



## Effect of Contaminated Surface Water by Residents of Brick Kilns on Antioxidants and Lipid Profile of Rats

Duaa R.M. Al-Safi<sup>1</sup>, Zahraa N.K. Al Aboudi<sup>2</sup>

<sup>1</sup>Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Wasit, Wasit, Iraq

<sup>2</sup>Department of Biology, College of Education for Pure Sciences, University of Wasit, Wasit, Iraq

\*Corresponding Author: Duaa R.M. Al-Safi

Email: [duaarasool@uowasit.edu.iq](mailto:duaarasool@uowasit.edu.iq)



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### Abstract

Antioxidants and lipid markers have gained significant attention in recent decades for their pivotal role in promoting overall health and well-being. On other hand, the improper disposal of industrial effluents has emerged as a major environmental challenge leading to contamination of water sources that subsequently impact public health. Estimation of the effect of contaminated water by the residents of brick kilns on the level of antioxidants (CAT, GPX, and SOD), lipid peroxidation MDA, and lipid profile (TC, HDL, LDL, and TG). A total of 30 surface water samples were collected from the areas near the brick kilns located in Al-Hai, Al-Kut, and Badra cities into labeled plastic containers. Then, overall 60 rats were divided equally into two groups; the 1st was given tap water (negative control), and the 2nd was given the contaminated water (experimental). After 75 days, blood was collected from all animals to obtain the sera that tested using the specific species quantitative enzyme-linked sorbent assay (ELISA) kits to measure the concentration of antioxidants, lipid peroxidation and lipid profile. In comparison to values of control group, results of antioxidants including CAT ( $5.419 \pm 0.363$  pg/ml), GPX ( $24.533 \pm 1.317$  IU/ml) and SOD ( $4.013 \pm 0.479$  U/ml) were reduced significantly; while significant increases were shown in results of lipid peroxidation MDA ( $187.333 \pm 10.402$  ng/ml), as well as in LDL ( $641.611 \pm 54.809$  ng/ml). However, insignificant variation between the values of experimental and control groups was seen in values of TC ( $3.528 \pm 0.332$  nmol/ml), HDL ( $36.283 \pm 1.881$  ng/ml), and TG ( $567.556 \pm 72.724$  ng/ml).

## Introduction

Across the world, the improper disposal of industrial effluents has emerged as a major environmental challenge leading to contamination of water resources and the subsequent impact on public health (Chowdhary et al., 2020). Brick kiln industry, a crucial contributor to the construction sector, has long been a fixture in many developing nations; however, the consequences have become increasingly apparent due to the energy-intensive that can contribute to apparent to health issues and environmental degradation (Bashir et al., 2023; Parvez et al., 2023). The brick-making processes include the burning of fossil fuels associated with releasing greenhouse gases in addition to various harmful pollutants (e.g. carbon dioxide, nitrogen oxides, and sulfur dioxide) which linked to development of different health issues due to increasing of oxidative stress among populations living in close proximity to brick kilns (Asif et al., 2021; Zangana & Yusof, 2024).

Oxidative stress, a phenomenon marked by an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them, has emerged as a central focus

in contemporary biomedical research (Powers et al., 2016; Checa & Aran, 2020). ROS is defined as a highly reactive molecule that can cause oxidative damage and development a wide range of chronic diseases such as cancer, Alzheimer disease, Parkinson disease, diabetes mellitus and rheumatoid arthritis (Sharifi-Rad et al., 2020; Leyane et al., 2022).

Naturally, antioxidants occurring compounds are found in a variety of plant-based foods (fruits, vegetables, seeds and grains) and play a crucial role in counterbalancing the harmful effects of free radicals and ROS (Sundaram Sanjay & Shukla, 2021; Zhao et al., 2021). Fortunately, the bodies of animals and humans have developed various antioxidant defense mechanisms, enzymatic (CAT, GSH-PX and SOD) and non-enzymatic, to combat the detrimental effects of these radicals (Goc et al., 2017; Mironczuk-Chodakowska et al., 2018). Numerous epidemiological studies demonstrated that antioxidants can block the cell cycle and trigger apoptotic cell death and simultaneously protecting the normal cells from oxidative damage and fortifying the body's defense systems against degenerative diseases (Sadiq, 2023; An et al., 2024).

One of the keyways in which oxidative stress can impact health is through its effect on lipid metabolism through a process known as lipid peroxidation, leading to formation of oxidized lipid that resulting changes in the lipid profile (Barrera, 2012; Borza et al., 2013). Furthermore, oxidative stress can influence the activity of enzymes involved in lipid synthesis and metabolism leading to dysregulation of lipid homeostasis (Kleiboeker & Lodhi, 2022). Thus, the assessment of lipid profile is a crucial component of comprehensive healthcare as it provides valuable insights into the overall health and risk of development various metabolic disorders (Clemente-Suárez et al., 2023; Wazir et al., 2023).

In Iraq, online search revealed the absence of data about the effect of brick kilns on surface water and the impact of surface water on animals and/or humans. Hence, the current study aims to estimate the effect of contaminated water by the residents of brick kilns, located in Wasit province (Iraq), on the level of antioxidants (CAT, GSH-PX, and SOD), lipid peroxidation MDA, and lipid profile (TC, HDL, LDL, and TG).

## **Methods**

The research received institutional approval through the Scientific Committees of the College of Veterinary Medicine together with the College of Education for Pure Sciences at the University of Wasit where ethical practices were followed. The approval procedure assessed animal procedures to guarantee humane practices and institutional guidelines adherence.

### **Water samples**

Thirty surface water samples were collected aseptically from brick kiln site surroundings within three primary districts of Wasit province, Iraq encompassing Al-Hai, Al-Kut, and Badra. The collection tubes were labeled beforehand and used sterile plastic to stop contamination from happening. The laboratory received the samples at proper temperatures after they received immediate transport under cooled conditions.

### **Study designs and sera's collection**

A total of sixty healthy young female albino rats received housing at a university animal facility under established environmental conditions. Standard pellets and tap water became available to the rats for one week during their acclimation period before exposure under a 12-hour cyclic controlled lighting system. The rat population underwent acclimatization before the researchers divided them randomly into two homogeneous groups containing thirty subjects each. During the 75-day research duration the experimental group consumed the surface water sample that

was collected while the control group continuously consumed tap water. During the exposure duration researchers put all rats under anesthesia before taking blood samples through cardiac puncture. The researchers obtained blood specimens in uncoagulated gel tubes before performing 5-minute centrifugation at 5000 rpm. The laboratory technicians moved the separated serum to 1.5 mL Eppendorf tubes for preservation at  $-4^{\circ}\text{C}$  storage using opaque bags until biochemical testing took place.

### ***Analysis of antioxidants and lipid profile***

Analysis of laboratory biomarkers in serum was achieved through enzyme-linked immunosorbent assay (ELISA) kits specifically designed for different species that SunLong Biotech (China) supplied. Measurement included three antioxidant enzymes (catalase [CAT], glutathione peroxidase [GPX] and superoxide dismutase [SOD]) together with malondialdehyde [MDA] and four lipid profile markers such as total cholesterol (TC) and triglycerides (TG) high-density lipoprotein (HDL) and low-density lipoprotein (LDL). The preparation process followed explicit instructions from the manufacturer while performing each kit. The ELISA plates received their standards and serum samples followed by optical density (OD) value detection through microplate reader after blank well regulation to zero level. The mathematical analysis of serum analyte levels happened through examination of their detected OD readings against standard curves made from known concentrations.

### **Statistical analysis**

Statistical differences between control and experimental groups were evaluated with the two-tailed independent Student's t-test while data remained displayed as mean  $\pm$  standard error (SE). The statistical tests performed with GraphPad Prism software identified significance when  $p < 0.05$ .

### **Results and Discussion**

In current study, the findings of antioxidants, lipid peroxidation and lipid profile for rats of experimental group were varied significantly ( $p < 0.05$ ). In experimental group, the results of antioxidant including CAT ( $5.419 \pm 0.363$  pg/ml), GPX ( $24.533 \pm 1.317$  IU/ml) and SOD ( $4.013 \pm 0.479$  U/ml) were showed a significant reduction ( $p < 0.0018$ ,  $0.0001$ , and  $0.0008$ , respectively) when compared to those of control group ( $7.406 \pm 0.426$  pg/ml,  $33.833 \pm 1.136$  IU/ml, and  $7.387 \pm 0.757$  U/ml, respectively), (Figures 1-3)

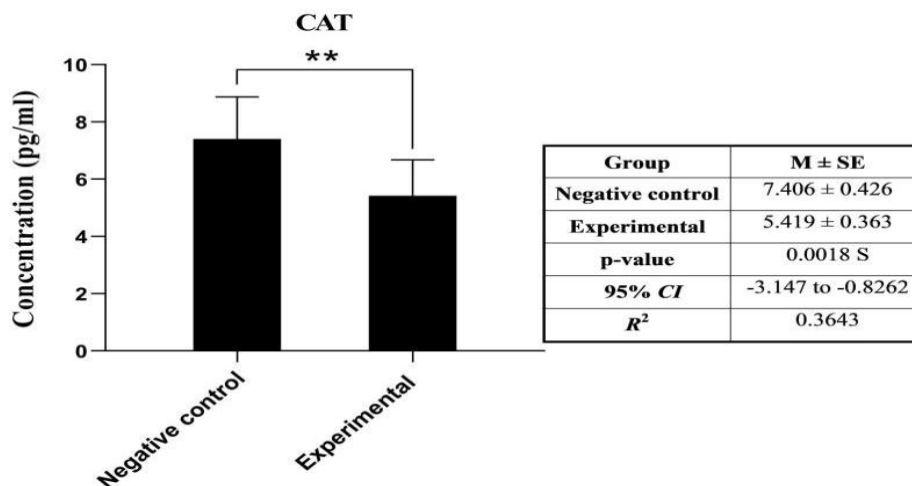


Figure 1. Levels of CAT among the study rats of experimental and negative control groups

The data presented in Figure 1 show a statistically significant reduction in catalase activity in the experimental group ( $5.419 \pm 0.363$  pg/ml) compared to the control group ( $7.406 \pm 0.426$  pg/ml;  $p < 0.0018$ ). This decline highlights a compromised antioxidant defense system in rats exposed to contaminated surface water. Catalase plays a pivotal role in neutralizing hydrogen peroxide, a reactive oxygen species (ROS). Its suppression implies that oxidative burden exceeded the enzymatic capacity of the cells, leading to possible accumulation of peroxides and increased risk of oxidative damage. Critically, the findings suggest that environmental contaminants in the water—possibly heavy metals or organic toxins—may have interfered with the transcriptional regulation or activity of catalase, thereby amplifying cellular vulnerability to oxidative stress.

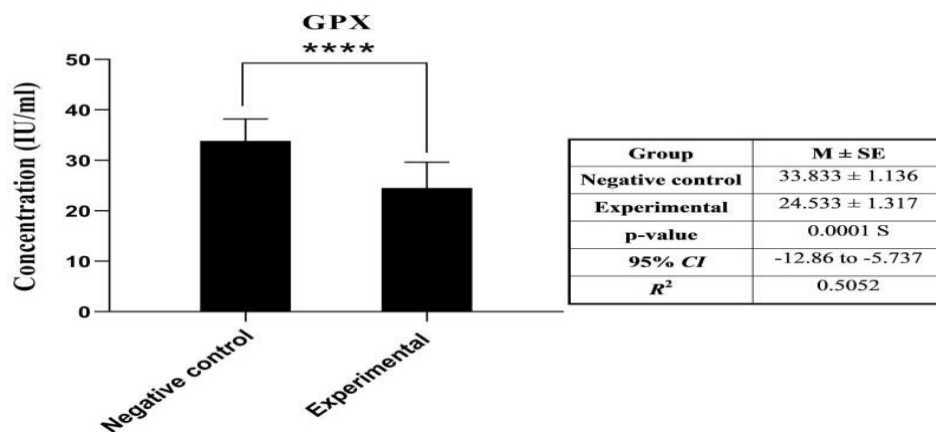


Figure 2. Levels of GPX among the study rats of experimental and negative control groups

The experimental group displayed a deep reduction in GPX activity at  $24.533 \pm 1.317$  IU/ml as compared to the control group at  $33.833 \pm 1.136$  IU/ml ( $p < 0.0001$ ). As a selenium-dependent enzyme GPX serves to detoxify peroxides through the reduced glutathione substrate. A significant decrease in enzyme activity shows that both enzyme depletion and reduced glutathione content and selenium accessibility face challenges as toxic chemicals found in the environment. Rats that drank contaminated water developed oxidative stress which heavily impacted their enzymatic detoxification pathway as shown by the significant reduction in GPX activity.

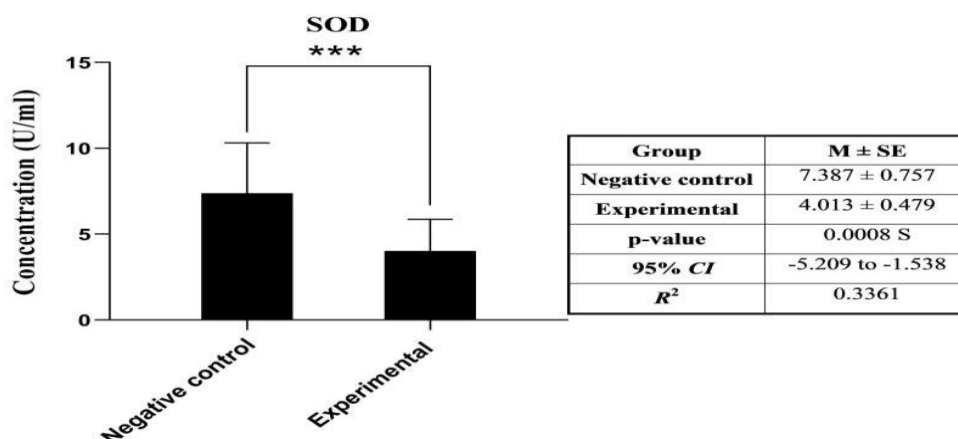


Figure 3. Levels of SOD among the study rats of experimental and negative control groups

The experimental group-maintained SOD activity at reduced levels of  $4.013 \pm 0.479$  U/ml compared to the control group with  $7.387 \pm 0.757$  U/ml as demonstrated by the  $p < 0.0008$  value. SOD works as the first-line antioxidant enzyme to convert superoxide radicals into

oxygen and hydrogen peroxide through the process of dismutation. A marked reduction in redox balance exists in experimental animals because this imbalance makes them more likely to suffer cellular and mitochondrial damage from superoxide radicals. Experimental contaminants seem to restrict SOD synthesis and enable enzyme breakdown according to critical evaluation. Oxidative injury becomes more severe because antioxidant network failures build upon each other due to this reduction.

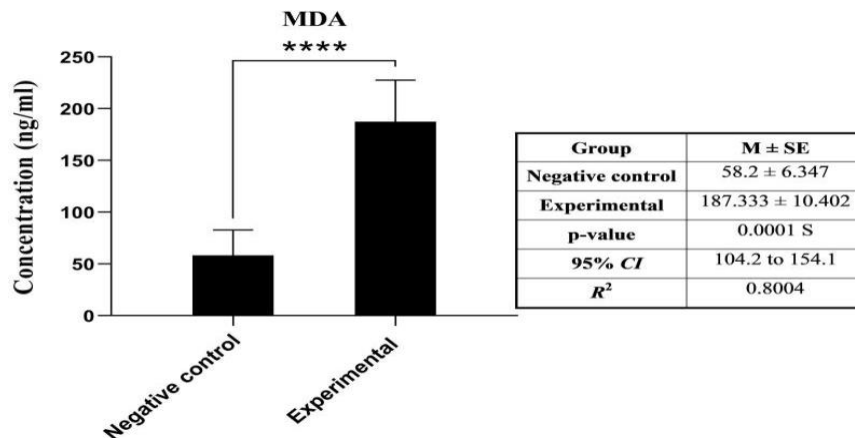


Figure 4. Levels of MDA among the study rats of experimental and negative control groups

The MDA levels differed substantially between the experimental group at  $187.333 \pm 10.402$  ng/ml and the control group at  $58.2 \pm 6.347$  ng/ml with a highly significant statistical value of  $p < 0.0001$ . Research has firmly established MDA as a well-accepted indicator of cell membrane damage through lipid peroxidation processes. Excessive lipid peroxidation developed from contaminated water consumption because these results indicate substantial MDA elevation. Cell structures remain unstable and cell communication becomes disrupted due to membrane disruptions that result from high MDA levels which simultaneously indicate continuous oxidative damage to cellular components. The nearly threefold increase emphasizes the toxic potential of the brick kiln-contaminated water, possibly through a synergistic mix of heavy metals and organic pollutants.

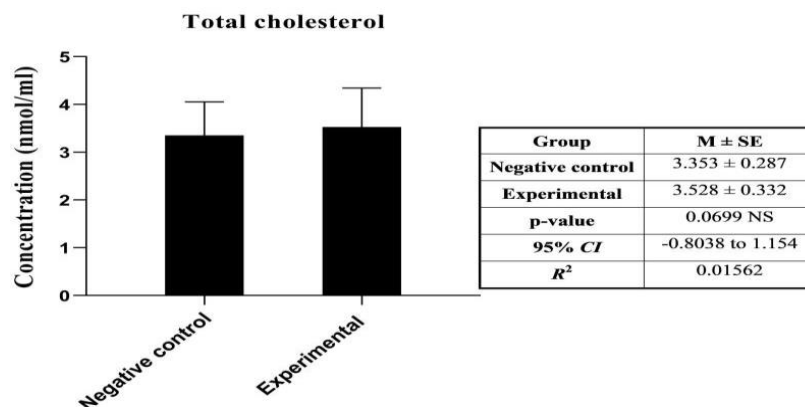


Figure 5. Levels of TC among the study rats of experimental and negative control groups

Total cholesterol levels in experimental group ( $3.528 \pm 0.332$  nmol/ml) surpassed control group measurements ( $3.353 \pm 0.287$  nmol/ml) yet the variance remained insignificant according to statistical analysis ( $p = 0.0699$ ). The absence of significant changes in total cholesterol reading suggests minimal disturbance in hepatic lipid synthesis occurs. Viewed from a critical angle this result could reflect two possible compensatory mechanisms which might include

interrupted lipid transport or feedback control over cholesterol production. The response of cholesterol concentrations might not be quick when exposed to oxidative stress but patients should monitor their cholesterol levels regularly after a period of exposure. The modest increase, albeit insignificant, warrants cautious monitoring in future longitudinal studies.

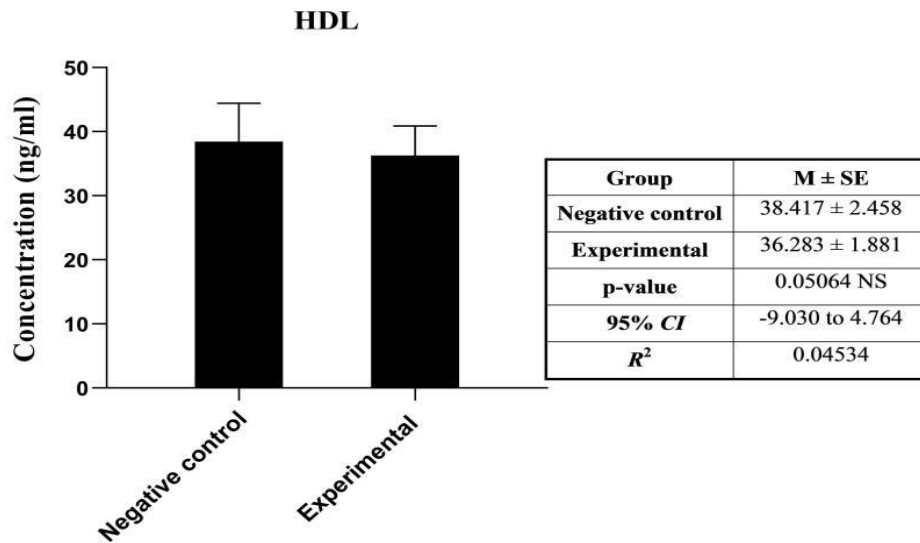


Figure 6. Levels of HDL among the study rats of experimental and negative control groups

The experimental group under oxidative stress had a minimal yet statistically insubstantial decrease in HDL levels than the control group ( $36.283 \pm 1.881$  ng/ml compared to  $38.417 \pm 2.458$  ng/ml;  $p = 0.05064$ ). Studies have shown that the protective cardiovascular mechanism and antioxidant characteristics belong to HDL. Statistically the decline in levels was not significant yet observations suggest that the downward direction might show biological importance for beginning dyslipidemia. Minimal changes in HDL levels might reveal weakened abilities for reverse cholesterol transport and systemic antioxidative defense mechanisms especially when oxidative stress is high like in this study.

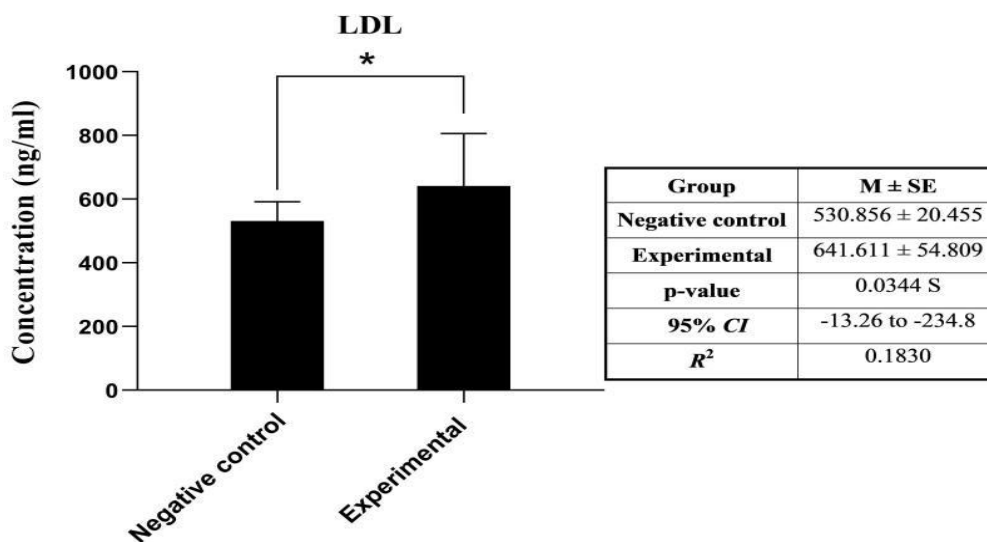


Figure 7. Levels of LDL among the study rats of experimental and negative control groups

The experimental group revealed elevated LDL levels at  $641.611 \pm 54.809$  ng/ml compared to the control group at  $530.856 \pm 20.455$  ng/ml according to the  $p < 0.0344$  value. There is

significant concern about elevated LDL because oxidized LDL (oxLDL) contributes extensively to atherogenesis while sustaining systemic inflammation. The elevated LDL levels indicate hepatic lipid metabolism and lipoprotein clearance mechanisms faced some kind of disruption. Research data implies that environmental toxins either disrupted hepatic lipids regulatory pathways or caused hepatic oxidative injuries which resulted in increased LDL production and defective LDL receptor activity for clearance.

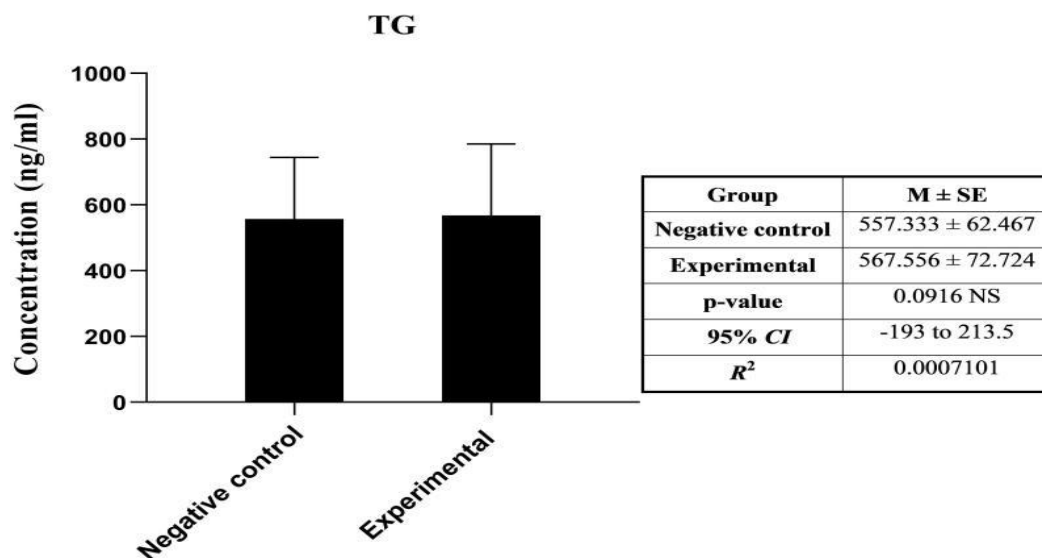


Figure 8. Levels of TG among the study rats of experimental and negative control groups

According to Figure 8 the experimental group exhibited a slight elevation in triglycerides compared to the control at  $567.556 \pm 72.724$  ng/ml against  $557.333 \pm 62.467$  ng/ml ( $p = 0.0916$ ). An increasing trend in triglyceride levels showed similarities between hepatic fat production and dysfunctional lipoprotein lipase function under stressful conditions although the results remained non-significant. The obtainable results from short-term exposure or biological compensation likely explain why investigators did not detect significant differences. The increasing oxidative conditions in the environment might result in hypertriglyceridemia when exposure extends for a prolonged period. The findings indicate that researchers need to extend their studies to measure how multiple waterborne pollutants affect the body's metabolic system.

The brick kiln industry plays a crucial role in the economic development of many regions, providing employment and contributing to the construction of essential infrastructure. However, the industry's impact on the environment and public health particularly through the contamination of water resources has emerged as a significant concern (Hossain et al., 2019). In this study, the findings revealed that the contaminated water by the residents of brick kilns can effectively cause oxidative stress by decreasing the concentrations of antioxidants and increasing the levels of lipid peroxidation MDA and LDL. Worldwide, several researchers have been demonstrated the association of environmental contamination to oxidative stress and the effect of environmental contaminants on the body's functions (Mathur & D'cruz, 2011; Fathy et al., 2012; Navid et al., 2021; Shen et al., 2022; Christensen & McCormick, 2023).

However, data concerning the risk of contaminated environments were varied significantly between the various studies in following the type of industry emissions, area / country, and demographic data. Occupational agents may also act great roles through their affecting on growth and body systems of human populations (Wilcock and Hocking, 2024). Evidences suggested that agents such as types of burned fuels, types of works, loss of protective tools,

and periods of works are correlated with the pulmonary diseases in various workers (Vlahovich & Sood, 2021; Nishida & Yatera, 2022).

In Croatia, Zuskin et al. (2008) reported that the workers of brick kilns are greatly exposed to the heavy metals with large incidence of respiratory diseases like chronic cough (31.8%), chronic phlegm (26.2%), and chest tightness (24%). In Pakistan, Shahid et al. (2015) reported that the almost brick kilns used the woody materials or coaled-origin substances to bake of brick that making brick kilns occupations are high susceptibility for great exposing to air pollutants as well as their adversely healthy impacts. Kim et al. (2018) evaluated that exposing woody smoke is correlated to ~70% of increasing hazardous for populations that have chronically obstructive respiratory disorders. The lack of protective equipment during work may effectively increase the risk of exposure to brick kilns (Gaviola et al., 2024). For this reason, workers whose coming in direct contacts with xenobiotic by any routes like skin, respiratory, gastrointestinal, and mucous membranes may combine with various degrees of poor conditions as well as for a gradient heat were having an elevated risk for health of populations (Riviere, 2011; Marimuthu & Schätzlein, 2013; Gomes & Silva, 2021).

The hierarchy of hazard controls emphasizes the importance of utilizing, removal, and alternative tools that are available possibly prior to search of engineering and governmental controlling basis (Kousar et al., 2025). Personal protective equipment existed at the low level of sequence due to users should be actively engaged in prevention. This equipment could be unprofitable for wearing, reducing efficacy of working processes, and wore incompletely (Saha et al., 2020; Gaviola et al., 2024). Nonetheless, workers must be subjected for suitable education about the methods that properly provide protective equipment for effective controls of existed risks (Kekana, 2021).

Burned fossil fuel was detected as the main source for pollution of atmosphere and different types of environments (Perera, 2018). In developed cities, brick kilns represented the main significant sources for air pollutants and summarized that the potential air pollutants caused by brick kilns among rural regions represented actual big trouble for the health of humans, animals, and plants (Rajarithnam et al., 2014). Heavy metals appeared to be significantly very important as a source of toxics originated from brick kilns and greatly still exist in natural environments causing a wide degradation in biological materials and serious threats for living organisms (Parvez et al., 2023).

In general, heavy metal is systemically has high toxicity for nervous system, fetus and reproductive system, and have effectively a carcinogenic impact (Goswami & Neog, 2023). Heavy metals can be influenced on behaviors through impairment of mentality and intervention with nervous functions, altering many metabolites in body's system, and influences on synthesis and using of neural transmitters (Khalid et al., 2021). Heavy metals could be caused high toxicity to different body systems as it initiate a significant impairments or dysfunctions for many body systems like urinary and reproductive system, peripheral and central nervous system, immunity, digestive system, ATP-generator pathways, hormonal endocrine system, elimination pathways (skin, kidney, liver, and colon), cardiovascular system, and blood components (Müller et al., 2019; David et al., 2022). Recently, elevated and frequent usage of refined petroleum and their derivatives as well as smelt process for sulfur materials of metal elements have been contributed largely to occurrence of imbalance on the levels of sulfur in environments (Staszak, 2018). Toxicities caused by sulfur are related mostly to the high concentrations of metallic minerals and their elements in addition to the toxicity of volatile materials among the environments (Nieder & Benbi, 2024). Remarkably, hydrogen sulfide has

been detected to be actually very important for physiological roles in many systems of the body (Cirino et al., 2023).

## Conclusion

This study clarified the significant impact of contaminated water by residents of brick kilns on the health status of study rats through significant decreasing of serum antioxidants and increasing of MDA and LDL. However, the resolution of this issue requires a holistic and collaborative approach that dresses the root causes of the problem. Also, government and authorities must develop and implement stringent environmental standards to ensure the effective monitoring and enforcement of these regulations

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