



Downregulation of GPX1 Gene Expression in Electronic Cigarette Users Compared to Non-Smokers

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HIGHLIGHTS

- ❖ GPX 1 gene expression is lower in e-cigarette users compared to non-smokers
- ❖ Antioxidant defense capacity is diminished in individuals exposed to e-cigarette aerosols
- ❖ Molecular oxidative stress is higher in young e-cigarette users despite their age

ABSTRACT

Electronic cigarette (e-cigarette) use is often perceived as a safer alternative to conventional smoking, but its biomolecular impact is poorly understood. Oxidative stress from aerosol exposure is detected through changes in antioxidant gene expression, like Glutathione Peroxidase 1 (GPX1). This quantitative cross-sectional study analyzed 72 blood-derived RNA isolates (36 e-cigarette users; 36 non-smokers) to evaluate peripheral blood leukocyte GPX1 gene expression as a marker of oxidative stress. Relative GPX1 expression was quantified via qPCR normalized to GAPDH using the $2^{-\Delta\Delta Ct}$ method. Groups were compared using the Mann–Whitney U test, unpaired t-test on log10-transformed data, and multivariable linear regression adjusted for age. E-cigarette users showed significantly lower GPX1 expression than non-smokers (median 0.92 vs. 1.65; Mann–Whitney U, $p=0.03$; t-test, $p=0.035$). This difference remained significant in the 18–24-year subgroup ($p=0.041$), with multivariable regression confirming e-cigarette use as an independent predictor of GPX1 downregulation ($\beta=-0.284$, $p=0.038$). These findings suggest that e-cigarette exposure may impair antioxidant defenses and disrupt systemic redox homeostasis.



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INTRODUCTION

Smoking is a primary risk factor for global morbidity and mortality, contributing significantly to preventable non-communicable diseases such as cardiovascular disease, cancer, and chronic respiratory disorders, according to the World Health Organization (WHO) (Roy et al., 2017). In Indonesia, the prevalence of active smokers remains high, particularly among the productive age group (Nugroho et al., 2023). Exposure to toxic substances in cigarettes—including nicotine, tar, and carbon monoxide—is known to increase the production of Reactive Oxygen Species (ROS), which triggers oxidative stress and plays a critical role in the pathogenesis of various chronic diseases (Seo et al., 2023). This condition identifies both conventional and electronic cigarette (e-cigarette) smokers as a biological risk group relevant for further biomolecular investigation.

With the rising use of e-cigarettes, categorized under Electronic Nicotine Delivery Systems (ENDS) and Electronic Non-Nicotine Delivery Systems (ENNDS), these products have gained popularity, especially among youth and non-smokers (World Health Organization, 2024). Globally, the number of e-cigarette users was estimated to reach 68–82 million in 2020–2021 (Jerzy & Stimson, 2023; Jerzyński et al., 2021). Meanwhile, in Indonesia, the prevalence increased significantly from 0.3% in 2011 to 3.0% in 2021 based on the Global Adult Tobacco Survey (CDC Stacks, 2021). The perception that electronic cigarettes are safer than conventional cigarettes, along with cost factors and aggressive marketing, is the primary driver of this increase (Camenga & Klein, 2016; Fadus et al., 2019; Ling et al., 2023). However, thermal degradation of e-liquid components (propylene glycol and vegetable glycerin) generates toxic compounds and pro-oxidants, such as acrolein, formaldehyde, and acetaldehyde, which can induce oxidative stress and tissue damage (Emma et al., 2022; Kosmider et al., 2020; Kuntic et al., 2023).

Oxidative stress is an imbalance between ROS production and the body's antioxidant capacity to neutralize them, resulting in damage to lipids, proteins, and DNA (Pizzino et al., 2017; Fleming & Luo, 2021). ROS accumulation plays a vital role in chronic inflammation and the pathogenesis of cardiovascular disease, cancer, and lung diseases, including Chronic Obstructive Pulmonary Disease (COPD) (Jelic et al., 2021; Miklós & Horváth, 2023; Sies et al., 2025). Specifically, unneutralized ROS reduce Nitric Oxide (NO) bioavailability in the endothelium and activate matrix metalloproteinases (MMPs) in airway tissues. Several studies indicate that e-cigarette users experience increased oxidative stress compared to non-smokers, with biological damage profiles potentially comparable to those of conventional smokers (Carnevale et al., 2016; Christensen et al., 2021). Chronic e-cigarette aerosol exposure is reported to exacerbate redox imbalance through sustained inflammatory mechanisms (Addissouky et al., 2024).

A major component of the cellular antioxidant defense system is the enzyme Glutathione Peroxidase 1 (GPX1), which reduces hydrogen peroxide and lipid peroxides into harmless compounds using reduced glutathione (Handy & Loscalzo, 2022; Lubos et al., 2011). While Superoxide Dismutase (SOD) converts superoxide and Catalase operates primarily at high H_2O_2 concentrations in peroxisomes, GPX1 is a cytosolic selenoprotein with high affinity for baseline H_2O_2 and membrane lipid hydroperoxides (Sharifi-Rad et al., 2020). Alongside Superoxide Dismutase (SOD) and Catalase (CAT), GPX1 forms the first line of defense against ROS and plays a crucial role in maintaining cellular redox homeostasis (Ighodaro & Akinloye, 2018).

Decreased GPX1 expression has been linked to increased susceptibility to oxidative damage and risk of chronic diseases, including cardiovascular, cancerous, and neurodegenerative disorders (Handy & Loscalzo, 2022). While GPX1 gene expression typically increases adaptively under oxidative stress through transcription factors such as Nrf2, this response can be impaired and lead to transcriptional exhaustion

under long-term oxidant exposure (Lubos *et al.*, 2011). Furthermore, electrophilic aldehydes like acrolein directly deplete cellular "GSH" pools via Michael addition, depriving GPX1 of its essential co-substrate.

Most previous research regarding oxidative stress and GPX1 expression has focused on conventional smokers, with evidence showing that tobacco smoke exposure decreases GPX1 expression and worsens oxidative imbalance (Al-Zahrani & Almutairi, 2023). Conversely, data on the effect of electronic cigarettes on GPX1 gene expression remain limited and inconsistent, particularly at the biomolecular level (Vlahos & Bozinovski, 2013). This limitation highlights a research gap concerning the impact of e-cigarette use on endogenous antioxidant systems, specifically GPX1, essential for understanding the long-term biological risks of e-cigarette aerosol exposure. As mature erythrocytes lack nuclei, measuring whole-blood GPX1 mRNA reflects transcript levels in circulating leukocytes, exposed to inhaled aerosol toxicants via direct pulmonary capillary diffusion and indirect systemic inflammatory cytokine release (Gharib *et al.*, 2021).

Therefore, this study aims to analyze differences in Glutathione Peroxidase 1 (GPX1) gene expression in peripheral blood leukocytes between electronic cigarette users and non-smokers using quantitative real-time PCR (qPCR) to identify potential oxidative stress and its contribution to health risks from electronic cigarette use.

MATERIALS AND METHODS

Materials and equipment

The materials used in this study include stored biological materials (SBM) in the form of total RNA isolates (A260/A280 ratio 1.8–2.0) derived from peripheral blood (predominantly leukocyte populations, as mature erythrocytes lack nuclear DNA and mRNA transcription machinery) of e-cigarette users and non-smokers, sourced from the Biomolecular Laboratory Biobank, Faculty of Medicine, Universitas Trisakti. The molecular reagents consist of the SensiFAST cDNA Synthesis Kit (Bioline, Meridian Bioscience Inc., USA) for reverse transcription and the SensiFAST SYBR No-Rox Kit (Bioline, Meridian Bioscience Inc., USA) for real-time PCR amplification. Specific primers were utilized for the target gene Glutathione Peroxidase 1 (GPX1) and the reference gene Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as written in **Table 1**. Additional laboratory consumables include nuclease-free water (NFW), 0.2 mL PCR tubes, filter pipette tips (10 µL, 20 µL, 200 µL, and 1000 µL), and disposable nitrile gloves.

The equipment employed in this research consists of a deep freezer (-80°C) for long-term storage of biological samples, a NanoDrop spectrophotometer (or similar micro-volume UV-Vis spectrophotometer) to assess RNA concentration and purity, and a refrigerated centrifuge for processing molecular samples. The amplification and detection were performed using the Rotor-Gene 3000 (Qiagen, Germany) real-time PCR thermal cycler. Other essential laboratory tools include a vortex mixer for homogenizing reagents, a minicentrifuge for quick spin-downs, and calibrated micropipettes (One Touch, Sorenson BioScience, USA) for precise volume handling. Data analysis was conducted using a workstation equipped with Rotor-Gene Software and SPSS Statistics.

Table 1. Sequence of forward and reverse primer pairs used in this study

Primer	Sequence (5' → 3')
GPX1 F1	GTG CTC GGC TTC CCG TGC AAC
GPX1 R1	CTC GAA GAG CAT GAA GTT GGG C

Primer	Sequence (5' → 3')
GAPDH F	GTC TCC TCT GAC TTC AAC AGC G
GAPDH R	ACC ACC CTG TTG CTG TAG CCA A

Methods

This study is a quantitative non-experimental study with a cross-sectional design. Research was conducted at the Biomolecular Laboratory, Faculty of Medicine, Universitas Trisakti, West Jakarta, from September to December 2025, utilizing stored biological materials (SBM) in the form of RNA isolates derived from a previous study. The study analyzed 72 peripheral blood RNA isolates obtained from subjects of productive age (20–55 years), comprising 36 e-cigarette users and 36 non-smokers. All samples were obtained from the biological biobank of the Biomolecular Laboratory, Faculty of Medicine, Universitas Trisakti, and stored at -80°C. Inclusion criteria included RNA quality meeting biomolecular standards (A260/A280 ratio of 1.8–2.0) and, for the e-cigarette group, a history of e-cigarette use for at least 6 months. Subjects with hypertension, diabetes mellitus, or a history of heart disease or kidney failure were excluded from the study. Sample size calculation was performed a priori using G*Power software version 3.1.9.6 based on an independent t-test approach, assuming a medium-to-large effect size ($d = 0.736$), $\alpha = 0.05$, and 80% power. Accounting for a 15% dropout rate, the final sample size was set at 36 subjects per group. The sampling technique employed was consecutive sampling based on the availability of stored biological materials.

cDNA synthesis was performed using the SensiFAST cDNA Synthesis Kit from total RNA that met the purity criteria. Expression analysis of the Glutathione Peroxidase 1 (GPX1) gene was conducted using quantitative real-time PCR (qPCR) with the SensiFAST SYBR No-Rox Kit on a Rotor-Gene 3000 (Qiagen). The GAPDH gene served as the reference gene for data normalization. To verify that observed changes were not driven by fluctuations in reference gene expression under ROS stress, internal reference gene stability was rigorously evaluated prior to relative quantification; raw quantification cycle (CT) values for GAPDH were verified across all samples ($CT = 21.42 \pm 0.48$), with no significant difference between e-cigarette users (21.45 ± 0.45) and non-smokers (21.39 ± 0.51 ; $p = 0.624$). Each sample was analyzed in duplicate to minimize technical variation.

Gene expression data were analyzed using the $2^{-\Delta\Delta Ct}$ method to determine the relative expression of GPX1. Intra-assay variation between duplicates was maintained at $\Delta Ct < 0.2$. Statistical analysis was performed using SPSS Statistics (IBM Corp., Armonk, NY, USA). Data distribution was assessed using the Shapiro–Wilk test, which revealed a non-normal distribution ($p < 0.001$). Consequently, primary between-group comparisons of relative expression were evaluated using the non-parametric Mann–Whitney U test. Logarithmic transformation ($\log_{10}$) was additionally performed on fold-change values to satisfy normality assumptions, followed by a parametric independent two-sample unpaired t-test.

Furthermore, a subgroup analysis was conducted specifically within the age-matched young adult cohort (aged 18–24 years; $n = 35$ e-cigarette users vs. $n = 12$ non-smokers) using the Mann–Whitney U test to eliminate baseline generational bias. Finally, to control for potential age-related confounding across the entire sample size, multiple linear regression analysis was performed on $\log_{10}$ -transformed relative expression values ($\log_{10}(2^{-\Delta\Delta Ct})$), utilizing group status as the primary independent variable and age as a continuous covariate. Statistical significance was defined as $p < 0.05$.

RESULTS

Participant characteristics

A total of 72 subjects were included in this study, divided equally into e-cigarette users ($n = 36$) and non-smokers ($n = 36$). The demographic profile is summarized in **Table 2**. In the e-cigarette group, most participants were aged 18–24 years (97.2%), whereas the non-smoker group showed a more distributed age range, with 47.2% of subjects aged over 35 years. Both groups were predominantly male, representing 88.9% of the e-cigarette group and 77.8% of the non-smoker group.

Table 2. Baseline demographic characteristics of e-cigarette Users and non-smokers

Characteristics	Electric Smokers (n=36)		Non-smokers (n=36)	
	n	%	n	%
Age				
18 – 24 years	35	97.2	12	33.3
25 – 34 years	1	2.8	7	19.4
> 35 years	0	0	17	47.2
Gender				
Male	32	88.9	28	77.8
Female	4	11.1	8	22.2

Differential GPX1 gene expression

Prior to relative quantification, internal reference gene stability was verified across all subjects. Raw quantification cycle (CT) values for GAPDH showed no statistically significant difference between e-cigarette users (21.45 ± 0.45) and non-smokers (21.39 ± 0.51 ; $p=0.624$), confirming that GAPDH expression remained stable and unaffected by aerosol exposure or pro-oxidant status.

Relative GPX1 gene expression levels were normalized against GAPDH and calculated using the $2^{(-\Delta\Delta CT)}$ method. Because of individual variability and extreme biological values—specifically an outlier sample in the e-cigarette group with a minimum relative expression of 0.00026 (**Figure 1**)—the data departed significantly from a normal distribution (Shapiro–Wilk test, $p<0.001$). Primary statistical evaluation using the non-parametric Mann–Whitney U test demonstrated a statistically significant downregulation of GPX1 gene expression in the electronic cigarette group compared to non-smokers ($p=0.030$). The e-cigarette user group had a lower median relative expression level of 0.92 (range: 0.00026–11.47; IQR: 0.28–2.85), whereas the non-smoker group had a median relative expression level of 1.65 (range: 0.000003–1239.03; IQR: 0.41–8.92). These findings are detailed in **Table 3** and visually depicted in the box plot in **Figure 1**.

Table 3. Comparison of relative GPX1 gene expression levels between groups

Electric Smokers	Non Smokers	P Value
0.92 (0.00026 – 11.47)	1.65 (0.000003 – 1239.03)	0.030

Note: Data were presented as median (Min-Max). Mann-Whitney test was used for statistical analysis. P<0.05 was considered significant.

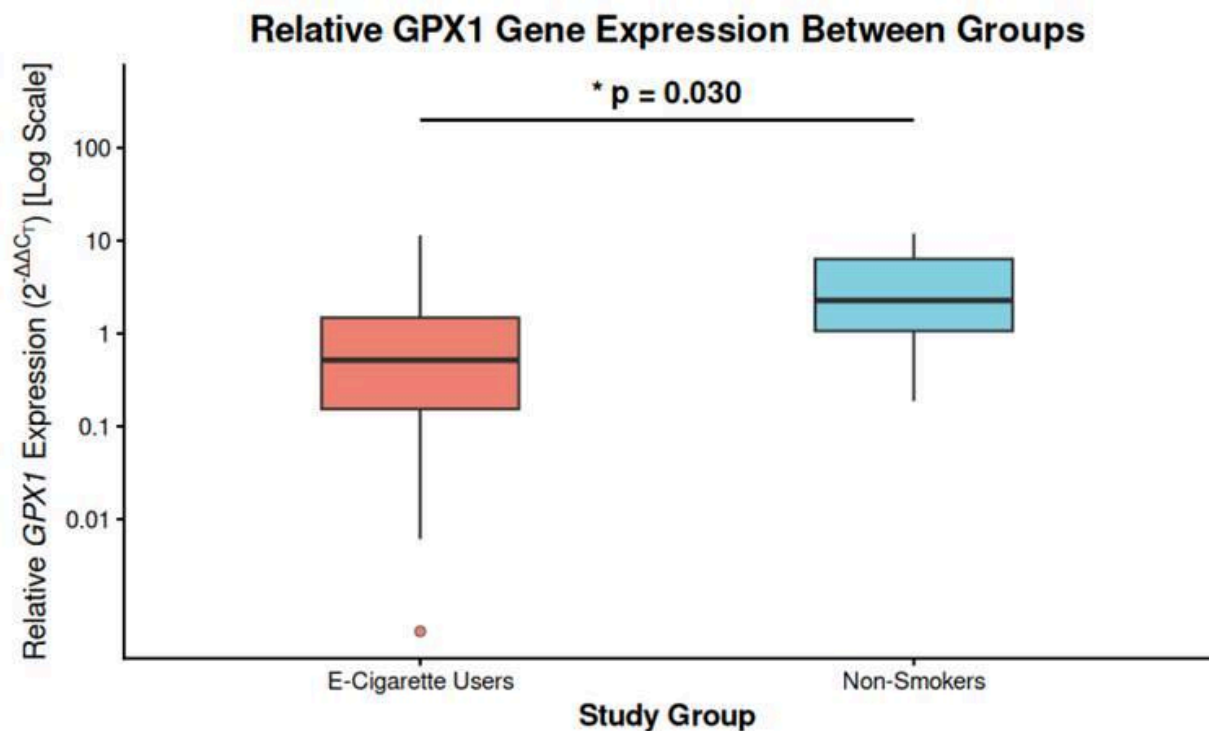


Figure 1. Relative GPX1 gene expression ($2^{-\Delta\Delta C_T}$) in peripheral blood leukocytes between electronic cigarette users (n=36) and non-smokers (n=36). Data are presented on a logarithmic scale ($\log_{10}$) due to non-normal distribution. The horizontal solid line inside each box represents the median relative expression level (0.92 in the e-cigarette group vs. 1.65 in non-smokers). The lower and upper box boundaries denote the 25th and 75th percentiles (Interquartile Range, IQR), respectively. Whiskers represent 1.5×IQR. The individual plotted dot below the e-cigarette box plot represents an extreme biological sample outlier exhibiting marked transcriptional suppression (0.00026). Statistical significance was evaluated using the non-parametric Mann–Whitney U test (*p=0.030).

To address analytical biases and statistical concerns regarding data skewness and demographic variation, we conducted additional parametric and subgroup analyses. First, we performed the parametric comparison on transformed data by logarithmically transforming ($\log_{10}$) the $2^{-\Delta\Delta C_T}$ values to normalize the data distribution. An independent two-sample Unpaired t-test on $\log_{10}$ -transformed expression confirmed a statistically significant reduction in GPX1 expression among e-cigarette users compared to non-smokers (-0.24 ± 0.81 vs. 0.18 ± 0.89 ; $t=-2.146$, $p=0.035$).

Next, we analyzed the age-matched subgroup to eliminate age mismatch as a potential confounder. The subgroup analysis was conducted exclusively within the young adult cohort aged 18–24 years (n=35 e-cigarette users; n=12 non-smokers). Within this age-matched group, GPX1 expression remained significantly suppressed in e-cigarette users compared to non-smokers (Median: 0.90 vs. 2.11; Mann–Whitney U test, $p=0.041$).

Lastly, we performed a multiple linear regression on $\log_{10}(2^{-\Delta\Delta CT})$ values, incorporating age as a continuous covariate, which demonstrated that e-cigarette use was a statistically significant independent predictor of lower GPX1 gene expression ($\beta=-0.284$, $p=0.038$). Age showed no statistically significant association with GPX1 expression ($\beta=0.102$, $p=0.412$), confirming that transcript suppression is driven by vaping exposure rather than age differences.

DISCUSSION

This study investigated how electronic cigarette use influences the endogenous antioxidant system to understand the molecular implications of vaping. Our primary finding confirms a statistically significant downregulation of GPX1 gene expression in e-cigarette users compared to non-smokers ($p = 0.030$). As depicted in **Figure 1**, the median relative expression of GPX1 in the e-cigarette group was significantly lower (0.92; IQR: 0.28–2.85) compared to the non-smoker control group (1.65; IQR: 0.41–8.92). This reduction indicates diminished cellular defense against oxidative stress and suggests that chronic exposure to e-cigarette aerosols may compromise the molecular mechanisms responsible for maintaining redox homeostasis (Chitteti et al., 2025).

Biologically, GPX1 acts as a primary antioxidant enzyme that neutralizes hydrogen peroxide and lipid hydroperoxides using glutathione as an electron donor (Lubos et al., 2011). Because mature erythrocytes lack nuclei, whole-blood GPX1 transcripts predominantly reflect gene expression within circulating peripheral blood leukocytes. The downregulation of GPX1 observed here may be linked to the postulated impairment of the Nrf2 (Nuclear factor erythroid 2-related factor 2) signaling pathway. [n6.1]While Nrf2 was not directly assayed in this study, existing literature indicates that it normally upregulates antioxidant response elements (ARE) during acute stress but can become suppressed or "exhausted" under chronic chemical exposure such as reactive aldehydes from e-liquids. Lower GPX1 expression in e-cigarette users potentially signifies chronic oxidative stress that exceeds the antioxidant system's ability to compensate optimally (Geraghty et al., 2016). This reduction in enzymatic defense specifically within the pulmonary environment and systemic circulation may increase the vulnerability of airway epithelial cells and leukocytes to oxidative damage and subsequent inflammatory remodeling. This interpretation aligns with our objective to identify biological risks, indicating that vapers may exist in a state of persistent oxidative vulnerability, increasing their risk for inflammatory airway remodeling.

The age distribution in this study reveals a clear distinction between the two groups. The e-cigarette group is dominated by respondents aged 18–24 years (97.2%), whereas the non-smoker group exhibits a more heterogeneous age distribution, with 47.2% of subjects aged over 35 years. This pattern aligns with previous reports that e-cigarette use is most prevalent among youth, influenced by social factors, media exposure, and a lower perception of risk compared with conventional cigarettes (Jerzy & Stimson, 2023; Jerzyński et al., 2021; McCauley et al., 2022). Notably, downregulation of antioxidant genes in a predominantly young e-cigarette cohort is particularly concerning, as younger individuals typically possess a more robust adaptive capacity compared to older populations. To ensure that age discrepancy did not bias our findings, multivariable linear regression incorporating age as a covariate confirmed that e-cigarette use remained a significant independent predictor of lower GPX1 expression ($\beta=-0.284$, $p=0.038$).

Age and gender modulate oxidative stress and cellular responses to pro-oxidant exposure (Donohue, 2006; Halstead et al., 2023); thus, these demographic factors provide critical context for interpreting the observed biological risks. Regarding gender, the majority of respondents in the e-cigarette group were male (88.9%), with a similar pattern observed in the non-smoker group. These findings align with literature showing that the prevalence of tobacco and e-cigarette product use is consistently higher

among males, driven by social, psychological, and environmental factors (El Shahawy et al., 2020). These results align with previous findings reporting decreased expression or activity of the antioxidant system in e-cigarette users compared with non-smokers (Cichońska et al., 2021; Emma et al., 2022). Although e-cigarette aerosols produce fewer free radicals than conventional cigarettes, repeated exposure can still increase reactive oxygen species (ROS) production and disrupt the regulation of antioxidant biomarkers, including GPX1 (Seo et al., 2023).

Despite these findings, this study has limitations that must be acknowledged. There is a notable age discrepancy between the groups; the e-cigarette cohort was predominantly young adults (97.2% aged 18–24), whereas the non-smoker group was more heterogeneous and generally older. While older age is typically associated with increased oxidative stress, the younger group (vapers) exhibited significantly lower GPX1 levels, which is particularly striking, suggesting that chronic aerosol exposure induces profound transcriptional exhaustion and overwhelms an antioxidant system that should naturally operate at peak capacity. Additionally, this study evaluated GPX1 strictly at the transcriptomic level without measuring systemic selenium cofactors, differential leukocyte counts, or downstream functional enzymatic activity.

Overall, this study demonstrates that e-cigarette use is associated with a downregulation of GPX1 gene expression, reflecting a potential disruption of oxidative homeostasis at the cellular level. While many perceive e-cigarettes as a "harm reduction" tool, this study provides evidence that they are not biologically neutral and can disrupt gene expression at a fundamental level. For clinical practice and policy, these results support more stringent public health warnings and suggest that GPX1 could serve as a molecular biomarker for early-stage lung injury in vapers. These findings confirm that e-cigarettes are not a neutral exposure regarding the body's antioxidant system and support the need for further longitudinal research and evaluation of additional oxidative stress biomarkers to strengthen the biological implications of these findings.

Future research should address the gaps left by this cross-sectional study. A longitudinal approach is needed to determine if this downregulation is reversible upon cessation of e-cigarette use. Furthermore, investigating the protein levels and enzymatic activity of GPX1 and other phase II enzymes (such as SOD and Catalase), alongside differential leukocyte profiling and systemic selenium status, would provide a more comprehensive view of the systemic antioxidant capacity. Investigating different e-liquid flavors and nicotine concentrations would also help clarify which specific components are most responsible for this molecular suppression.

CONCLUSION

In conclusion, this study demonstrates a statistically significant transcriptional downregulation of the Glutathione Peroxidase-1 (GPX1) gene expression in peripheral blood leukocytes of e-cigarette users compared with non-smokers ($p=0.030$; median expression: 0.92 vs. 1.65). This significant reduction remained consistent across multiple analytical frameworks, including parametric comparison of transformed data ($p=0.035$), an age-matched subgroup analysis among young adults ($p=0.041$), and multivariable linear regression controlling for age ($\beta=-0.284$, $p=0.038$). Mechanistically, these findings indicate that chronic exposure to electronic cigarette aerosols—and the resulting reactive aldehyde-induced depletion of intracellular reduced glutathione ("GSH")—impairs essential enzymatic hydroperoxide detoxification capacity, leading to transcriptional exhaustion. Overall, this research provides concrete biomolecular evidence that vaping disrupts systemic redox homeostasis in circulating leukocytes, posing potential long-term risks for cardiovascular and respiratory pathogenesis.

AUTHOR CONTRIBUTIONS

JSK: Formal analysis, Investigation, Writing – Original Draft. **MDH:** Data curation, Supervision, Writing – Review and editing.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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ADDITIONAL INFORMATION

None.

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