Al-Nisour Journal for Medical Sciences

Manuscript 1159

Environmental Impact of Benzo[a]anthracene Exposure on Liver Biomarkers and COVID-19 Severity: Evaluation of Combined Clinical and Molecular Modeling

Osamah Alkilidar

Hayder G. Jawad

Hasan K. Nimr

Environmental Impact of Benzo[a]anthracene Exposure on Liver Biomarkers and COVID-19 Severity: Evaluation of Combined Clinical and Molecular Modeling

Osamah Alkilidar *, Hayder G. Jawad, Hasan K. Nimr

Scientific Research Commission, Baghdad, Iraq

Abstract

This study aimed to evaluate the relationship between exposure to environmental pollutants, particularly benzo[a]anthracins (BaA), and the severity of the inflammatory response and liver dysfunction in COVID-19 patients, using a combination of statistical analysis and molecular modeling. Molecular docking studies revealed stable and robust interactions between benzo[a]anthracins and cytochrome P450 (CYP450) enzymes, suggesting that these interactions may contribute to increased oxidative stress and hepatocyte damage.

The statistical analysis included 70 COVID-19 patients, stratified by benzo[a] anthracene exposure. The results showed statistically significant associations between exposure to this pollutant and elevated C-reactive protein (CRP) levels, as well as a significant increase in aspartate aminotransferase (AST). Alanine aminotransferase (ALT) showed an upward trend, but this increase did not reach statistical significance. These findings suggest that exposure to benzo[a]anthracens may exacerbate systemic inflammation and negatively affect liver function, potentially contributing to the increased severity of clinical symptoms of COVID-19.

The study emphasizes the public health importance of reducing exposure to environmental pollutants, particularly among the most vulnerable population groups during health crises. It also highlights the role of bioinformatics and computational modeling in enhancing our understanding of the molecular mechanisms underlying the effects of environmental pollutants and supporting prevention strategies and early intervention.

Keywords: Molecular docking, Systemic inflammation (CRP), COVID-19 severity, Cytochrome P450 (CYP450), Environmental pollutants in Iraq, Benzo[a]anthracene (BaA), Liver enzymes

1. Introduction

Investigating the effect of environmental pollutants on enzyme levels and their ability to predict the severity of viral infection by using bioinformatics techniques is an essential topic in the fields of public health and molecular biology. While the interaction between environmental pollutants and viral infection has become an area of increasing study and interest, Exposure to some pollutants, such as volatile organic compounds, is definitely associated with increased susceptibility to viral infection. The mechanisms via which this association occurs affect the human immune system and virus behaviour (Ibrahim et al., 2020).

This study aims to investigate the effect of oil industrial emissions, with a deep focus on chemical pollutants, on the levels of specific enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Emissions from oil industries release a diversity of chemical pollutants into the environment, which can significantly impact the immune system. Exposure to these pollutants can cause acute and chronic inflammatory responses in the respiratory system, leading to systemic effects that

Received 31 December 2025; accepted 14 February 2026.
Available online 20 May 2026

* Corresponding author.
E-mail address: osamah.alkilidar@mohesr.edu.iq (O. Alkilidar).

https://doi.org/10.70492/2664-0554.1159

alter enzyme levels and other physiological processes (Ruiz-Lara et al., 2023).

Alveolar macrophages and epithelial cells release pro-inflammatory cytokines such as IL-6, IL-8, and TNF-α upon exposure to petroleum emissions, contributing to inflammation. Chronic exposure worsens conditions such as asthma, chronic obstructive pulmonary disease (COPD), and bronchitis (Ishii et al., 2005).

Moreover, these pollutants yield reactive oxygen species (ROS), which conduct oxidative stress that damages cells, proteins, and DNA, harming immune function and increasing susceptibility to illness (Juan et al., 2021). Chronic exposure to petroleum emissions is also associated with immunosuppression, which decreases the body's ability to resist infection and raises the incidence of respiratory diseases (Kurt et al., 2016). Furthermore, these pollutants are related to the evolution of autoimmune diseases, maybe through molecular mimicry or the release of self-antigens. Systemic effects occur when these pollutants enter the bloodstream, causing systemic inflammation and contributing to cardiovascular disease, diabetes, and systemic autoimmune conditions (Gan et al., 2011). While more significant pollutants primarily cause local irritation in the upper respiratory tract, smaller particles from petroleum emissions penetrate deeper into the lungs and bloodstream, causing more severe health issues. These smaller particles, with their increased surface area-to-mass ratio, are highly reactive and competent in transporting toxic materials deep into the lungs and beyond (Rückerl et al., 2006).

Regulatory standards concentrate on decreasing exposure to these pollutants, with the World Health Organization (WHO) advising inflexible guidelines to mitigate these health risks (World Health Organization, 2021). This research paper assesses the synergistic impact of long-term exposure to the pollutant benzanthracin[A] on the clinical outcomes of COVID-19 patients. The methodology is based on a retrospective review of 70 cases, in which liver function indicators were analyzed as biomarkers of viral infection severity and compared with those of patients who were not exposed to the pollutant. To understand the molecular mechanisms, the study integrated environmental and biological data using bioinformatics techniques to explore the relationship between this environmental pollutant and treatment resistance. This integrated approach aims to develop predictive models for disease severity and mortality, thereby contributing to the enhancement of public health protocols and mitigating the dual impact of pollution and the pandemic.

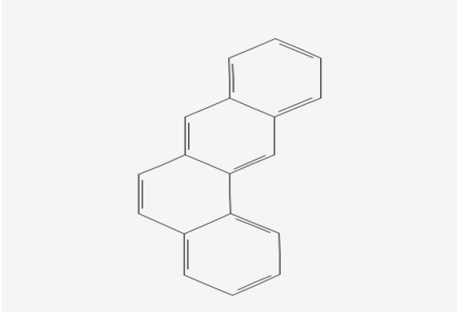

Fig. 1. chemical structure of benzo[a]anthracene (CID: 5954).Source: National Center for Biotechnology Information (2026). PubChem Compound Summary for CID 5954, Benzo[a]anthracene. Retrieved February 18, 2026, from https://pubchem.ncbi.nlm.nih.gov/compound/5954.

2. Bioinformatics in environmental health research

Bioinformatics plays a pivotal role in studying the relationship between environmental pollutants and viral infections by analyzing and evaluating large-scale biological data. It helps identify molecular pathways affected by environmental pollutants and reveals their impact on viral efficacy and immune responses. Tools such as genomic and transcriptomic analysis (e.g., next-generation sequencing, RNA-Seq) are used to track genetic changes and expression patterns. Databases (e.g., NCBI GEO, KEGG) help access and understand relevant data and information in detail. Computational modelling programs predict pollutant-protein interactions, while machine learning systems analyze large datasets for patterns. Data integration and visualization tools, such as Cytoscape, provide insights into pollutant-virus interactions, which is especially important for public health research. Together, these methodologies provide comprehensive insights into how environmental pollutants modulate enzyme activity and expression levels, elucidating their role in environmental health and disease pathogenesis.

2.1. In vitro studies

Research on Organisms yields important information about how environmental contaminants impact the biology of enzymes in intricate physiological settings. It provides information on toxicity methodologies, the identification of biomarkers, and potential treatment strategies (Chauhan & Johnston, 2003).

These approaches advance our knowledge of environmental health and support evidence-based regulations to control pollutants and protect public health. Research in living organisms yields important information about how environmental pollutants affect

enzyme biology in complex physiological settings, providing insights into toxicological processes, the identification of biomarkers, and possible remediation approaches (Lai et al., 2021).

This study was conducted at Al-Shifa Crisis Hospital in Baghdad. The initial survey involved reviewing clinical and laboratory data (including C-reactive protein and liver enzymes) from 244 patients admitted as COVID-19 emergency cases.

Although direct environmental measurements were not available, which represents a limitation, the final research sample was selected based on a detailed questionnaire assessing occupational and environmental exposure, with strict exclusion criteria applied for chronic diseases (hypertension, cardiovascular disease, and asthma).

The selected cohort consisted of 70 patients, all male smokers, equally divided into two groups (n = 35 per group):

1. The Exposed Group: This group included workers in sectors with high exposure to organic pollutants, such as oil facilities, gas stations, industrial areas (vehicle repair and painting workshops), and drivers. The cases within this group (n = 35) were classified as moderate to severe, as all patients required supplemental oxygen to control the shortness of breath caused by COVID-19. Furthermore, many patients suffered complications that led to respiratory failure, forcing medical providers to admit them to the intensive care unit (ICU), reflecting the large clinical burden in this sample.
2. The Control Group: This group included patients working in environments free from chronic exposure to these pollutants, matching the first group in demographic characteristics and health status (smokers and free of chronic diseases), They were provided with oxygen masks as a precautionary measure during the treatment phases without the need for ICU.

2.2. *Bioinformatics tools*

The study was conducted using PyrX and DS Visualizer Client softwires.

- Ligand: Benzo[a]anthracene
- Protein: CYP450, specifically the structure encoded by 3ua5.pdb.
- Docking software: Docking was performed using the PyRx simulation tool, which is capable of using the universal force field (UFF) principal force.
- Metrics; Binding affinity (measured in terms of energy E), the square of the upper mean deviation (RMSD/ub), and the square of the lower mean deviation (RMSD/lb) were used to determine the docking results. These methodologies advance our understanding of pollutant toxicity mechanisms and support regulatory decisions aimed at mitigating environmental health risks.

3. Results and discussion

The findings of the molecular docking test between benzo[a]anthracene and CYP450 provide valuable information about the binding affinity (E) and differences in Mean Radical Deviation (RMSD) values (root difference) values between the binding molecules, as shown in Table 1.

The binding affinity (E) values ranged from –10.2 to –8.9 kilocalories/mol (measured by negative energy values), which indicated a strong interaction between benzo[a]anthracene and CYP450. More negative values indicate a tight interaction (Blaney & Dixon, 1993).

The RMSD /ub (root mean difference for the upper part of the chain) values ranged from 0 to 14.865 Angstrom (Å), which indicated changes in the structural conformations of the binding molecules (Blaney & Dixon, 1993).

Table 1. Molecular docking results showing binding affinity (kcal/mol) and RMSD values (Å) between BaA and CYP450 protein.

rmsd/lb	*rmsd/ub*	Relational affinity (E)	*Ligand*
0	0	–10.2	*3ua5.pdb_mod2_5954_uff_E = 209.55*
0.76	2.6	–9.8	
0.883	6.428	–9.7	
0.783	5.805	–9.6	
1.755	5.771	–9.4	
2.395	4.864	–9.3	
2.396	4.415	–9.1	
13.294	14.865	–9	
13.755	14.9	–8.9	

RMSD /lb values also range from 0 to 13.294 (Å), reflecting the flexibility of the structure and the degree of displacement between different structures.

Dock simulations revealed several key insights into the interaction between benzo[a]anthracene and CYP450, as shown in Fig. 2.

According to Trott and Olson (2010) Binding affinity: Binding affinity (measured by negative energy values) indicates that the ligand forms a stable complex with the protein. The most favorable binding affinity was observed at –10.2 kcal/mol, indicating a strong interaction. RMSD values: RMSD values provide insight into conformational changes upon binding. Lower RMSD values typically indicate a more stable and predictable binding mode. The lowest RMSD values were observed at a binding affinity

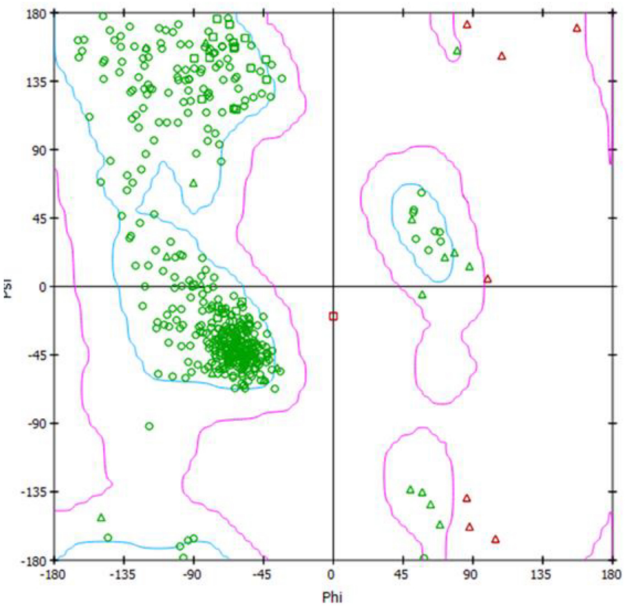

Fig. 2. Shows insights of Dock simulations into the interaction between BaA and CYP450.

of –10.2 kcal/mol, indicating a very stable complex formation at this energy level.

Interaction analysis, the lower the binding affinity (less negative), the higher the RMSD values, indicating less stable binding interactions. Higher RMSD values at the binding affinity of –9.1 (Å) to –8.9 (Å) indicate greater flexibility or less stable binding conformations. The molecular docking study between benzo[a] anthracene and CYP450 reveals a range of binding affinities, with the interaction being most stable at binding affinity –10.2 kcal/mol. The low RMSD values at this energy level support the stability of the ligand-protein complex.

These results contribute to the understanding of how BaA interacts with CYP450, which may have implications for its metabolic processing capacity and potential effects on human health.

3.1. Statistical analysis

Statistical analysis was performed using one-way ANOVA and independent samples t-test with Welch correction via SPSS version 30. The results showed highly significant differences among patients based on their level of exposure to BaA, indicating a strong correlation between exposure level, elevated C-reactive protein (CRP) levels, and COVID-19 severity. This supports the hypothesis of a negative impact of environmental pollutants on clinical outcomes (Chart 1).

ANOVA revealed significant differences in mean CRP levels among the three exposure categories (low, medium, and high). CRP values increased progressively with increasing exposure level, reflecting a dose-response relationship. The ANOVA value reached a highly significant level of statistical significance ($p < 0.001$).

To verify the consistency of the results, an independent samples t-test was performed after combining the moderate and high-exposure groups into a single group (exposed) and comparing them with the low-exposure group (non-exposed). The results showed that the mean CRP level in exposed patients was 119.38, while it was 11.68 in non-exposed patients. Levene's test also revealed heterogeneity of variance between the two groups, with a variance of 4127.53 in the exposed group compared to 2376.55 in the non-exposed group, indicating greater dispersion of CRP values among exposed patients.

Consequently, Welch's independent t-test was used, which showed a very high statistical significance difference between the two groups ($t = 10.68$, $df \approx 35.3$, $p < 0.001$). These results clearly indicate that patients exposed to BaA have significantly higher levels of C-reactive protein compared to non-exposed patients, reflecting an increase in the severity of the inflammatory response associated with environmental exposure.

Based on a p-value of less than 0.05, the results of this study showed a strong statistically significant difference in CRP protein levels between the groups according to their degree of exposure to BaA. These results support a clear association between environmental exposure to the compound and an increased severity of the inflammatory response, indicating that the observed differences are not due to statistical chance (Chart 2).

3.1.1. ALT-test

The results showed that mean levels of alanine aminotransferase (ALT) were higher in patients exposed to BaA compared to unexposed patients. However, this difference did not reach statistical significance using a two-way independent samples t-test ($p > 0.05$), indicating that the observed increase in ALT levels represents a statistically inconclusive trend. Therefore, a clear effect of exposure on ALT levels cannot be definitively established in the current study sample.

3.1.2. AST test

The independent samples t-test showed a statistically significant difference in aspartate aminotransferase (AST) levels between patients exposed to and unexposed to benzo[a]anthracins, with mean AST levels significantly higher in the exposed group

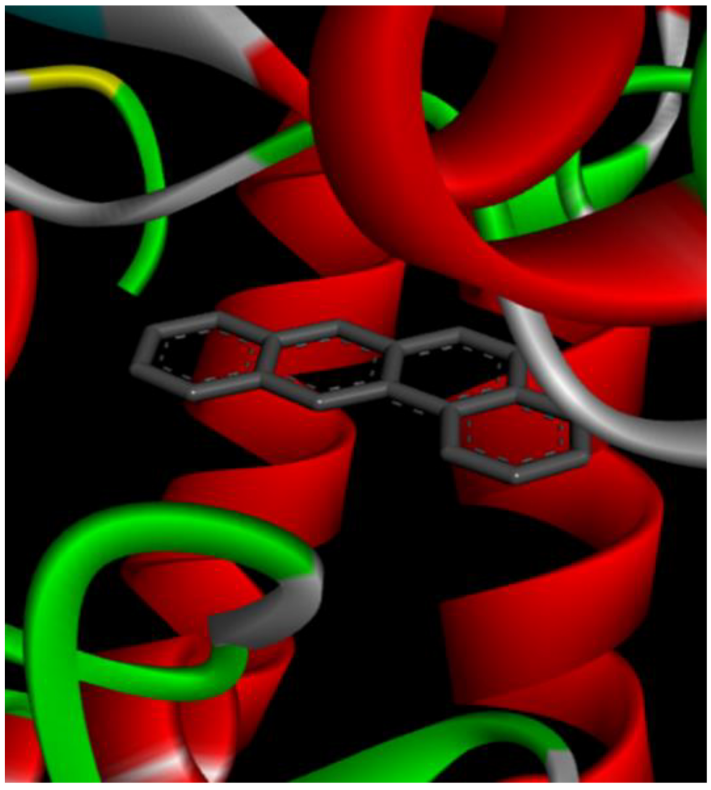

Fig. 3. Docking interaction of benzo[a]anthracene with CYP450 generated using PyRx and visualized with Discovery Studio Visualizer Client.

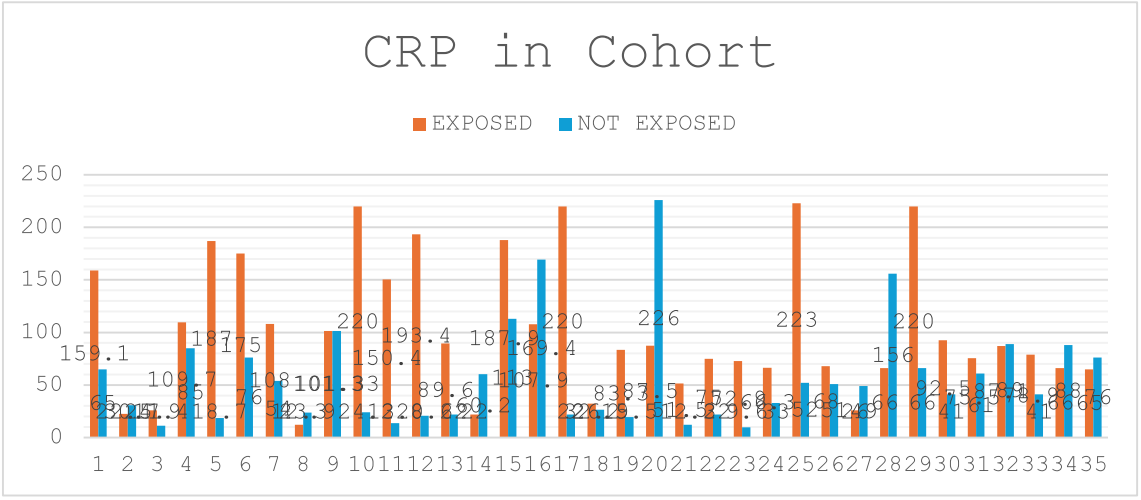

Chart 1. Shows the cohort CRP elevation.

($p < 0.05$). Increased variability in AST levels was also observed among these exposed patients, suggesting greater variation in the hepatic response associated with environmental exposure. These findings support a possible association between benzo[a]anthracin exposure and impaired liver function.

3.2. Cohort study and research findings

The results of this study showed that exposure to BaA may be associated with marked disturbances in inflammatory markers and liver function in COVID-19 patients. A significant increase in C-reactive protein (CRP) levels was observed with increasing exposure, suggesting an exacerbation of the inflammatory response in patients with higher exposure to environmental pollutants. AST levels also showed a statistically significant increase in the exposed group, while ALT levels showed an upward trend but did not reach statistical significance, which may reflect a difference in the sensitivity of liver markers to inflammatory response and environmental influences.

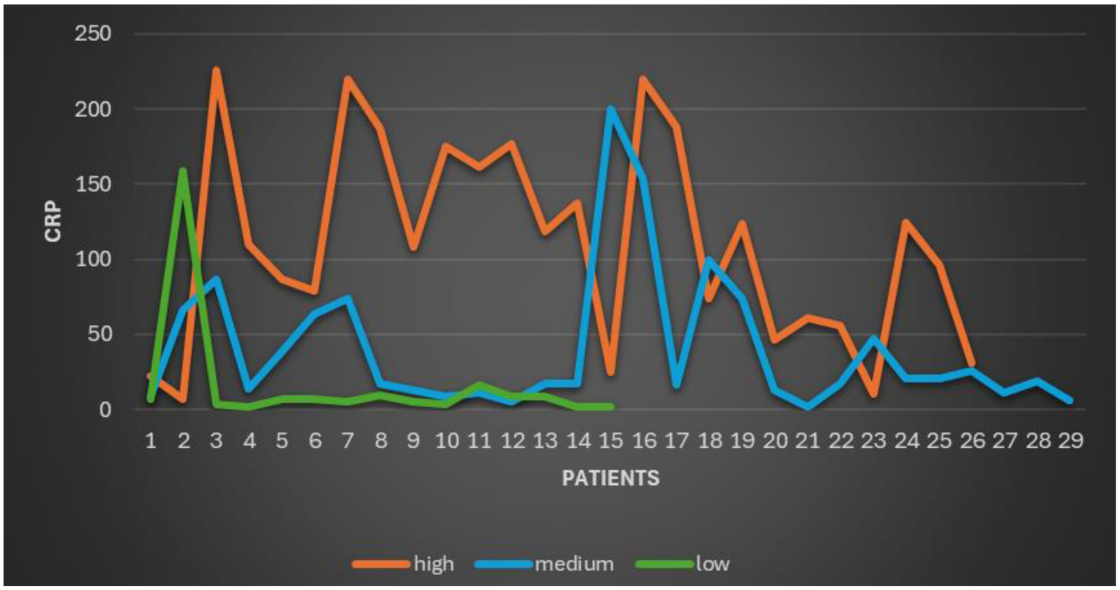

Chart 2. Shows the elevation in CRP levels due to exposure ratio.

Metabolically, BaA is metabolized in the liver by cytochrome P450 enzymes, particularly CYP1A1 and CYP1B1, generating reactive oxygen species (ROS) as byproducts. This oxidative stress leads to cellular damage in the liver and elevated levels of AST and ALT enzymes, a mechanism exacerbated in the context of COVID-19 infection, which is characterized by systemic inflammation, hypoxia, and immune dysfunction.

The results of this study are consistent with previous research indicating an association between chronic exposure to polycyclic aromatic hydrocarbons (PAHs) and elevated liver enzymes and inflammatory (Jabir et al., 2016; Ruiz-Lara et al., 2023).

Recent studies have also supported the potential role of environmental pollutants in amplifying systemic inflammation and raising CRP levels in COVID-19 patients, which may explain the poor clinical outcomes in areas with high air pollution (Cekerevac et al., 2021; Wang et al., 2025).

Despite the significance of these findings, studying the effects of environmental pollutants faces several limitations, most notably the challenges of accurately quantifying exposure, the overlap between different pollutants, and the individual variability in biological responses. However, the study's findings underscore the importance of reducing exposure to environmental pollutants, particularly during viral epidemics, to prevent exacerbation of inflammation and liver dysfunction in high-risk populations.

3.3. Future directions and implications for public health

The results of this study indicate the importance of directing future researchs toward longitudinal designs and larger sample sizes, integrating polymorphic methods and computational modeling, to understand the cumulative effects of environmental pollutants such as BaA on inflammation severity and liver function, particularly in the context of viral diseases. At the public health level, the findings highlight the need to integrate environmental factors into risk assessment and epidemic preparedness strategies, and to strengthen pollution reduction policies in areas with high industrial burdens, given their potential to reduce the severity of inflammatory responses and health complications in high-risk populations.

4. Conclusion and summary of findings

This study, combining statistical analysis and molecular modeling, demonstrates that exposure to BaA is associated with increased inflammatory response and impaired liver function indices in COVID-19 patients. Molecular docking results showed a strong interaction between BaA and cytochrome P450 enzymes, supporting a potential oxidative stress-based mechanism. Clinically, exposure to these pollutants was associated with a significant increase in C-reactive protein levels and a statistically significant increase in AST, while ALT showed an upward trend, though not statistically significant. Taken together, these findings confirm that exposure to environmental pollutants may exacerbate inflammation and negatively impact liver function, contributing to poor clinical outcomes in COVID-19 patients.

References

Blaney, J.M. & Dixon, J.S. (1993) A good ligand is hard to find: Automated docking methods. *Perspectives in Drug Discovery and Design*, 1(2), 301–319.

Cekerevac, I., Turnic, T.N., Draginic, N., Andjic, M., Zivkovic, V., Simovic, S., Susa, R., Novkovic, L., Mijailovic, Z., Andjelkovic, M., Vukicevic, V., Vulovic, T., & Jakovljevic, V. (2021) Predicting severity and intrahospital mortality in CovID-19: The place and role of oxidative stress. *Oxidative Medicine and Cellular Longevity*, 2021, 15.

Chauhan, A.J. & Johnston, S.L. (2003) Air pollution and infection in respiratory illness. In *British Medical Bulletin*, 68, 95–112.

Gan, W.Q., Koehoorn, M., Davies, H.W., Demers, P.A., Tamburic, L., & Brauer, M. (2011) Long-term exposure to traffic-related air pollution and the risk of coronary heart disease hospitalization and mortality. *Environmental Health Perspectives*, 119(4), 501–507.

Ibrahim, M., MacFarlane, E.M., Matteo, G., Hoyeck, M.P., Rick, K.R.C., Farokhi, S., Copley, C.M., O'Dwyer, S., & Bruin, J.E. (2020) Functional cytochrome P450 1A enzymes are induced in mouse and human islets following pollutant exposure. *Diabetologia*, 63(1).

Ishii, H., Hayashi, S., Hogg, J.C., Fujii, T., Goto, Y., Sakamoto, N., Mukae, H., Vincent, R., & van Eeden, S.F. (2005) Alveolar macrophage-epithelial cell interaction following exposure to atmospheric particles induces the release of mediators involved in monocyte mobilization and recruitment. *Respiratory Research*, 6(1), 87.

Jabir, M.S., Khalil, O.A., Taqi, Z.J., & Hussain, H.A. (2016) Biochemical changes in hepatic function of petrol station attendants in basrah. *Journal of Al-Nahrain University-Scienc*, 19(4), 135–138.

Juan, C.A., de la Lastra, J.M.P., Plou, F.J., & Pérez-Lebeña, E. (2021) The chemistry of reactive oxygen species (ROS) revisited: Outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. *International Journal of Molecular Sciences*, 22(9), 4642.

Kurt, O.K., Zhang, J., & Pinkerton, K.E. (2016) Pulmonary health effects of air pollution. *Current Opinion in Pulmonary Medicine*, 22(2), 138.

Lai, C.H., Ho, S.C., Pan, C.H., Chen, W.L., Wang, C.C., Liang, C.W., Chien, C.Y., Riediker, M., Chuang, K.J., & Chuang, H.C. (2021) Chronic exposure to metal fume PM2.5 on inflammation and stress hormone cortisol in shipyard workers: A repeat measurement study. *Ecotoxicology and Environmental Safety*, 215, 112144.

Rückerl, R., Ibald-Mulli, A., Koenig, W., Schneider, A., Woelke, G., Cyrys, J., Heinrich, J., Marder, V., Frampton, M., Wichmann, H.E., & Peters, A. (2006) Air pollution and markers of inflammation and coagulation in patients with coronary heart disease. *American Journal of Respiratory and Critical Care Medicine*, 173(4), 432–441.

Ruiz-Lara, K., García-Medina, S., Galar-Martínez, M., Parra-Ortega, I., Morales-Balcázar, I., Hernández-Rosas, N.A., Moreno-Vázquez, S.E., Hernández-Díaz, M., Cano-Viveros, S., Olvera-Roldán, E.O., Gómez-Oliván, L.M., Gasca-Pérez, E., & García-Medina, A.L. (2023) The evaluation of liver dysfunction and oxidative stress due to urban environmental pollution in Mexican population related to Madin Dam, State of Mexico: a pilot study. *Environmental Science and Pollution Research*, 30(3), 6950–6964.

Trott, O. & Olson, A.J. (2010) AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry*, 31(2), 455–461.

Wang, Y., Zhu, E., Zhu, X., Li, X., He, M., Zhai, R., Wu, X., Hu, D., & Han, X. (2025) Exposure to polycyclic aromatic hydrocarbons and risk of abnormal liver function: The mediating role of C-reactive protein. *Ecotoxicology and Environmental Safety*, 298.

World Health Organization. (2021) What are the WHO air quality guidelines?. World Health Organization. https://www.who.int/news-room/feature-stories/detail/what-are-the-who-air-quality-guidelines