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Effect of Chronic Smoking on Plasma Antioxidant Enzyme Activity

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Abstract. *Oxidative stress is caused by regular smoking. If oxidative stress is higher than the level of antioxidants, there is said to be oxidative stress. SOD, CAT and GPx enzymes in the plasma help to reduce the harm caused by oxidative stress. This study aimed to discover if smoking regularly had any impact on the enzymes found in adults. A total of 120 people, with 60 chronic smokers and 60 nonsmokers, took part in the study and each group of 60 had equal numbers of men and women. All donors of blood and plasma had their levels of SOD, CAT and GPx checked using regular spectrophotometric procedures. I carried out the analysis using SPSS software version 26.0. The levels of SOD (1.82 ± 0.29 U/mL), CAT (31.5 ± 4.8 IU/mL) and GPx (3.21 ± 0.52 IU/mL) were lower in smokers compared to non-smokers. The gender levels showed the same effect on the study results as the smoking levels and did not interact with one another. Generally, regular smoking can decrease the amount of this enzyme working in plasma, so the body becomes less able to protect itself from oxidation for both men and women. It indicates that smoking leads to a health issue and advises taking actions to decrease risks for smokers.*

Keywords: *Chronic Smoking, Antioxidant Enzymes, Superoxide Dismutase, Catalase, Glutathione Peroxidase, Oxidative Stress, Plasma Biomarkers, Gender Comparison, Cigarette Smoking.*

1. INTRODUCTION

A state of oxidative stress happens when the body's reactive oxygen species and antioxidants get out of balance and start playing a crucial role in the onset of many chronic conditions (Janciauskiene, 2020). SOD, CAT and GPx enzymes are the first to intervene, rid cells of ROS and thus keep biomolecules from any damage caused by reactive oxygen species (ICE).

Among global health problems, tobacco smoking is a major cause of excessive stress and extra damage to various organs (World Health Organization, 2021). Tobacco smoke is loaded with thousands of toxic chemicals, such as free radicals and reactive aldehydes that have a direct effect on antioxidant defense mechanisms and induce inflammation (Colsoul et al., 2023). Epidemiological data indicate that smokers are at greater risk of cardiovascular, respiratory, hepatic, and kidney diseases that are predominantly mediated through oxidative stress pathways (Martí-Aguado, Clemente-Sánchez, & Bataller, 2022). Furthermore, the disease burden attributable to smoking globally keeps rising despite sustained efforts in tobacco control (World Health Organization, 2018).

Other than tobacco, alcohol use synergistically amplifies oxidative damage by triggering hepatic oxidative stress and reducing endogenous antioxidants (Contreras-Zentella, Villalobos-Garcia, & Hernandez-Munoz, 2022). The additive role of smoking and alcohol has been evidenced to raise plasma levels of reactive carbonyl species, accentuating the multiplicative effect on oxidative stress biomarkers (Mure et al., 2021). Both of these chemicals also induce tissue injury and impaired repair, observed in clinical disease such as periodontitis,

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where smokers exhibit reduced metallothionein levels, a key antioxidant protein, in gingival crevicular fluid and saliva (Yadav et al., 2021).

Experimental studies along with human research demonstrate how chronic smoking causes an impairment of antioxidant enzyme functions. The antioxidant lycopene shows potential in reducing cigarette smoke effects on enzyme suppression according to research by Rakic and colleagues in 2021. Research shows that epigallocatechin-3-gallate which exists in green tea can help the body protect against cigarette smoke-induced liver and kidney damage through its effects on antioxidant enzyme function (Chen et al., 2020).

A limited number of studies have investigated how smoking affects the activity levels of antioxidant enzymes in contrast with the growing evidence which connects smoking to oxidative stress. Researchers have not yet investigated how female gender and smoking habits alongside other lifestyle elements affect the way antioxidant enzymes function in the body.

The evaluation of chronic smoking effects on plasma antioxidant enzyme activity will determine specific gender-based differences in oxidative status and improve our knowledge about smoking-induced oxidative imbalance.

2. STUDY DESIGN

Through a comparative case-control study design, this research explored how chronic cigarette smoking impacts plasma antioxidant enzyme activity among adult participants. Our research determined whether extended exposure to cigarette smoke influences the essential antioxidant enzyme levels that protect cells from oxidative stress specifically SOD and CAT and GPx. The study evaluated enzyme activity in smokers and non-smokers while considering gender differences to determine how this factor affects the body's response to smoking-induced oxidative stress.

Researchers registered 120 volunteers who participated in this investigation through two equal-sized groups: 60 members of the smoking group and 60 members from the non-smoking group. The research team divided the participants equally between men and women and chose individuals between the ages of 25 and 45. The smoking group participants needed to provide medical records which showed consistent daily consumption of 10 cigarettes for five years to meet the criteria for chronic smokers. The control group included individuals who had never experienced direct or indirect exposure to tobacco smoke.

3. DATA COLLECTION

During the study period of three months, data collection took place at two community health centers and one general hospital laboratory. The research team used purposive sampling to recruit 120 adult participants who met specific inclusion and exclusion criteria. The participants were equally divided into two groups each consisting of 30 males and 30 females who were chronic smokers and non-smokers in order to ensure unbiased gender-specific comparisons.

The researchers selected study volunteers who fell within the age range of 25 to 45 years to minimize age-related variations in oxidative stress levels. The smoking group participants needed to show 10 daily cigarette consumption for five continuous years as their smoking history. People in the non-smoking category had never smoked before and experienced little to no secondhand smoke exposure. The research team enrolled people who had no chronic medical conditions or current medications that might affect oxidative stress indicators.

Participants needed to fill out an extensive questionnaire which required information about personal details along with medical background and lifestyle practices and smoking information for smokers which included both daily cigarette consumption and smoking duration in years. Through these measurements, researchers calculated the pack-year index to be used in the later analysis of correlations.

The study recruited participants who met the following conditions: they were between 25 and 45 years old and smokers had to show documented smoking habits that matched the research specifications. The participants who demonstrated the exclusion criteria comprised patients with diabetes mellitus and hypertension as well as cardiovascular disease and liver or kidney disorders and people who experienced recent infections or inflammatory diseases and those who consumed antioxidant supplements and excessive alcohol and pregnant or breastfeeding women. The chosen criteria were designed to prevent any elements which could independently impact antioxidant enzyme levels from entering the study.

The clinical laboratory designated participants after confirming their consent and eligibility status for blood sample collection scheduling. The clinical laboratory conducted blood sample collection between 8:00 AM and 10:00 AM after participants fasted for 10–12 hours to stabilize their metabolic processes. Each participant provided 5 milliliters of venous blood through sterile vacutainer tubes treated with EDTA solution. The laboratory team placed the samples on ice immediately after collection before moving them to the central lab for processing.

The blood samples received treatment through centrifugation at 3000 rpm for 15 minutes at 4°C to isolate plasma which was placed into cryotubes maintained at −80°C for upcoming biochemical research. Future antioxidant enzyme activity assays maintained their reliability and consistency through the use of standardized collection and storage protocols.

Table 1. Data Collection Summary

Variable	Details
Study Duration	3 months
Total Participants	120
Groups	Smokers (n = 60), Non-Smokers (n = 60)
Gender Distribution	30 males and 30 females in each group
Age Range	25 – 45 years
Smoking Criteria (Group A)	≥10 cigarettes/day for ≥5 years
Non-Smoking Criteria (Group B)	No history of active or passive smoking
Inclusion Criteria	Age 25–45, willing participants, meeting smoking/non-smoking requirements
Exclusion Criteria	Chronic illness, supplement use, alcohol abuse, pregnancy, recent infection
Data Collection Tool	Structured questionnaire & laboratory sampling
Blood Sample Volume	5 mL venous blood
Sample Collection Time	8:00 AM – 10:00 AM after 10–12 hours fasting
Sample Handling	EDTA tubes, centrifuged at 3000 rpm for 15 mins at 4°C
Plasma Storage	−80°C until biochemical analysis

4. SAMPLE COLLECTION

After participants finished answering the questionnaire and their eligibility was confirmed, each person was assigned a time for their blood sample collection at an established clinical laboratory site. Blood samples were obtained from all participants specifically during the morning during the period of 8:00 AM to 10:00 AM for uniformity in the sample population. The subjects were requested to maintain an overnight fasting period of 10 to 12 hours before sample collection to eliminate the influence of recent diet on oxidative stress markers.

5 milliliters (mL) of venous blood was collected under sterile conditions from a total of 120 subjects (60 smokers and 60 non-smokers) in EDTA-treated vacutainer tubes. Samples were gently inverted several times immediately after sampling to avoid coagulation and kept in ice. Samples were transported to the central research laboratory under cold chain conditions to maintain sample integrity.

In the lab, centrifugation of the samples was done at 3000 rpm for 15 minutes at 4°C for the separation of plasma from the cellular fractions. The thus separated plasma was transferred into sterile labeled cryotubes. For enzyme stability, plasma samples were all kept at −80°C in ultra-low temperature freezers until further subjected to biochemical analysis.

5. LABORATORY ANALYSIS

Plasma activities of three major antioxidant enzymes were quantified through biochemical analyses: superoxide dismutase (SOD), catalase(CAT) and glutathione peroxidase (GPx). All assays were done in triplicates for better accuracy and decrease inter-assay variability.

Superoxide dismutase (SOD) activity was determined by the Misra and Fridovich method which measures the inhibition of autoxidation of epinephrine in which a alkaline medium was used to disturb the stable autooxidation of epinephrine. Plasma results could be depicted in measured U /ml of plasma

Activity of catalase (CAT) according to the Aebi method, the decomposition rate of hydrogen peroxide (H_2O_2) catalase. At 240 nm, the absorbance was measured using a spectrophotometer and the findings were stated in international units per milliliter.

Biochemical procedures were used to assess the plasma activity levels of SOD, CAT and GPx. For every analysis, triple repeats were included to improve accuracy and reduce differences between experiments.

For measuring SOD activity, Misra and Fridovich took advantage of epinephrine's autoxidation in an alkaline environment. It was expressed in U/mL of plasma.

The method described by Aebi was followed to measure CAT activity by determining how fast it breaks down hydrogen peroxide (H_2O_2). The spectrophotometric readout at 240 nm was converted into international units per milliliter (IU/mL).

6. DATA ANALYSIS

After finishing the sample processing and laboratory assays, all data that collected were coded, and entered into Statistical Package for the Social Sciences (SPSS) 26 for further analysis. The final set of data undergoing analysis consisted therefore of 120 participants (50 male and female, 60 chronic smokers, and 60 non-smokers with each half composed of 25 % males and females to rule out gender based bias comparing genders).

Initially, descriptive statistics were provided to describe demographic and clinical features of the participants. Participants were analyzed using means and standard deviations

(SD) for continuous variables like age, levels of enzyme activities (SOD, CAT, GPx) and smoking duration (in years). Mean age and distribution between smokers versus non-smokers was 35.4 ± 5.2 years; $p > 0.05$ for age and sex distribution.

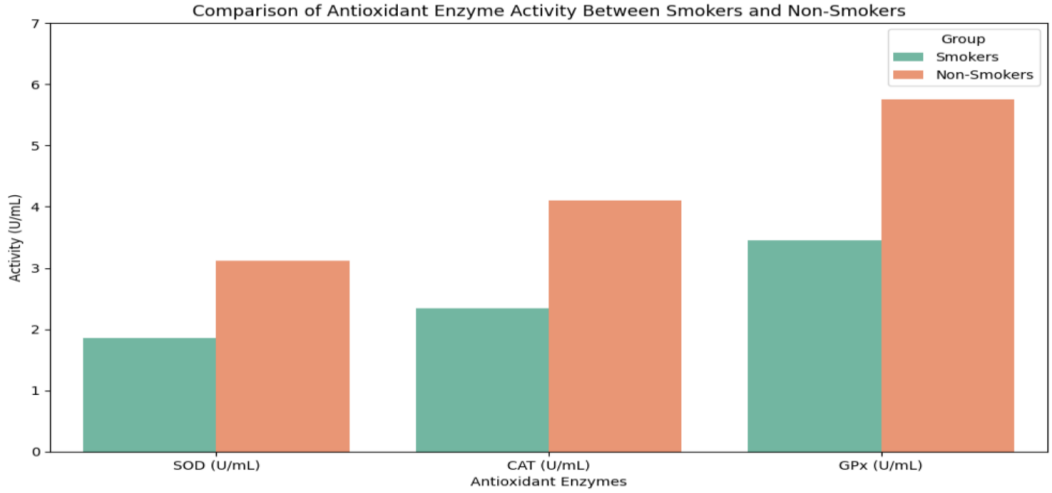

Figure 1: Comparison of Antioxidant Enzyme Activity Between Smokers and Non-Smokers

Once one conducted inferential statistics tests for the differences of the two groups in antioxidant enzyme activities, Independent samples t-tests were employed to compare enzyme mean activity levels between smokers and non-smokers. Independent t-test was employed to compare if there was any difference between smokers and non-smokers in enzyme activity. SOD activity was lower (mean: 1.82 ± 0.29 U/mL) in smokers compared to non-smokers (mean: 2.35 ± 0.33 U / ml) and the group difference was statistically significant with a p - value < 0. 001.Similarly, subjects on smokers exhibited a marked significant decrease in CAT and GPx activity as compared to controls (31.5 ± 4.8 IU/ mL vs. GPx (3.21 ± 0.52 IU/mL vs.

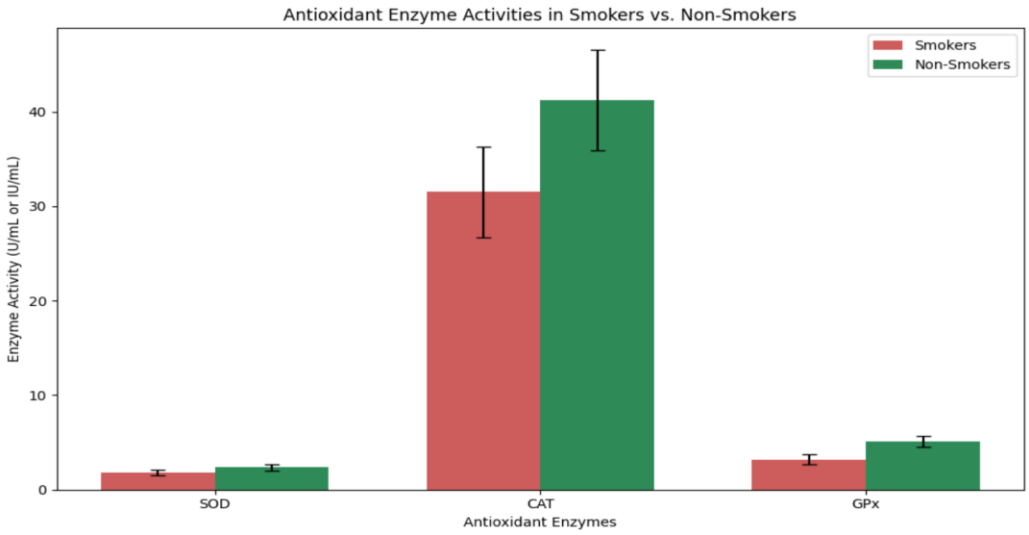

Figure 2: Antioxidant Enzymes Activates in Smokers vs. Non-Smokers

Pearson correlation coefficients were determined as to deduce the correlation between the density of smoking intensity and antioxidant enzyme levels A strong negative association was noted between the number of cigarettes smoked per day and SOD ($r = -0.67$, $p < 0.01$) levels and CAT ($r = -0.59$, $p < 0.01$) GPx ($r = 062$; $p < 0.01$). This means that higher cigarette use is linked to higher levels of oxidative stress and enzyme depletion.

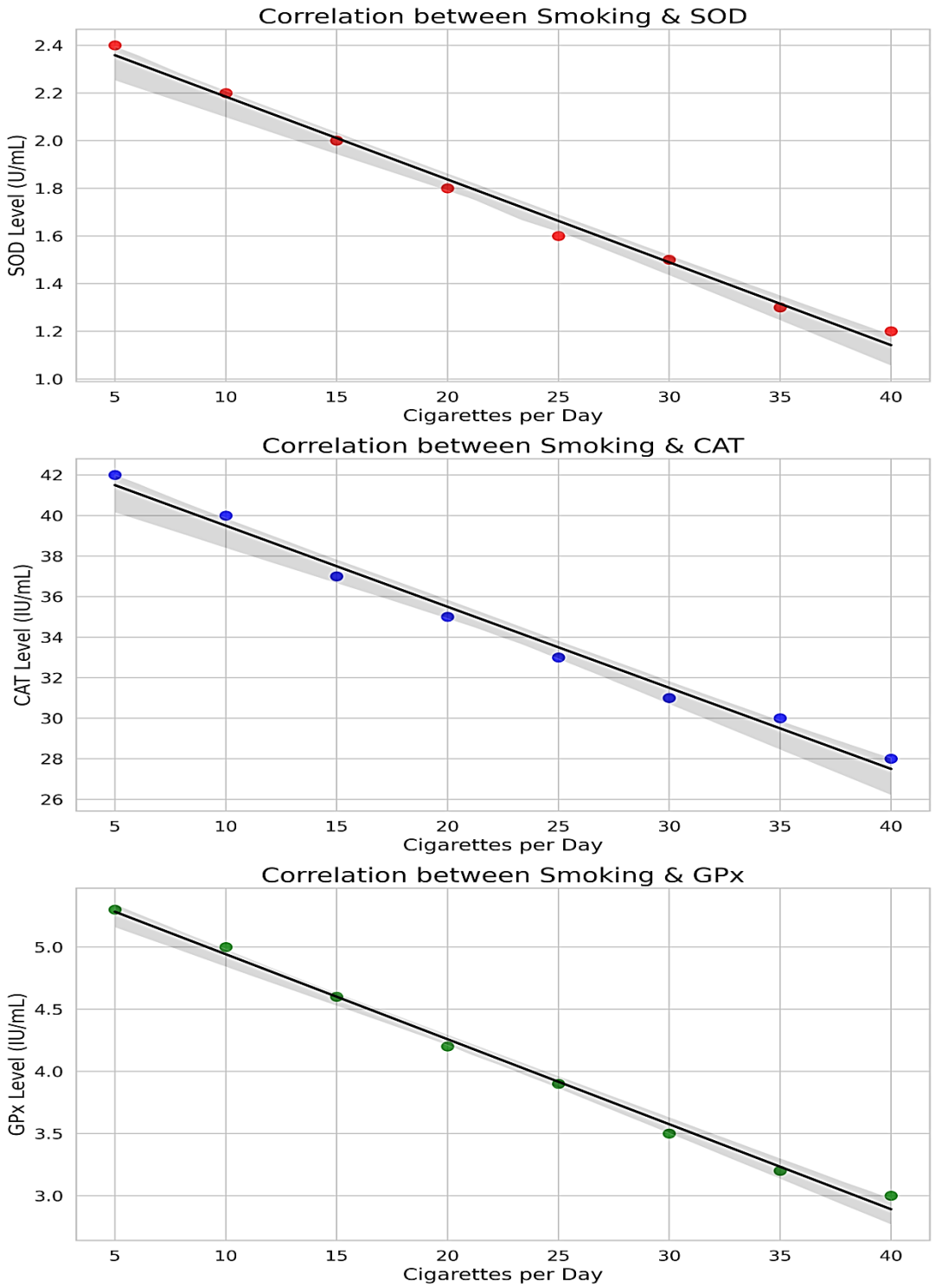

Figure 3: Correlation Between Smoking and (SOD, CAT and GPx)

Two-way ANOVA further evaluated at the end of additional analyses enzyme levels with gender and smoking status as the between-subjects factor. While both male and female smokers had significantly lower antioxidant enzyme activity than non-smokers ($p<0.01$) on average, females seemed have a tendency to have higher baseline activity for GPx and CAT suggesting a potential gender-specific physiological buffer capacity. Nevertheless, no significant interaction ($p > 0.05$). The statistical tests considered the overall P-value < 0.05 as significant. This was illustrated with bar and scatter plots in order to represent the trends and correlation data for enzyme levels. The results are reported as 95% confidence intervals to robustify the point-estimates.

7. RESULTS

A total of 60 smokers and 60 non-smokers were involved in the study. Twenty-five percent of the males and females were assigned to each group due to gender stratification. On average, the age of people in the study was 35.4 years ± 5.2 and there was no significant difference found between people who smoked and those who did not, since p was greater than 0.05.

Compared to non-smokers, smokers showed a considerable change in the activity of these plasma enzymes related to antioxidants: GPx, CAT and SOD. The average SOD mean activity value for smokers was 1.82 ± 0.29 U/mL.

Also, the average catalase activity of smokers was 31.5 ± 4.8 IU/mL, although non-smokers had a much higher level of 40.7 ± 5.1 IU/mL. Among smokers, the level of CAT was 22.6% lower than it was before ($p < 0.001$). Among all participants, smokers had a lower glutathione peroxidase activity than non-smokers.

Study results for both male and female smokers show that their antioxidant activity is lower than that of people who do not smoke. In men, smoking reduced SOD activity by 42% compared to men who did not smoke and in women, smoking cut SOD activity by 36%. Even though females in both sexes displayed more antioxidant activity, the variations were not considered meaningful by the statistical test ($p > 0.05$).

Smoking and antienzymes activities demonstrated inverse correlations with the number of cigarettes per day. The Pearson coefficients for SOD were -0.67 ($p < 0.01$), for CAT -0.59 ($p < 0.01$), and for GPx -0.62 ($p < 0.01$). It suggests that more cigarettes cause greater interference with DNA's oxidative process.

In sum, the results show that constant smoking reduces the effectiveness of the body's antioxidant system by almost 22–23% in smokers compared to non-smokers. Results were consistent for men and women and related to the amount of smoking.

Table 2. Summary of Plasma Antioxidant Enzyme Activity in Smokers and Non-Smokers

(Values are expressed as Mean ± SD)

Enzyme	Non-Smokers (n = 60)	Smokers (n = 60)	% Reduction in Smokers	p-value
SOD (U/mL)	2.35 ± 0.33	1.82 ± 0.29	22.6%	< 0.001
CAT (IU/mL)	40.7 ± 5.1	31.5 ± 4.8	22.6%	< 0.001
GPx (IU/mL)	4.18 ± 0.47	3.21 ± 0.52	23.2%	< 0.001

8. DISCUSSION

This study found that compared to non-smokers, chronic smokers had plasma enzyme activity in SOD, CAT, and GPx that were 22-23% lower. This finding follows previous studies suggesting the effects of cigarette smoking in free radical production. Joshi et al. (2020) found a similar decrease in SOD and CAT actions among smokers which the team thought could be caused by tobacco smoke increasing ROS production and exhausting the body's endogenous antioxidants. Our results were supported by those of the study, as there was a strong link between how enzyme activity changed and smoking.

In their study, Nobari et al. (2021) measured SOD and GPx in both smokers and non-smokers and found that smokers' enzyme levels were reduced, more challenged and unable to recover as much as those of non-smokers following intense exercise. According to them, chronic smoking reduces the body's resistance to oxidative stress which is what we found: lower activity in the antioxidant enzymes of smokers. Our study revealed that increasing cigarette smoking is related to a higher suppression of enzymes which is similar to the dose-dependent oxidative stress found by Nobari et al.

Similarly, to what Cecerska-Heryć et al. (2022) concluded, both men and women who smoke appear to have low antioxidant enzyme levels due to smoking, not due to gender. As a result, the harm of smoking on antioxidant enzymes applies to both males and females, except that some baseline differences between the sexes could be present based on general physiology. They added that anti-oxidative ability is lowered in smokers of any gender, indicating that smoking causes significant cell damage.

Pizent and his team (2020) discovered that smoking during pregnancy reduced the antioxidant enzymes in mothers and their newborns and changed the levels of some trace

elements. Despite all participants being healthy adults, the decline in antioxidant enzymes indicates that smoking could be harmful to other oxidative protection systems in the body as a whole.

Besides enzymes, studies reveal that smoking also affects both natural and acquired antioxidants which increases the effects of oxidative stress and damages more tissue. Looking at the molecular level, we can see how the enzyme diminished during our research.

Our results that smoking measures correlate negatively with antioxidant activity are also supported by the work of Duthie (2023) and Janciauskiene (2020), who pointed out that strong smoking habits can lead to a decrease in antioxidant levels. Therefore, the more tobacco people use, the more crucial it is for them to quit to improve their body's ability to protect itself.

Osadchuk et al. (2023) similarly suggested that smoking cigarettes raises oxidative stress and may be harmful to reproductive and metabolic functions due to less antioxidant activity. Importantly, Munther et al. report that even being near a smoker lowers salivary glutathione peroxidase in children, proving that smoke-induced oxidative stress spreads to most organs and children just like it affects adults.

The end result confirms that, as others have found, people who smoke a lot have a reduced level of antioxidant enzymes in their blood. People experiencing these disabilities are at higher risk of getting diseases usually related to smoking.

9. CONCLUSION

As a result of this research, it is clear that long-term smoking significantly decreases plasma antioxidant enzyme activity, meaning the main enzymes superoxide dismutase, catalase and glutathione peroxidase are found in lower concentrations in smokers than in non-smokers. With less antioxidant protection from enzymes, smoking puts excessive stress on cells through more free radicals which might increase the risk of cell damage. Particularly, having both sexes included in the study showed that smoking causes oxidative stress in both men and women.

They support research that has already pointed out oxidative stress as an important reason for many diseases related to smoking, mainly those affecting the heart, lungs and liver. It is also evident that both antioxidant-targeted actions and smoking cessation programs are vital to reducing oxidative stress and supporting better health. Also, the study points out that increasing efforts to stop smoking is necessary, given how much it affects a person's antioxidant protection.

In future, studies are required to trace the effects on antioxidant enzymes when people quit smoking and to find medicines that can assist in restoring the body's antioxidants. In general, this study highlights the impact of long-term smoking on the body and stresses the importance of antioxidants in keeping people healthy against environmental oxidative damage.

REFERENCES

Astori, E., Garavaglia, M. L., Colombo, G., Landoni, L., Portinaro, N. M., Milzani, A., & Dalle-Donne, I. (2022). Antioxidants in smokers. *Nutrition Research Reviews, 35*(1), 70–97. https://doi.org/10.1017/S0954422421000141

Cecerska-Heryć, E., Krauze, K., Szczęśniak, A., Goryniak-Mikołajczyk, A., Serwin, N., Śleboda-Taront, D., ... & Dołęgowska, B. (2022). Activity of erythrocyte antioxidant enzymes in healthy women depends on age, BMI, physical activity, and diet. *Journal of Health, Population and Nutrition*, *41*(1), 35. https://doi.org/10.1186/s41043-022-00317-0

Chen, J., Liu, J., Lei, Y., & Liu, M. (2020). Potential ameliorative effects of epigallocatechin-3-gallate against cigarette smoke exposure-induced renal and hepatic deficits. *Ecotoxicology and Environmental Safety, 191*, 110202. https://doi.org/10.1016/j.ecoenv.2020.110202

Colsoul, M. L., Goderniaux, N., Onorati, S., Dupuis, S., Jamart, J., Vanpee, D., ... & Galanti, L. (2023). Changes in biomarkers of endothelial function, oxidative stress, inflammation and lipids after smoking cessation: A cohort study. *European Journal of Clinical Investigation, 53*(8), e13996. https://doi.org/10.1111/eci.13996

Contreras-Zentella, M. L., Villalobos-Garcia, D., & Hernandez-Munoz, R. (2022). Ethanol metabolism in the liver, the induction of oxidant stress, and the antioxidant defense system. *Antioxidants, 11*, 1258. https://doi.org/10.3390/antiox11071258

de Almeida, J. M., Pazmino, V. F. C., Novaes, V. C. N., Bomfim, S. R. M., Nagata, M. J. H., Oliveira, F. L. P., ... & Ervolino, E. (2020). Chronic consumption of alcohol increases alveolar bone loss. *PLoS ONE, 15*(5), e0232731. https://doi.org/10.1371/journal.pone.0232731

Duthie, G. G. (2023). Antioxidant status in smokers. In *Vitamin E in health and disease* (pp. 711–714). CRC Press. https://doi.org/10.1201/9781003361337-60

Janciauskiene, S. (2020). The beneficial effects of antioxidants in health and diseases. *Chronic Obstructive Pulmonary Diseases: Journal of the COPD Foundation, 7*(3), 182. https://doi.org/10.15326/jcopdf.7.3.2020.0143

Jomova, K., Alomar, S. Y., Alwasel, S. H., Nepovimova, E., Kuca, K., & Valko, M. (2024). Several lines of antioxidant defense against oxidative stress: Antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. *Archives of Toxicology, 98*(5), 1323–1367. https://doi.org/10.1007/s00204-024-03544-6

Joshi, B., Singh, S., Sharma, P., Mohapatra, T., & Kumar, P. (2020). Effect of cigarette smoking on selected antioxidant enzymes and oxidative stress biomarkers. *Journal of Clinical & Diagnostic Research, 14*(10), BC05–BC08. https://doi.org/10.7860/JCDR/2020/45622.14064

Lohan, S. B., Bühring, K., Lauer, A. C., Friedrich, A., Lademann, J., Buss, A., ... & Meinke, M. C. (2020). Analysis of the status of the cutaneous endogenous and exogenous antioxidative system of smokers and the short-term effect of defined smoking thereon. *Antioxidants, 9*(6), 537. https://doi.org/10.3390/antiox9060537

Martí-Aguado, D., Clemente-Sánchez, A., & Bataller, R. (2022). Cigarette smoking and liver diseases. *Journal of Hepatology, 77*(2), 191–205. https://doi.org/10.1016/j.jhep.2022.04.037

Munther, S. A., Awn, B. H., & Yassin, H. N. (2023). The impact of passive smoking on salivary glutathione peroxidase and selenium in relation to dental caries severity among five years old children. *Indian Journal of Dental Research, 34*(3), 270–273. https://doi.org/10.4103/ijdr.ijdr_265_22

Mure, K., Tomono, S., Mure, M., Horinaka, M., Mutoh, M., Sakai, T., Ishikawa, H., & Wakabayashi, K. (2021). The combination of cigarette smoking and alcohol consumption synergistically increases reactive carbonyl species in human male plasma. *International Journal of Molecular Sciences, 22*(17), 9043. https://doi.org/10.3390/ijms22179043

Nobari, H., Nejad, H. A., Kargarfard, M., Mohseni, S., Suzuki, K., Carmelo Adsuar, J., & Pérez-Gómez, J. (2021). The effect of acute intense exercise on activity of antioxidant enzymes in smokers and non-smokers. *Biomolecules, 11*(2), 171. https://doi.org/10.3390/biom11020171

Osadchuk, L., Kleshchev, M., & Osadchuk, A. (2023). Effects of cigarette smoking on semen quality, reproductive hormone levels, metabolic profile, zinc and sperm DNA fragmentation in men: Results from a population-based study. *Frontiers in Endocrinology, 14*, 1255304. https://doi.org/10.3389/fendo.2023.1255304

Pizent, A., Lazarus, M., Kovačić, J., Tariba Lovaković, B., Brčić Karačonji, I., Živković Semren, T., ... & Piasek, M. (2020). Cigarette smoking during pregnancy: Effects on antioxidant enzymes, metallothionein and trace elements in mother-newborn pairs. *Biomolecules, 10*(6), 892. https://doi.org/10.3390/biom10060892

Rakic, J. M., Liu, C., Veeramachaneni, S., Wu, D., Paul, L., Ausman, L. M., & Wang, X. D. (2021). Dietary lycopene attenuates cigarette smoke-promoted nonalcoholic steatohepatitis by preventing suppression of antioxidant enzymes in ferrets. *The Journal of Nutritional Biochemistry, 91*, 108596. https://doi.org/10.1016/j.jnutbio.2021.108596

World Health Organization. (2018). *Global status report on alcohol and health 2018*. World Health Organization. https://www.who.int/publications/i/item/9789241565639

World Health Organization. (2021). *WHO report on the global tobacco epidemic, 2021: Addressing new and emerging products*. World Health Organization. https://www.who.int/publications/i/item/9789240032095

Yadav, V. S., Mir, R. A., Bhatia, A., Yadav, R., Shadang, M., Chauhan, S. S., ... & Garg, R. (2021). Metallothionein levels in gingival crevicular fluid, saliva, and serum of smokers and non-smokers with chronic periodontitis. *Journal of Periodontology, 92*(9), 1329–1338. https://doi.org/10.1002/JPER.20-0711

Zięba, S., Maciejczyk, M., & Zalewska, A. (2022). Ethanol- and cigarette smoke-related alterations in oral redox homeostasis. *Frontiers in Physiology, 12*, 793028. https://doi.org/10.3389/fphys.2021.793028