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The Effect of Green Tea (Camellia Sinensis) Infusion on Leydig Cell Count in Testis of Male Balb/C Mice Following Electronic Cigarette Exposure

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ABSTRACT

Exposure to electronic cigarette aerosol increases reactive oxygen species (ROS) production, leading to oxidative stress and potential damage to Leydig cells, which are crucial for testosterone synthesis. Green tea, particularly its polyphenol epigallocatechin gallate (EGCG), may protect testicular cells from oxidative damage. This study aimed to evaluate the effect of green tea (Camellia sinensis) infusion at two doses (0.03 g/mouse/day and 0.06 g/mouse/day) on Leydig cell count in male BALB/c mice exposed to electronic cigarette vapor. A randomized posttest-only control group design was used with 28 male BALB/c mice divided into four groups: negative control (KN), positive control with vitamin C (KP), treatment group 1 (P1: 0.03 g/mouse/day), and treatment group 2 (P2: 0.06 g/mouse/day). Mice were exposed to electronic cigarette vapor for 30 minutes daily over 30 days. Leydig cell counts were assessed with image raster analysis and HE staining. Data were analyzed using robust ANOVA and Tamhane's post-hoc test. Significant differences were found among the groups ($p = 0.000$). P2 had the highest Leydig cell count (44.54 cells/field), followed by P1 (30.05 cells/field), KP (14.80 cells/field), and KN (13.40 cells/field). Significant differences were observed between KN–P1 ($p = 0.004$), KN–P2 ($p = 0.008$), KP–P1 ($p = 0.006$), and KP–P2 ($p = 0.010$), but no difference was found between P1–P2 ($p = 0.251$). Green tea infusion dose-dependently increases Leydig cell counts in exposed mice, outperforming vitamin C, though doses did not differ significantly.

Keywords: *Green Tea; Leydig Cells; Electronic Cigarettes; Oxidative Stress; Antioxidants.*

INTRODUCTION

Cigarettes are products made from processed and packaged tobacco leaves derived from plants *Nicotiana tabacum*, *Nicotiana rustica*, and other species (Lewis, 2020; Liu et al., 2022; Popova et al., 2020). Cigarettes contain nicotine and tar, where nicotine is an addictive pyrrolidine compound that can cause dependence, while tar is a polycyclic aromatic hydrocarbon compound that is carcinogenic in nature. Cigarettes have become a global health problem, with an estimated 2.5 billion smokers worldwide, two-thirds of whom come from developing countries. Indonesia ranks third with the highest smoking rate in the world among the 10 countries monitored. Based on data from Basic Health Research in 2007, active smokers in the adult population group in Indonesia comprise 46.8% of men and 3.1% of women (Srivastava et al., 2022).

Based on data from the Global Youth Tobacco Survey in 2006, Indonesia had a prevalence of smokers in the adolescent population group aged 13–15 years of 23.9% among males and 1.9% among females (Tanuwihardja et al., 2012). One of the most popular ways to use smokeless tobacco and nicotine delivery systems is e-cigarettes. As an alternative to conventional cigarettes, electronic cigarettes (e-cigarettes) are battery-powered devices that deliver vaporized nicotine, usually in propylene glycol or glycerin. E-cigarette exposure has become a significant concern in recent years. Although considered a safer alternative to conventional tobacco cigarettes, recent research suggests that e-cigarette exposure can also negatively impact health, including the testicular organs (Elsa & Nadjib, 2019).

The gaseous components in e-cigarettes have the potential to produce free radicals. These free radicals can damage three major molecular components in the body's cells: lipids, proteins, and DNA. Damage to lipids during the oxidation process and to DNA during base oxidation disrupts cell integrity, which can ultimately lead to cell death. Excessive production of free radicals or reactive oxygen species (ROS) can damage sperm. When ROS levels increase beyond the body's defense capacity, oxidative stress occurs, leading to infertility through negative impacts on spermatozoa, such as increased motility loss, membrane damage, decreased morphology, viability, and ability (Isasari, 2017).

The testicular organ serves as a center for sperm production and male reproductive hormones, where Leydig cells play a key role in the synthesis of the hormone testosterone. Leydig cells, located in the testicular interstitium, are responsible for producing testosterone in response to luteinizing hormone (LH) from the anterior pituitary. However, exposure to cigarette smoke, which contains nicotine and other harmful substances, can damage Leydig cells and interfere with testosterone production. Nicotine causes oxidative stress that damages the Leydig cell membrane and interferes with the hormonal feedback mechanism between the hypothalamus, anterior pituitary, and testicles, thereby lowering LH and follicle-stimulating hormone (FSH) levels (Kim et al., 2020).

Antioxidant substances can inhibit or suppress ROS formation. Antioxidants are compounds that suppress or reduce the formation of ROS or free radicals. One beneficial type of antioxidant is catechins, the main polyphenols found in green tea (Thun-Hohenstein et al., 2021). Catechins, polyphenol compounds found in green tea, positively affect the male reproductive organs, especially by improving sperm quality and testicular function.

Research shows that catechins can increase testosterone hormone levels and protect Leydig cells from damage caused by oxidative stress. One study found that green tea extract administration increased spermatozoa count and motility in ethanol-induced mice, as well as testicular weight, suggesting that catechins act as antioxidant agents protecting the reproductive organs from damage (Opuwari & Monsees, 2020). This indicates that green tea consumption can help maintain spermatozoa quality by preventing damage caused by ROS.

Based on the background presented, this study addresses the following problem formulations: first, whether administration of green tea infusion at a dose of 0.03 g/head/day can inhibit the decrease in Leydig cell count in the testicles of male BALB/c mice due to e-cigarette exposure; and second, whether administration at a dose of 0.06 g/head/day can achieve the same result. The general purpose of this study is to demonstrate that green tea infusion can inhibit the decrease in Leydig cells in the testicles of male BALB/c mice exposed to e-cigarettes.

Specifically, the study seeks to prove that doses of 0.03 g/head/day and 0.06 g/head/day can inhibit the decrease in Leydig cell counts due to e-cigarette exposure. The theoretical benefits of this research include providing deeper insights into the effects of e-cigarette exposure on reproductive health, particularly on Leydig cells in male BALB/c mice, and offering a scientific reference for further studies on green tea’s potential in reducing oxidative stress from histological and biochemical perspectives. The practical benefits include raising awareness among e-cigarette users about the oxidative stress risks to reproductive health and informing the public about green tea’s potential as a natural remedy to mitigate damage caused by free radicals.

METHOD

The design of this study is experimental analytical research using the randomized posttest-only control group method with the research subjects being BALB/c mice. The study included four groups: two control groups, one with exposure to e-cigarette smoke and vitamin C (positive control), and the other with exposure to e-cigarette smoke only (negative control). Two treatment groups were administered green tea infusion at doses of 0.03 g/head/day and 0.06 g/head/day, while all groups were exposed to e-cigarette smoke for 30 minutes daily over 30 days. After exposure, the testicular organs of the mice were removed for histological examination using Hematoxylin-Eosin (HE) staining to assess the number of Leydig cells.

The objective of the study was to evaluate the potential of green tea infusion in mitigating oxidative stress caused by e-cigarette smoke exposure, focusing on Leydig cell health. The use of BALB/c mice as the experimental subjects aligns with the inclusion criteria, and the experimental groups were formed based on random allocation.

The research was conducted at the LBT Histology Division of the Faculty of Medicine, Udayana University, located in Denpasar, Bali. The study took place over 30 days starting in January 2025. The sample consisted of 28 male BALB/c mice, aged 3-4 months, with a weight of 20 grams. Inclusion criteria for the sample were active mobility and good health, while exclusion criteria excluded unhealthy or defective mice.

The experimental design used Federer's formula to determine the number of mice per group, ensuring a sufficient sample size to account for potential dropouts. The independent variable in this study was the dose of green tea infusion, while the dependent variable was the number of Leydig cells in the testicular tissue post-exposure. Control variables included the sex, age, and health status of the mice. Data collected were analyzed using SPSS, and normality and homogeneity tests were performed before applying One Way Anova for significance testing.

RESULTS AND DISCUSSION

This study aims to analyze the effect of giving green tea infusion (Camellia sinensis) on the number of leydig cells in the testicles of male mice BALB/c exposed to e-cigarettes. The number of leydig cells was measured using the variable of the average number of leydig cells obtained through raster image analysis. The data used were the result of statistical analysis with the Statistical Package for the Social Sciences (SPSS) of four treatment groups, where each group consisted of seven mice (N = 7).

The four groups include: Negative Control Group (KN), namely mice that are only given exposure to e-cigarettes; Positive Control Group (KP), namely mice exposed to e-cigarettes and given vitamin C at a dose of 0.26 mg/head/day; Treatment Group 1 (P1), namely mice exposed to e-cigarettes and given green tea infusion at a dose of 0.03 g/head/day; and Treatment Group 2 (P2), namely mice exposed to e-cigarettes and given green tea infusion at a dose of 0.06 g/head/day.

Figure 1 shows a histopathological picture of the testicles with Hematoxilin-Eosin (HE) staining in each treatment group with a magnification of 400×. In the image, it shows leydig cells located in the interstitial tissue between the seminiferous tubules, which are marked with a blue arrow. The difference in the number of leydig cells between groups is visually visible.

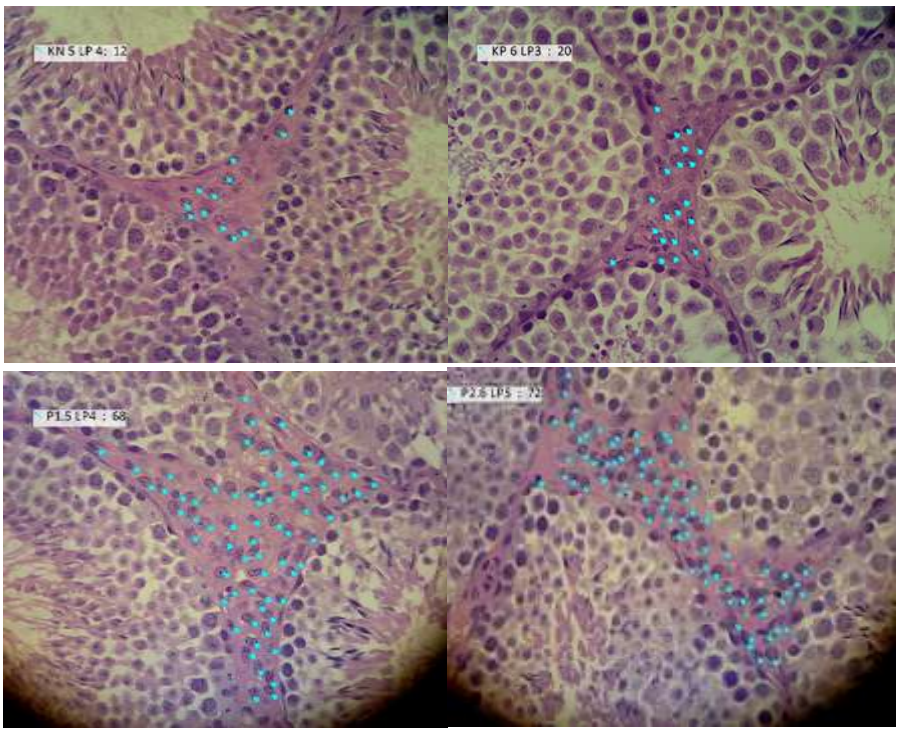

Figure 1. Number of Leydig Cells in Each Group (arrows) with 400x magnification

Overview of the Average Number of Leydig Cells

Descriptive analysis was carried out to see an overview of the mean (mean), standard deviation (standard deviation) of the number of leydig cells in each treatment group. Table 5.1 illustrates the results.

Table 1. Results of Descriptive Analysis of the

Treatment Groups	N	Rerata (Mean)	Simpang Baku (Std. Deviation)
KN	7	13,40 sel/sl	1,51
KP	7	14,80 sel/LP	2,40
P1	7	30,05 sel/LP	7,24
P2	7	44,54 sel/LP	14,89

Average Number of Leydig Cells

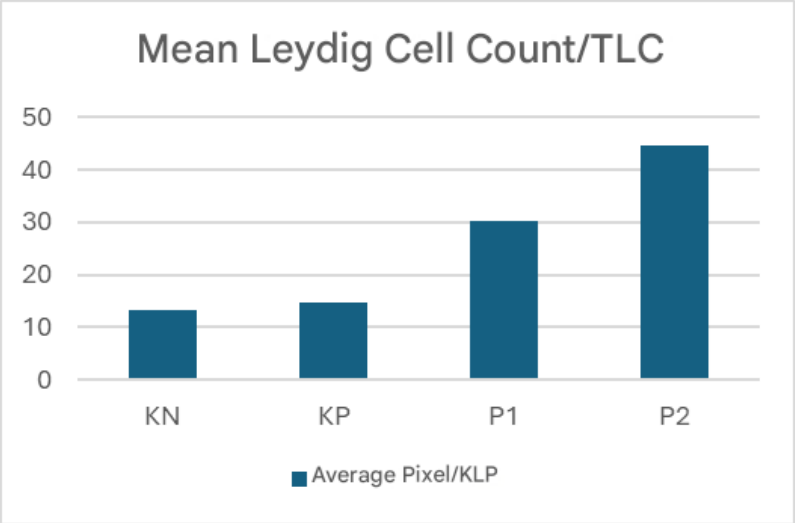

Figure 2. Average Graph of Number of Leydig Cells/Group

Based on Table 1, the average number of leydig cells in male mice showed clear variation between treatment groups. Treatment group 2 (P2), which was given green tea infusion at a dose of 0.06 g/head/day, had the highest average of 44.54 cells/LP (SD = 14.89). This value was followed by treatment group 1 (P1), which was given green tea infusion at a dose of 0.03 g/head/day, with an average of 30.05 cells/LP (SD = 7.24). Meanwhile, the positive control group (KP), which was given vitamin C at a dose of 0.26 mg/head/day, showed an average of 14.80 cells/LP (SD = 2.40).

The lowest value was found in the negative control group (KN), which received only exposure to e-cigarettes, which was 13.40 cells/LP (SD = 1.51). The average increase pattern from KN to P2 showed that the increase in the dose of green tea infusion was directly proportional to the increase in the number of leydig cells. In addition, the largest standard deviation was found in the P2 group (SD = 14.89), indicating a higher variation in biological responses in the group with the highest dose of green tea infusion.

Inferential Analysis Essay Testing

A prerequisite test was performed to determine the most appropriate method of inferential analysis (parametric or non-parametric) by verifying two main assumptions: the normality of the data distribution and the homogeneity of variance between groups. This step is essential to guarantee the validity and reliability of the research's statistical results.

a. Data Normality Test

In this study, the Normality test was performed using the Shapiro-Wilk Method, which is statistically more recommended for small sample sizes (n<50). The decision criteria set are: if the probability value (p) obtained is greater than 0.05 (p>0.05), then the null hypothesis (H0) is accepted, and it can be concluded that the data come from a normally distributed population.

Table 1. Shapiro-Wilk Normality Test Results

Treatment Groups	N	Statistic	*Sig. (p-value)*	Remarks
KN	7	0,934	0,584	Normal
KP	7	0,933	0,580	Normal
P1	7	0,927	0,525	Normal
P2	7	0,842	0,103	Normal

Based on the table of normality test results, Shapiro–Wilk showed that the distribution of the average data of the number of leydig cells in each group had a significance value (Sig.) for all groups of more than 0.05, so that the assumption of normality was met.

b. Variant Homogeneity Test

Table 2. Homogeneity Test Results *Levene Statistic*

		Lavene Staristic	df1	df2	Say.
Rerata Jumlah Sel Leydig	Based on Mean	12,208	3	24	0,000
	Based on Median	3,321	3	24	0,037
	Based on Median and with adjusted df	3,321	3	7,887	0,078
	Based on trimmed mean	11,535	3	24	0,000

Based on the table of variance homogeneity test results *Levene's test* (α = 0,05). If p is less than 0.05, the variance is considered inhomogeneous, then the ANOVA robust test (*Welch / Brown–Forsythe*) is used as confirmation.

Leydig Cell Number Hypothesis Testing

Because the data is normally distributed, a One-Way ANOVA test was performed to test the mean difference between the 4 groups at α = 0.05. Given that the Levene test showed inhomogeneous variance, the ANOVA results were corroborated by the robust test (*Welch dan Brown–Forsythe*).

Table 3. Test Results *Anova Robust* (*Welch dan Brown-Forsythe*)

	Statistic	df1	df2	Sig. (p-value)
Between Groups	19,322	3	11,758	0,000
Within Groups	21,285	3	9,202	0,000

The results of the two robust tests, namely *Welch* and *Brown-Forsythe*, showed a significance value of. A value well below 0.05 indicates a very significant difference in the average number of leydig cells between the analyzed groups. These findings suggest that giving green tea infusion has a noticeable effect in maintaining the condition of leydig cells in mice exposed to e-cigarettes.$p = 0{,}00p$

Post-Hoc Tamhane's test

Because the results of the homogeneity test showed that the variance between groups was not homogeneous, the *post-hoc* Tamhane's test was used, which is indeed recommended for data with unequal variance.

Table 5. Test Results *Post-Hoc* Tamhane's test

Comparison (I) vs (J)	Mean Difference (I-J)	Std. Error	Sig. (p-value)	Remarks
KN vs KP	-1,400	1,0717	0,775	Insignificant
KN vs P1	-16,6571	2,7989	0,004	Signifikan
KN vs P2	-31,1429	5,6585	0,008	Signifikan

Comparison (I) vs (J)	Mean Difference (I-J)	Std. Error	Sig. (p-value)	Remarks
KP vs P1	-15,2571	2,8863	0,006	Signifikan
KP vs P2	-29,7429	5,7022	0,010	Signifikan
P1 vs P2	-14,4857	6,2611	0,251	Insignificant

Post-hoc *analysis* using Tamhane's T2 (because variance between groups is not homogeneous) showed that the difference in the average number of leydig cells between group pairs was different. There was no significant difference between KN and KP (p = 0.775) and between P1 and P2 (p = 0.251). In contrast, KN differed significantly with P1 (p = 0.004) and P2 (p = 0.008). KP differed significantly with P1 (p = 0.006) and P2 (p = 0.010). However, the average P2 (44.54) was higher than P1 (30.05), indicating a trend of increasing effects with higher doses of green tea. These results indicated that the administration of green tea infusion significantly increased the average number of leydig cells compared to controls and vitamin C, and that the effect was increased at higher doses.

The main finding of this study is that the administration of green tea infusion (Camellia sinensis) to male mice of BALB/c exposed to e-cigarettes showed a higher increase in the number of leydig cells compared to the group that was not given green tea infusion. The treatment group with a dose of 0.06 g/head/day (P2) had the highest average number of leydig cells (44.54 cells/LP), followed by the P1 group with a dose of 0.03 g/head/day (30.05 cells/LP). Meanwhile, the positive control group (KP) given vitamin C showed a much smaller increase (14.80 cells/LP), and the negative control group (KN) that was only exposed to e-cigarettes had the lowest average (13.40 cells/LP).

These results support the research hypothesis (H1) which states that there is a significant effect of green tea infusion on the number of leydig cells in mice exposed to e-cigarettes. This pattern suggests that the higher the dose of green tea infusion given, the greater the protective effect it exerts on leydig cells.

The findings of this study are in line with the theory that exposure to e-cigarettes can increase the formation of Reactive Oxygen Species (ROS) through the content of heavy metals and carbonyl compounds contained in their aerosols (Emma et al., 2022). ROS then activates oxidative stress pathways that can damage cell membranes, disrupt mitochondrial function, and decrease testosterone biosynthesis in leydig cells (Darbandi et al., 2018). Uncontrolled oxidative stress can also increase the apoptosis of leydig cells, which ultimately decreases the number of those cells significantly (Zhang et al., 2024). This explains the low average of leydig cells in the KN group that did not receive antioxidant interventions.

Other studies have also shown that the toxicity effects of e-cigarettes can be influenced by small variations in exposure distribution, inhalation intensity, as well as differences in metabolism between animals, resulting in differences in oxidative stress levels that impact the number of leydig cells (Wang et al., 2023) stated that exposure to cigarette aerosols caused testicular dysfunction through an increase in ROS, which then activated the apoptosis pathway and decreased the number of leydig cells, but the rate of such a decrease was not uniform in every mice.

In addition, Mamdouh's research et al. (2020) regarding the effects of cigarettes on the reproductive system of mice also showed that exposure to smoke can cause varying histological changes, such as germ cell degeneration and interstitial damage, which differ between

individuals despite being given the same exposure. Differences in cellular adaptation capacity between mice in the face of oxidative stress are thought to be one of the main causes of variance inhomogeneity in this study. Thus, these findings are in line with the literature that states that exposure to e-cigarettes and conventional cigarettes can cause varying degrees of testicular damage between individuals, resulting in inhomogeneous group variance.

The variance of leydig cell count data in this study was not homogeneous, which was most likely due to biological variation between mice in response to exposure to e-cigarettes and green tea infusion. The response to oxidative stress in testicular tissue is strongly influenced by genetic factors, endogenous antioxidant capacity, and the degree of susceptibility of cells to free radicals. This causes some mice to experience heavier leydig cell damage than others, resulting in a wider spread of data.

This phenomenon is in accordance with research (Löscher, 2024), which suggests that exposure to cigarettes and e-cigarettes in mice causes testicular damage such as disorganization of seminiferous tubules, necrosis, and increased lipid peroxidation, but the degree of such damage is not uniform between individuals. Such disparity in responses contributes to the high variability of the data. In addition, another study by (Rahali et al.,2018) reported that exposure to e-cigarette aerosols can increase reactive oxygen species (ROS), decrease the activity of steroidogenesis enzymes, and trigger mitochondrial damage, but the extent of damage varied between test animals. The findings of the study support that exposure to e-cigarettes has toxic effects that are not always constant, resulting in high data variance in the group.

The results of this study showed that the administration of vitamin C as a positive control provided an increase in the number of leydig cells, but the increase was not as high as in the green tea infusion treatment group (P1 and P2). When compared to the negative group (KN), vitamin C still shows a protective effect because it is able to resist the decrease in the number of leydig cells due to exposure to e-cigarettes, although the magnitude of the increase is relatively limited. This can happen because vitamin C is a single antioxidant that works primarily in aqueous environments (Hydrophilic Antioxidant), while green tea polyphenols are multipotent and can work on a variety of cellular compartments (Chrysikopoulou et al., 2025). Thus, the protective effect of green tea infusion which is stronger than vitamin C is a result of its ability to act on many oxidative stress pathways at once.

Green tea infusion is believed to be able to overcome the effects of oxidative stress because the main content is in the form of polyphenols, especially epigallocatechin gallate (EGCG), which has strong antioxidant activity. EGCG works by capturing free radicals, increasing the activity of endogenous antioxidant enzymes such as superoksida dismutase (SOD) and glutathione peroxidase (GPx), as well as inhibiting lipid peroxidation on cell membranes (Devika et al., 2008). In addition, the polyphenols in green tea have been shown to decrease mitochondrial damage, improve testicular microcirculation, as well as inhibit the activation of apoptosis pathways through caspase-3 modulation (Articles, 2020). This antioxidant activity is strongly suspected to play a role in increasing the number of leydig cells in the treatment group.

Although there were statistically significant differences between the group given the green tea infusion and the control group, post-hoc analysis showed that the P1 and P2 groups did not differ significantly from each other. This can be due to the dose difference between P1 and P2 being relatively close together so that they are not strong enough to produce different

biological responses to the number of leydig cells. In other words, increasing the dose from 0.03 g/head/day (P1) to 0.06 g/head/day (P2) has not been able to cause statistically significant changes. Chances are, the new difference will be significant if the dose is increased further, for example up to three times the P1 dose, so that the physiological response to green tea infusion can appear more clearly. However, the tendency to increase the average from P1 to P2 indicates a *dose-dependent trend* that illustrates the antioxidant protective power that increases as the dose of green tea infusion increases.

This study provides a clear picture of the protective effect of green tea infusion on leydig cells in mice exposed to e-cigarettes. However, there are limitations that need to be considered in interpreting the results of the study. These limitations are related to the validity of the method of calculating the number of leydig cells with the raster image used. Assessment using raster images is only able to describe the density or area of the leydig cell, not the absolute number of cells, so the accuracy of the quantification is limited. This method is certainly less accurate when compared to more standardized stereologic techniques, such as *Optical Fractionator*, so the quantitative interpretation of the results of this study must be done with great caution (Liu Z, 2009).

CONCLUSION

This study concludes that green tea infusion doses of 0.03 g/head/day and 0.06 g/head/day effectively inhibited the decline in Leydig cell counts in the testicles of male BALB/c mice exposed to e-cigarettes, with the higher dose (0.06 g/head/day) yielding a more pronounced increase. For future research, employing advanced quantitative histology like the Optical Fractionator technique would provide more precise Leydig cell assessments, while testing lower doses could pinpoint the minimum effective dose for optimal protective effects against oxidative stress.

REFERENCES

Articles, R. (2020). *The Role of Epigallocatechin-3-Gallate in Autophagy and Endoplasmic Reticulum Stress (ERS) -Induced Apoptosis of Human Diseases*. 1–12. https://doi.org/10.12659/MSM.924558

Chrysikopoulou, V., Rampaouni, A., Koutsia, E., Ofrydopoulou, A., Tsoupras, A., & Mittas, N. (2025). *Anti-Inflammatory , Antithrombotic and Antioxidant Efficacy and Synergy of a High-Dose Vitamin C Supplement Enriched with a Low Dose of Bioflavonoids ; In Vitro Assessment and In Vivo Evaluation Through a Clinical Study in Healthy Subjects*.

Darbandi, M., Darbandi, S., Agarwal, A., Sengupta, P., & Durairajanayagam, D. (2018). *Reactive oxygen species and male reproductive hormones*. 1–14.

Devika, P. T., & Stanely Mainzen Prince, P. (2008). Protective effect of (-)-epigallocatechin-gallate (EGCG) on lipid peroxide metabolism in isoproterenol induced myocardial infarction in male Wistar rats: a histopathological study. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 62(10), 701–708.

Elsa, M. S., & Nadjib, M. (2019). Electric cigarette determinants in Indonesia: using SUSENAS (National Social Economic Survey) data in 2017. *Journal of Community Medicine and Public Health*, *35*(2), 441–448.

Kim, U., Kim, C. Y., Lee, J. M., Ryu, B., Kim, J., Bang, J., Ahn, N., & Park, J. H. (2020). Loss

of glutathione peroxidase 3 induces ROS and contributes to prostatic hyperplasia in Nkx3.1 knockout mice. *Andrology*, *8*(5), 1486–1493. https://doi.org/10.1111/andr.12828

Lewis, R. S. (2020). Nicotiana tabacum L.: Tobacco. In *Medicinal, aromatic and stimulant plants* (pp. 345–375). Springer.

Liu, Z., Chang, Q., Xu, Z. L., & Zhang, Z. G. (2009). Stereological measurement of rat's seminiferous tubule. Chinese medical journal, 122(21), 2643–2646.

Liu, H., Xiao, X., Liu, H., Li, Z., & He, X. (2022). Research and Application of Tobacco Based on Leaf Nicotine Value to Realize Redrying Homogenization Processing. *International Journal of Reliability, Quality and Safety Engineering*, *29*(05), 2240003.

Löscher, W. (2024). *Of Mice and Men : The Inter-individual Variability of the Brain ' s Response to Drugs*. *11*(February), 1–10.

Mantayborbir, V. (2014). *Eksplorasi Pengaruh Pemaparan Laserpunktur Pada Titik Reproduksi Terhadap Peningkatan Jumlah Sel Leydig Ikan Lele (Clarias sp)*. http://repository.ub.ac.id/id/eprint/157870/

Opuwari, C., & Monsees, T. (2020). Green tea consumption increases sperm concentration and viability in male rats and is safe for reproductive, liver and kidney health. *Scientific Reports*, *10*(1), 1–14. https://doi.org/10.1038/s41598-020-72319-6

Rahali, D., Jrad-Lamine, A., Dallagi, Y., Bdiri, Y., Ba, N., El May, M., El Fazaa, S., & El Golli, N. (2018). Semen Parameter Alteration, Histological Changes and Role of Oxidative Stress in Adult Rat Epididymis on Exposure to Electronic Cigarette Refill Liquid. The Chinese journal of physiology, 61(2), 75–84.

Tanuwihardja, R. K., & Agus Dwi Susanto. (2012). Rokok Elektronik (Electronic Cigarette). *J Respir Indo*, *32*(1), 53–61. http://arsip.jurnalrespirologi.org/wp-content/uploads/2012/01/jri-2012-32-1-531.pdf

Thun-Hohenstein, L., Höbinger-Ablasser, C., Geyerhofer, S., Lampert, K., Schreuer, M., & Fritz, C. (2021). Burnout in medical students. *Neuropsychiatrie*, *35*(1), 17–27. https://doi.org/10.1007/s40211-020-00359-5

Zhang, M., Tang, Y., Li, H., Zhou, G., & Zeng, C. (2024). *Oxidative Stress and Antioxidation in Testicular Leydig : Implications for Male Fertility Leydig : Implications for Male Fertility*. https://doi.org/10.20944/preprints202410.1855.v1

Wang, H., Tian, Y., Fu, Y., Ma, S., Xu, X., Wang, W., Lu, F., Li, X., Feng, P., Han, S., Chen, H., & Hou, H. (2023). *Testicular tissue response following a 90-day subchronic exposure to HTP aerosols and cigarette smoke in rats*. *September*, 902–912.

Zhang, M., Tang, Y., Li, H., Zhou, G., & Zeng, C. (2024). *Oxidative Stress and Antioxidation in Testicular Leydig : Implications for Male Fertility Leydig : Implications for Male Fertility*. https://doi.org/10.20944/preprints202410.1855.v1