treatment with 6GR significantly ameliorated these changes in a dose-dependent manner, indicating enhanced antioxidant defense and reduced lipid peroxidation compared to the Cd-alone group ($p < 0.05$).

Table 1: Effects of 6GR on splenic enzymic and non-enzymic antioxidants

Parameter	Control	Negative Control	6GR 100	Cd	6GR 50 + Cd	6GR 100 + Cd
SOD (U/mg protein)	26.08 ± 2.47	25.19 ± 1.84	26.87 ± 2.71	10.08 ± 2.68*	16.10 ± 2.21a	20.87 ± 1.94b
CAT (U/mg protein)	24.10 ± 2.64	23.96 ± 1.46	24.66 ± 2.14	7.08 ± 1.02*	11.88 ± 1.91*	18.18 ± 2.22*
GSH (µmol/g tissue)	6.17 ± 0.32	6.49 ± 0.29	6.87 ± 0.68	2.31 ± 0.38*	3.01 ± 0.60a	4.06 ± 0.21b
MDA (nmol/g tissue)	2.07 ± 0.12	2.10 ± 0.21	2.12 ± 0.10	8.17 ± 0.67*	5.07 ± 0.18a	4.04 ± 0.20b

Data were expressed as mean ± SD of 10 rats. *$p < 0.05$ vs control, a,b$p < 0.05$ vs Cd.

Cadmium is a well-established pro-oxidant that depletes endogenous antioxidant defenses by binding to sulfhydryl groups of key antioxidant enzymes, thereby impairing their catalytic activity (Casalino *et al*., 2002). In the present study, Cd exposure caused a significant reduction in SOD, CAT, and GSH levels in splenic tissue, consistent with previous reports of Cd-induced oxidative stress in various organs (Renugadevi and Prabu, 2010; Al-Zharani *et al*., 2024). SOD catalyzes the dismutation of superoxide anion radicals to H_2O_2, which is then neutralized by CAT; disruption of this enzymatic cascade by Cd allows accumulation of reactive oxygen species, leading to lipid peroxidation as evidenced in our study by the elevated MDA levels in the Cd-exposed group. GSH, a critical non-enzymatic antioxidant, serves as a substrate for glutathione peroxidase and acts as a direct scavenger of hydroxyl radicals; its depletion by Cd further compromises the cell's ability to neutralize oxidative insults. Treatment with 6GR dose-dependently restored the activities of SOD and CAT and the levels of GSH, while simultaneously reducing MDA concentrations. These findings are consistent with studies demonstrating that 6GR activates the Nrf2/ARE (nuclear factor erythroid 2-related factor 2/antioxidant response element) pathway, upregulating the expression of antioxidant enzymes and detoxification proteins (Yang *et al*., 2024). The catechol moiety in 6GR's structure also confers direct free radical scavenging capacity, allowing it to intercept ROS before they can initiate lipid peroxidation chain reactions (Dugasani *et al*., 2010).

6GR inhibited Inflammatory Markers in the Spleen of Cd-exposed Rats

Figure 1 depicts the effects of 6GR on biomarkers of inflammation in Cd-treated rats. Administration of Cd alone significantly increased inflammatory mediators, namely MPO activity and nitric oxide (NO) levels (measured as nitrite), in the spleen compared with controls. However, administration of 6GR at 50 and 100 mg/kg significantly reduced MPO activity and NO levels in the spleen compared with Cd alone ($p < 0.05$).

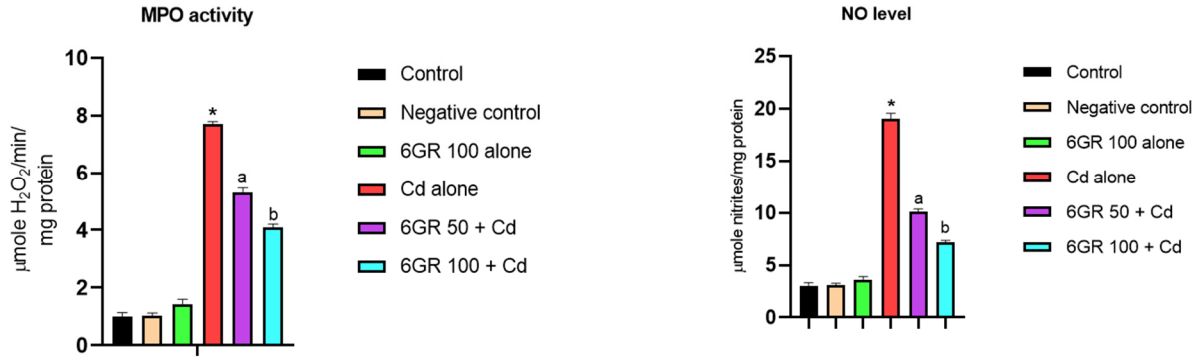

Fig. 1: Effect of 6GR on MPO activity and NO level in splenic tissues treated with Cd.

Data were expressed as mean ± SD of 10 rats. $^{*}p < 0.05$ vs control, $^{a,b}p < 0.05$ vs Cd.

The inflammatory response to cadmium observed in the current study was characterized by significant elevation of NO levels and MPO activity. Nitric oxide, produced in excess by inducible nitric oxide synthase (iNOS), acts as both an inflammatory mediator and a potent free radical that can combine with superoxide to form peroxynitrites, a highly reactive species capable of causing DNA damage and protein nitration (Pacher *et al*., 2007). MPO, a heme-containing peroxidase released by activated neutrophils, catalyzes the formation of hypochlorous acid and other oxidants, serving as a reliable histological and biochemical marker of neutrophil-mediated tissue inflammation. The concurrent elevation of NO and MPO in Cd-exposed rats in the present study indicates a robust inflammatory infiltration of splenic tissue, consistent with Cd's known ability to activate inflammatory signaling cascades (Charkiewicz *et al*., 2023). Co-administration of 6GR effectively suppressed both NO production and MPO activity in a dose-dependent fashion. These anti-inflammatory effects likely reflect 6GR's capacity to inhibit iNOS expression and NF-κB transcriptional activation, as reported in previous *in vitro* and *in vivo* studies (Akinyemi et al., 2015; Kim et al., 2005). By dampening NF-κB-driven transcription of pro-inflammatory genes, 6GR reduces the recruitment and activation of inflammatory cells in splenic tissue, thereby limiting the secondary oxidative damage associated with neutrophil degranulation.

6GR Reduced Histopathological Alterations in the Spleen of Cd-Challenged Rats

In Figure 2, histopathological examination of splenic sections using H&E staining revealed preserved lymphoid architecture and clearly demarcated red and white pulp in the control and 6GR alone groups. The Cd group showed lymphoid depletion, sinusoidal congestion, and diffuse inflammatory cell infiltration. These changes were markedly reduced in the 6GR 50 + Cd and 6GR 100 + Cd groups in a dose-dependent manner, with the higher dose demonstrating near-normal splenic architecture.

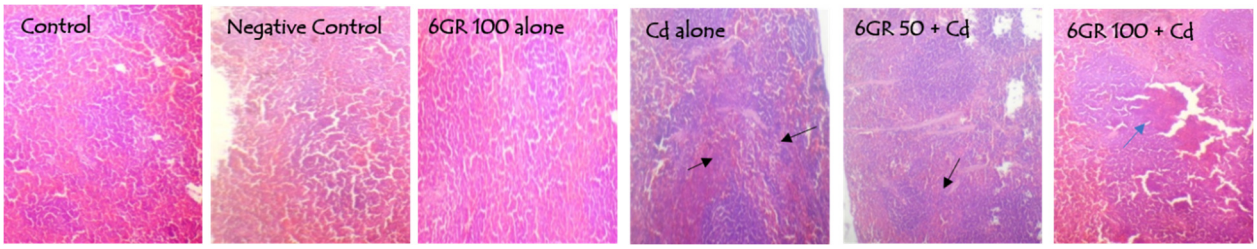

Fig. 2: Photomicrographs of haematoxylin and eosin-stained sections of splenic tissue from control and experimental rat groups (×40 magnification).

Group I (Control) and Group II (Negative Control, corn oil) display normal splenic architecture characterized by well-defined white pulp and red pulp, intact lymphoid follicles with visible germinal centres, a patent central arteriole, and clearly delineated periarteriolar lymphoid sheaths (PALS). Group III (6GR 100 mg/kg alone) similarly shows preserved lymphoid architecture with no observable histopathological changes compared to the control. Group IV (Cd alone) exhibits significant disruption of splenic architecture, including lymphoid depletion, sinusoidal congestion, diffuse infiltration of inflammatory cells, and loss of the normal white pulp/red pulp boundary, consistent with cadmium-induced splenotoxicity. Group V (6GR 50 mg/kg + Cd) displays partial restoration of splenic architecture with decreased inflammatory infiltration and less sinusoidal congestion relative to the Cd-only group, though mild lymphoid depletion remains. Group VI (6GR 100 mg/kg + Cd) shows close to normal splenic histoarchitecture, with largely preserved lymphoid follicles, reduced congestion, and minimal inflammatory cell infiltration, indicating dose-dependent splenoprotection provided by 6GR.

The histopathological findings corroborated the biochemical data, demonstrating that Cd exposure produced significant morphological disruption of splenic architecture, including lymphoid depletion and sinusoidal congestion, which were ameliorated by 6GR treatment. The dose-dependent nature of this protection, with the 100 mg/kg dose producing greater efficacy than the 50 mg/kg dose, suggests a pharmacokinetic concentration-response relationship that warrants further investigation.

Taken together, the present study demonstrates that 6GR exerts significant protective effects against cadmium-induced splenotoxicity through dual antioxidant and anti-inflammatory mechanisms, findings consistent with the broader pharmacological profile of 6GR as a potent cytoprotective agent. These results support the hypothesis that 6GR protects the spleen against Cd-induced injury and may be relevant for further studies in models of chronic low-level Cd exposure. However, several limitations must be acknowledged. The short duration of Cd exposure (7 days) may not fully recapitulate the pathophysiology of chronic cadmium accumulation. Additionally, the molecular targets of 6GR in splenic tissue, including potential modulation of the Nrf2 and NF-κB pathways, were not directly measured in this study and merit further investigation. Furthermore, the absence of direct tissue Cd measurement represents a limitation that should be addressed in future work.

CONCLUSION

In conclusion, the present study provides experimental evidence that 6-gingerol effectively attenuates cadmium-induced splenotoxicity in rats by restoring antioxidant enzyme activities, reducing lipid peroxidation, and suppressing pro-inflammatory mediators (NO and MPO) in a dose-dependent manner. These findings suggest that 6GR may serve as a promising natural splenoprotective agent

against heavy-metal-induced immunotoxicity.

REFERENCES

Aebi, H. (1984). Catalase *in vitro* Methods in *Enzymology*, 105: 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3.

Ajayi, B. O., Adedara, I. A. and Farombi, E. O. (2015). Pharmacological activity of 6-gingerol in dextran sulphate sodium-induced ulcerative colitis in BALB/c mice. *Phytotherapy Research*, 29(4): 566-572. https://doi.org/10.1002/ptr.5286.

Akinyemi, A. J., Thome, G. R., Morsch, V. M., Stefanello, N., Goularte, J. F., Belló-Klein, A., ... Schetinger, M. R. C. (2015). Effect of dietary supplementation of ginger and turmeric rhizomes on angiotensin-1 converting enzyme (ACE) and arginase activities in L-NAME induced hypertensive rats. *Journal of Functional Foods*, 17: 792-801. https://doi.org/10.1016/j.jff.2015.06.011.

Al-Zharani, M., Almuqri, E., Mubarak, M., Aljarba, N., Rudayni, H. and Yassen, K. (2024). Antioxidant effects of whey protein as a dietary supplement to alleviate cadmium-induced oxidative stress in male Wistar rats. *Current Research in Nutrition and Food Science Journal*, 12(1): 147-156. http://dx.doi.org/10.12944/CRNFSJ.12.1.12.

Bradley, P. P., Priebat, D. A., Christensen, R. D. and Rothstein, G. (1982). Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *Journal of Investigative Dermatology*, 78(3): 206–209. https://doi.org/10.1111/1523-1747.ep12506462.

Casalino, E., Calzaretti, G., Sblano, C. and Landriscina, C. (2002). Molecular inhibitory mechanisms of antioxidant enzymes in rat liver and kidney by cadmium. *Toxicology*, 179(1-2): 37-50. https://doi.org/10.1016/S0300-483X(02)00245-7.

Charkiewicz, A. E., Omeljaniuk, W. J., Nowak, K., Garley, M. and Nikliński, J. (2023). Cadmium toxicity and health effects - a brief summary. *Molecules*, 28(18): 6620. https://doi.org/10.3390/molecules28186620.

Davidova, S., Milushev, V. and Satchanska, G. (2024). The mechanisms of cadmium toxicity in living organisms. *Toxics*, 12(12): 875. https://doi.org/10.3390/toxics12120875.

Dugasani, S., Pichika, M. R., Nadarajah, V. D., Balijepalli, M. K., Tandra, S. and Korlakunta, J. N. (2010). Comparative antioxidant and anti-inflammatory effects of [6]-gingerol,[8]-gingerol,[10]-gingerol and [6]-shogaol. *Journal of Ethnopharmacology*, 127(2): 515-520. https://doi.org/10.1016/j.jep.2009.10.004.

Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*, 82(1): 70–77.

Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S. and Tannenbaum, S. R. (1982). Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. *Analytical Biochemistry*, 126(1):

131–138. https://doi.org/10.1016/0003-2697(82)90118-X.

Jomova, K., Alomar, S. Y., Nepovimova, E., Kuca, K. and Valko, M. (2025). Heavy metals: toxicity and human health effects. *Archives of toxicology*, 99(1): 153-209. https://doi.org/10.1007/s00204-024-03903-2.

Kang, D. Y., Chi, W. J., Cho, J. and Jang, K. J. (2026). 6-Gingerol alleviates high glucose-induced inflammation and cytotoxicity in THP-1 cells by inhibiting TLR4 signaling. Scientific Reports. https://doi.org/10.1038/s41598-025-34192-z.

Kim, S. O., Kundu, J. K., Shin, Y. K., Park, J. H., Cho, M. H., Kim, T. Y. and Surh, Y. J. (2005). [6]-Gingerol inhibits COX-2 expression by blocking the activation of p38 MAP kinase and NF-κB in phorbol ester-stimulated mouse skin. *Oncogene*, 24(15): 2558-2567. https://doi.org/10.1038/sj.onc.1208446.

Kruger, N. J. (2009). The Bradford method for protein quantitation. *The Protein Protocols Handbook*, 17-24. https://doi.org/10.1007/978-1-59745-198-7_4.

Li, R., Wang, X., Liu, W., Song, M., Zeng, T. and Zhang, C. (2025). Cadmium disrupts hepatic lipid homeostasis: multifaceted molecular mechanisms, unresolved controversies, and emerging therapeutic strategies. *iScience*, 114406. https://doi.org/10.1016/j.isci.2025.114406.

Misra, H. P. and Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry*, 247(10): 3170–3175. https://doi.org/10.1016/S0021-9258(19)45228-9.

Ohkawa, H., Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry, 95(2), 351–358. https://doi.org/10.1016/0003-2697(79)90738-3.

Pacher, P., Beckman, J. S. and Liaudet, L. (2007). Nitric oxide and peroxynitrite in health and disease. *Physiological Reviews*, 87(1): 315-424. https://doi.org/10.1152/physrev.00029.2006.

Peana, M., Pelucelli, A., Chasapis, C. T., Perlepes, S. P., Bekiari, V., Medici, S. and Zoroddu, M. A. (2023). Biological effects of human exposure to environmental cadmium. *Biomolecules*, 13(1): 36. https://doi.org/10.3390/biom13010036.

Qu, F. and Zheng, W. (2024). Cadmium exposure: mechanisms and pathways of toxicity and implications for human health. *Toxics*, 12(6): 388. https://doi.org/10.3390/toxics12060388.

Renugadevi, J. and Prabu, S. M. (2010). Cadmium-induced hepatotoxicity in rats and the protective effect of naringenin. *Experimental and Toxicologic Pathology*, 62(2): 171-181. https://doi.org/10.1016/j.etp.2009.03.010.

Xia, Y., Zhang, Y., Zhang, J., Du, Y., Wang, Y., Xu, A. and Li, S. (2023). Cadmium exposure induces necroptosis of porcine spleen via ROS-mediated activation of STAT1/RIPK3 signaling pathway. *Environmental and Molecular Mutagenesis*, 64(7): 382-392. https://doi.org/10.1002/em.22565.

Yang, K., Lu, Y., Yue, Z., Jin, S., Wang, P., Liu, C., ... & Chang, J. (2024). 6-Gingerol activates the Nrf2 signaling pathway to alleviate hydrogen peroxide induced oxidative stress on primary chicken embryo hepatocytes. *Journal of Functional Foods*, 122: 106535. https://doi.org/10.1016/j.jff.2024.106535.

Yu, Q., Li, J., Cui, M., Mei, C., He, Q. and Du, X. (2024). 6-Gingerol attenuates hepatic ischemia/reperfusion injury through regulating MKP5-mediated P38/JNK pathway. *Scientific Reports*, 14(1): 7747. https://doi.org/10.1038/s41598-024-58392-1.