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Effect of Steeping Green Tea (Camellia Sinensis L.) Against IL6 and IL 10 Levels in Rats Exposed to Cigarette Smoke

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ABSTRACT
The prevalence of smokers in Indonesia is more than 25% of the total population. Cigarette smoke contains numerous free radicals that trigger the formation and increase of Reactive Oxygen Species (ROS) within the body, leading to inflammation and cytokine production. Green tea contains catechins known for their potent antioxidant properties. This study aims to assess the levels of Interleukin 6 and Interleukin 10 in rats exposed to cigarette smoke. The research method used was the post-test only control group design, involving 24 male Wistar rats divided into 4 groups: Normal group (N), Control - (Cigarette Smoke), Control + (Cigarette Smoke + 1.44 mg/day Vitamin E), P. (Cigarette Smoke + 3.6 ml Green Tea Infusion). Interleukin 6 and Interleukin 10 levels were measured after 14 days of treatment. Data were analyzed using One Way Anova. The mean IL-6 level in the Control (-) group was the highest (141.75±6.24 pg/ml), while the Sham group had the lowest (73.58 ± 4.57 pg/ml) IL-6 level. One Way Anova results showed a p-value of <0.001 for both IL-6 and IL-10 levels, indicating a significant difference among the four groups. Post Hoc LSD test results indicated that the IL-6 levels in the K(+) group were relatively similar to the IL-6 levels in the P group (p=0.172). Post Hoc LSD test results for IL-10 levels between the two groups were all significant, with a p-value of <0.001. The administration of green tea infusion was shown to affect the Interleukin 6 levels, while green tea infusion did not have a significant impact on Interleukin 10 levels in white rats exposed to cigarette smoke.

Introduction

Cigarettes are one of the results of processed tobacco, besides that there are also cigars or other forms (Nugroho, 2017). Free radicals contained in cigarette smoke are a trigger for the formation and increase of *Reactive Oxygen Species* (ROS) in the body. *Reactive Oxygen Species* predominantly occur in the body (Endogenous ROS) due to the results of normal cell metabolism. While as small as it occurs due to exposure to other substances or radicals that are outside the body which results in inflammation or inflammation (Parwata, 2015) Inflammation due to cigarette smoke can trigger various cytokines (Strzelak et al., 2018) Cytokines that play a role are pro-inflammatory cytokines such as Interleukin 6 (IL 6) and anti-inflammatory cytokines such as Interleukin 10 (IL 10).

A 2015 study found that more than 25% of all Indonesians are smokers. The number of smokers in Indonesia has not decreased for 15 years from 1990 to 2015 (Holipah et al., 2020).A 2015 study showed that 925,611 men (93.27%) and 66,719 women (6.93%) in Indonesia were hospitalized due to smoking-induced diseases such as hypertension (42.6%), chronic obstructive pulmonary disease (COPD) (40.2%), and other stroke diseases (12%), smoking accounted for 21.05% of all chronic diseases in Indonesia (Holipah et al., 2020)

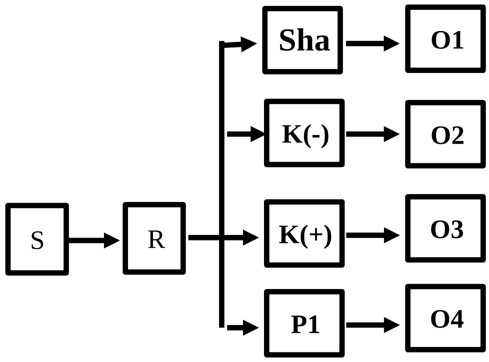
One strategy to overcome smoking and diseases that can be caused by smoking in Indonesia using various ways aligned with WHO is MPOWER strategy by monitoring tobacco use, enforcing cigarette advertising bans and warning about the dangers of cigarettes, regulation of cigarette prices by increasing cigarette prices by 10%, reducing cigarette sales outlets, enforcement of smoking bans in family and school environments, provide support to quit smoking, taking drugs such as varenicline to treat nicotine addiction (Nurwidya et al., 2014).

The strategy has not been satisfactory because cigarette advertisements in electronic media about the dangers of cigarettes are very rare, public awareness of a smoke-free environment is still lacking, anti-smoking laws only apply in certain provinces in Indonesia, drugs such as varenicline are still not covered by national health insurance (Nurwidya et al., 2014). So it needs alternative efforts that can reduce cigarette consumption and the dangers of smoking by using green tea which is used for drinks high in antioxidants, can be consumed in daily life, and is easy to obtain. Green tea contains natural antioxidants such as polyphenols and four main catechins, namely, epicatechin (EC), epicatechin-3-gallate (*ECG), epigallocatechin (*EGC) and *epigallocatechin-3-gallate* (*EGCG) which can prevent diseases caused by smoking* (Kochman et al., 2020).

The results of research Al-Awaida, Akash, Aburubaiha, Talib, & Shehadeh (2014) green tea can reduce oxidative stress, inflammation and tissue damage in rats exposed to cigarette smoke. The results of Septiana's research (2018), green tea can reduce the number of inflammatory cells and tnf-α pulmo rats (*Rattusnorvegicus*) after exposure to cigarette smoke. This study will investigate the effect of steeping green tea (*Camellia Sinensis L*.) on IL6 and IL 10 levels in rats exposed to cigarette smoke. This study is expected to reduce inflammation by looking at Interleukin 6 (IL 6) levels and increasing Interleukin 10 (IL 10) levels so that it can help control the prevalence of smoking and diseases that can be caused by smoking in Indonesia.

Research Methods

The type of research conducted was experimental laboratory with a *post test control group design* approach using male white rats of wistar strains given green tea and given exposure to cigarette smoke simultaneously for 14 days. The object of the study was a male rat wistar strain with a body weight of 200-250 gr which was declared healthy and suitable for use for research developed and maintained intensively at PAU UGM Yogyakarta. Test animals measured levels of interleukin-6 (IL 6) and levels of interleukin-10 (IL 10). The design of this study was divided into normal group, control group and test group shown in Figure 1.



S = Sampel
R = Randomisasi
O = Observasi
P = Perlakuan

Figure 1. Research design scheme

Sham was a group of healthy mice with no treatment given standard feed. K(-) was a control group given exposure to cigarette smoke 3 cigarettes per day for 14 days and given standard feed. K (+) is a treatment group given exposure to cigarette smoke 3 cigarettes per day for 14 days and given Vitamin E dose 1.44mg / day given 1 hour after exposure to cigarette smoke. P.I was the treatment group given exposure to cigarette smoke 3 cigarettes per day for 14 days and steeping green tea 3.6 ml given 1 hour after exposure to cigarette smoke, given standard feed. O1 was an observation of interleukin 6 and interleukin 10 in the healthy group without treatment. O2 was an observation of interleukin 6 and interleukin 10 in a control group given exposure to cigarette smoke 3 cigarettes per day for 14 days. O3 is an observation of interleukin 6 and interleukin 10 in the treatment group given exposure to cigarette smoke 3 cigarettes per day and Vitamin E dose 1.44 mg / day given 1 hour after exposure to cigarette smoke for 14 days. Finally, O4 was observed interleukin 6 and interleukin 10 in the treatment group given exposure to cigarette smoke 3 cigarettes per day and steeping green tea 3.6 ml / day given 1 hour after exposure to cigarette smoke for 14 days.

A total of 3 mL of blood samples of study subjects were taken on the 15th day of the orbital vein using a hematocrit tube without anesthesia. The blood sample is inserted into the EDTA vacutainer and allowed to stand at 2-8oC for 10 minutes and then centrifuge at a rate of 400 xG for 10 minutes. The blood plasma that has been separated from is carefully taken using a micropipette, then inserted into a 1.5 mL vial tube and stored at -80 hours until the analysis process is carried out. Blood plasma samples that have been obtained are liquefied first and then analyzed IL-6 and IL-10 levels using the ELISA method. Before the analysis process, the blood plasma is liquefied at room temperature and resuspended so that the plasma is evenly mixed. IL-6 and IL 10 readings were taken using a microplate reader with a wavelength of 450 nm.

Data analysis in the study using descriptive tests, normality and homogeneity, produced Interleukin-6 and Interleukin-10 data which were normally distributed and homogeneous ($p > 0.05$), so as to use the *One Way Anova* parametric test and continued with *the Post Hoc* LSD test because the analysis showed differences between groups ($p < 0.05$).

Results and Discussions

Research on the effect of steeping green tea (*Camellia Sinensis L.*) on IL-6 and IL-10 levels in rats exposed to cigarette smoke has been conducted at the Laboratory of the Center for Food and Nutrition Studies, Gajah Mada University Yogyakarta for 14 days. This study used 24 rats divided into 4 groups, namely Sham group with healthy mice without treatment, control group (-) rats exposed to cigarette smoke 3 cigarettes per day, control group (+), mice and rats exposed to cigarette smoke 3 cigarettes per day and vitamin E dose 1.44 mg / day, treatment group (P) rats given exposure to cigarette smoke 3 sticks per day and steeping tea 3.6 ml / day for 14 days. During the study, none of the mice developed an infection and died. After 14 days of the study, 24 rats measured IL-6 and IL-10 levels and obtained the following results.

The analysis of IL-6 levels carried out included descriptive analysis by presenting the mean value and standard deviation (SD), normality analysis of data distribution *with the Shapiro Wilk* test, analysis of variance homogeneity with *the Levene* test and comparison analysis of average IL-6 levels between 4 groups using *the One Way Anova* test. The test results are shown in Table 1.

Table 1. Analysis of IL-6 (pg/ml) levels between groups

	Group				
	Sham	K(-)	K(+)	P	*p-value*
Rerata±SD	73,58±4,57	141,75±6,24	86,52±2,57	90,17±3,62	
*Shaphiro wilk**	0,400	0,287	0,945	0,746	
Levene					0,065
One way anova					<0,001

**p-value*

The mean result of IL-6 levels in the Control (-) group was the highest (141.75±6.24 pg/ml) while in the sham group was the lowest (73.58±4.57 pg/ml). The results of the *Shapiro Wilk test in each group showed normal distributed results (p>0.05) and the results of the* Levene test obtained data variants of the four homogeneous groups *(p>0.05*). Variable data on Interleukin 6 levels can be concluded as normally distributed and homogeneous data, so that data analysis continues using *the One Way Anova* parametric test. The results of the average IL-6 comparison test between the *four groups with One Way Anova* obtained p < 0.001 showed that there was a significant difference in IL-6 levels between the four groups.

Post hoc LSD test to determine the comparison of IL-6 levels of various groups. These results can be seen in Figure 2.

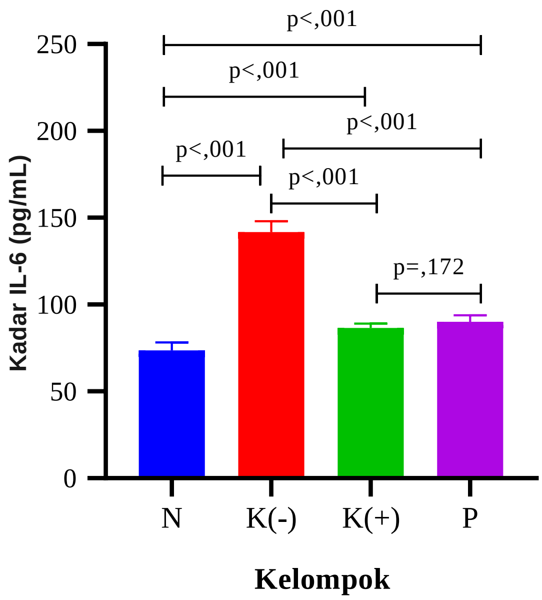


Figure 2. Comparison of IL-6 levels between two groups

The results of the comparison of IL-6 levels between groups were obtained almost all pairs of groups had a value of p < 0.001 except for the comparison between the K (+) group and P (p = 0.172). These results suggest that exposure to secondhand smoke causes an increase in IL-6 levels, and steeping green tea may decrease it. The effectiveness of steeping green tea on reducing IL-6 levels in rats exposed to cigarette smoke is relatively similar to vitamin E.

IL-10 levels were also tested similar to the IL-6 test with the results shown in Table 2.

Table 2. Analysis of IL-10 (pg/ml) levels between groups

	Group				
	N	K(-)	K(+)	P	*p-value*
Rerata±SD	35,87±2,09	110,97±3,06	44,25±3,12	53,43±2,39	
*Shaphiro wilk**	0,945	0,847	0,571	0,804	
Levene					0,671
One way anova					<0,001

**p-value*

The average result of IL-10 levels in the K(-) group was the highest (110.97±3.06 pg/ml) while in the N group was the lowest (35.87±2.09 pg/ml), while between the K(+) and P groups it was lower than in K(-) which was 44.25±3.12 pg/ml and 53.43±2.39 pg/ml, respectively. The results of the Shapiro Wilk test in each group showed normal distributed results (p>0.05) and the results of the Levene test obtained data variants of the four homogeneous groups (p>0.05). The results of the average IL-10 comparison test between the four groups with *One Way Anova* obtained p<0.001 showed that there was a significant difference in IL-10 levels between the four groups.

Significant results in the *One Way Anova* test were followed by *a post hoc* LSD test to determine the comparison of IL-10 levels between the two groups. These results can be seen in Figure 3.

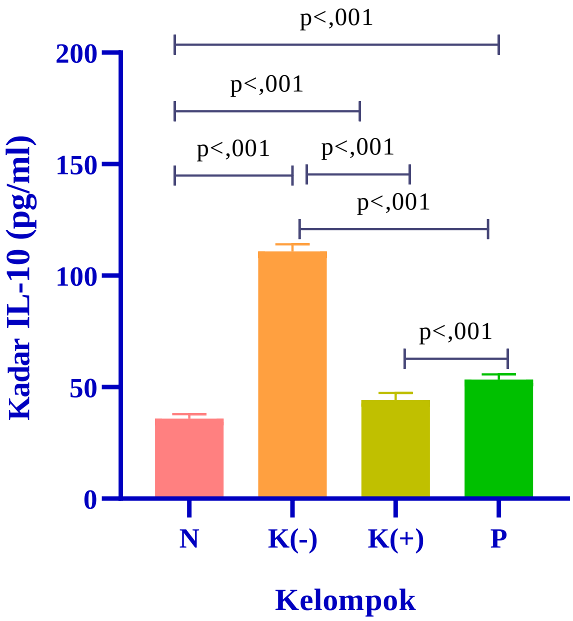


Figure 3. Comparison of IL-10 levels of various groups of rats

The results of the comparison of IL-10 levels between various groups are all significant, indicated by a $p<0.001$ value. These results suggest that secondhand smoke exposure causes an increase in IL-10 levels, and steeping green tea should have increased beyond the negative control group (-), so the results of the study did not match the expected theory.

The results of the examination of Interleukin 6 levels showed significant differences between the group that was not given green tea steeping and the group that was given green tea steeping. Between the group given steeping green tea with vitamin E obtained relatively similar results to reduce Interleukin 6, while for the group of Interelukin 10 levels the results were not proven in the group steeping green tea. Steeping green tea is expected to increase Interleukin 10 as an anti-inflammatory. It can be concluded that steeping green tea can reduce Interleukin 6 levels and cannot increase Interleukin 10 levels in the treatment group.

Tea contains polyphenols, polysaccharides, purine alkaloids, amino acids, and other compounds. Tea polyphenols are the main components of green tea composed of gallic acid, flavanols, flavonols and flavonol glycosides in green tea. Among flavanols, epigallocatechin gallate is the most abundant component (10%−14%) followed by epigallocatechin (5%−7%), epicatechin gallate (3%−4%), epicatechin (2%−3%) and catechins (0.5%−1.5%) (Sun et al., 2022). Epigallocatechin gallate functions as an anti-inflammatory by, decreasing IL-6 production through transcriptional downregulation of nuclear factor-KB (NF-KB). NF-κB plays a key role in regulating the immune response to infection. Epigallocatechin gallate also induces IL-10 release and reduces NF-κB expression, thereby increasing anti-inflammatory activity. In this study, IL 10 results could not increase anti-inflammatory activity. (Sun et al., 2022) This study is in line with the research of Al-Awaida et al. (2014) which explains that steeping Chinese green tea at a dose of 5 ml increases catalase activity, glutathione activity, G6PD activity in liver, kidney and lung tissue, histologically inflammatory changes occur in liver, lung, kidney tissue.

Green tea contains antioxidants, which are compounds that have a molecular structure that can give electrons to free radical molecules and can break the chain reaction of free radicals (Mane et al., 2019). Antioxidants produced in the human body are known as endogenous antioxidants or antioxidant enzymes consisting of the enzymes Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), and Catalase (CAT). The antioxidants in green tea can neutralize free radicals and can reduce or even help prevent some of the damage they cause (Emmadi et al., 2012).

Giving cigarettes can increase Interleukin 6 and decrease Interleukin 10 because cigarette smoke that contains large amounts of free radicals can cause tissue structure abnormalities that are closely related to the inflammatory response. The content of compounds contained in cigarette smoke, namely *Reactive Oxygen Species* (ROS) and phenol-rich glycoproteins, provides a direct stimulus to macrophages and triggers the production of pro-inflammatory cytokines such as interleukin-6 (IL-6). Tissue damage due to *ROS (Reactive Oxygen Species) is known as oxidative stress, while factors that can protect tissues against ROS (*Reactive Oxygen Species) are called antioxidants. Various tissues that can be damaged by ROS (*Reactive Oxygen Species*) include *Deoxyribo Nucleid Acid* (DNA), lipids and proteins. Free radicals cause cell membrane lipid peroxidation, DNA damage and apoptosis (Lohan et al., 2020).

Free radicals are very dangerous materials. These free radical materials are actually compounds or molecules with one or more unpaired electrons in their outer orbitals (Polidori &; Mecocci, 2022). Electrons continue to find their partners and some compounds are bound by free radicals in general large molecules such as lipids, proteins and DNA. If this happens, it will result in cell damage or uncontrollable growth (Bitzer et al., 2020).

Cigarette smoke causes recurrent inflammation leading to chronic and progressive activation of the immune system accompanied by an abnormal inflammatory response of the airways by various harmful gases and particles from cigarette smoke (Madani et al., 2018). Cigarette smoke crosses the alveolus-capillaries and spreads directly through the systemic bloodstream, activating NF-κB and followed by secretion of various pro-inflammatory cytokines specifically MMP-9 and -12, surfactant protein D, and IL-1, IL-6, IL-8, and IL-17 found in higher amounts in the lungs of long-term smokers with ongoing inflammation (Madani et al., 2018).

Vitamin E consists of several kinds of α-tocopherol, β-tocopherol, δ-tocopherol, and γ-tocopherol. The most commonly found component of vitamin E is α-tocopherol which has a substituted aromatic ring and on isoprenoid long chains that serve as side chains. The role of vitamin E in cells is by binding free radicals. In tissues, vitamin E can suppress unsaturated fatty acids found 33 in membranes, thereby being able to maintain or maintain membrane function (Niki, 2014). Vitamin E has anti-inflammatory effects through inhibition of NFκB, reduction of protein kinase (PKC) activity, as well as reduction of synthesis of adhesion molecules, such as vascular cell adhesion molecule-1, intercellular adhesion molecule 1, and E-selectin. The modulating effect of α-tocopherol during inflammation may decrease the secretion of pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and IL-8, possibly by inhibiting activation of 5-lipoxygenase, NF-κB, and protein kinase C (PKC) (Sozen et al., 2019).

Conclusion

Based on the research that has been done, several things can be concluded. Steeping green tea doses of 3.6 ml / day affected IL 6 levels in wistar rats exposed to cigarette smoke. Steeping green tea dose 3.6 ml / day had no effect on IL 10 levels in wistar rats exposed to cigarette smoke. Vitamin E dose 1.44 mg / day affects IL 6 levels in wistar strain rats exposed to cigarette smoke. Vitamin E dose of 1.44 mg / day had no effect on IL 10 levels in wistar strain rats exposed to cigarette smoke. Steeping green tea dose 3.6 ml / day and Vitamin E dose 1.44 IU / day relatively equivalent effect on IL 6 levels in wistar strain rats exposed to cigarette smoke. Vitamin E dose 1.44 mg / day with steeping green tea dose 3.6 ml / day can not affect IL 10 levels in wistar strain rats exposed to cigarette smoke.

Researchers did not measure levels of Interleukin-6 and Interleukin-10 before treatment, so the initial levels of Interleukin-6 and Interleukin-10 were not known before treatment. For further research, researchers suggest developing research in measuring ROS levels after steeping green tea, measuring SOD levels after steeping green tea, and comparing steeping green tea with anti-inflammatory drugs that have different mechanisms of action.

References

Al-Awaida, W., Akash, M., Aburubaiha, Z., Talib, W. H., & Shehadeh, H. (2014). Chinese Green Tea Consumption Reduces Oxidative Stress, Inflammation And Tissues Damage In Smoke Exposed Rats. Iranian Journal Of Basic Medical Sciences, 17(10), 740–746.

Bitzer, Z. T., Goel, R., Trushin, N., Muscat, J., & Richie, J. P. (2020). Free Radical Production And Characterization Of Heat-Not-Burn Cigarettes In Comparison To Conventional And Electronic Cigarettes. Chemical Research In Toxicology, 33(7), 1882–1887. Https://Doi.Org/10.1021/Acs.Chemrestox.0c00088

Emmadi, P., Krishna, P., Namasi, A., & Nugala, B. (2012). Role Of Green Tea As An Antioxidant In Periodontal Disease: The Asian Paradox. Journal Of Indian Society Of Periodontology, 16(3), 313. Https://Doi.Org/10.4103/0972-124x.100902

Holipah, H., Sulistomo, H. W., & Maharani, A. (2020). Tobacco Smoking And Risk Of All-Cause Mortality In Indonesia. In Plos One (Vol. 15, Issue 12 December). Https://Doi.Org/10.1371/Journal.Pone.0242558

I Made Oka Adi Parwata. (2015). Bahan Ajar Uji Bioaktivitas : Antioksidan. Universitas Udayana, April, 1–51.

Kochman, J., Jakubczyk, K., Antoniewicz, J., Mruk, H., & Janda, K. (2020). Health Benefits And Chemical Composition Of Matcha Green Tea: A Review. Molecules (Basel, Switzerland), 26(1). Https://Doi.Org/10.3390/Molecules26010085

Lohan, S. B., Bühring, K., Lauer, A., Friedrich, A., Lademann, J., Buss, A., Sabat, R., Wolk, K., & Meinke, M. C. (2020). Analysis Of The Status Of The Cutaneous Endogenous And Exogenous Antioxidative System Of Smokers And The Short-Term E Ff Ect Of Defined Smoking Thereon.

Madani, A., Alack, K., Richter, M. J., & Krüger, K. (2018). Immune-Regulating Effects Of Exercise On Cigarette Smoke-Induced Inflammation. Journal Of Inflammation Research, 11, 155–167. Https://Doi.Org/10.2147/Jir.S141149

Niki, E. (2014). Role Of Vitamin E As A Lipid-Soluble Peroxyl Radical Scavenger: In Vitro And In Vivo Evidence. Free Radical Biology And Medicine, 66, 3–12. Https://Doi.Org/10.1016/J.Freeradbiomed.2013.03.022

Nugroho, R. S. (2017). Perilaku Merokok Remaja (Perilaku Merokok Sebagai Identitas Sosial Remaja Dalam Pergaulan Di Surabaya). Jurnal Ilmiah Departemen Sosiologi Fisip Universitas Airlangga, 22.

Nurwidya, F., Takahashi, F., Baskoro, H., Hidayat, M., Yunus, F., & Takahashi, K. (2014). Possible Strategies For Future Tobacco Reduction Policy In Indonesia. Epidemiology And Health, E2014035. Https://Doi.Org/10.4178/Epih/E2014035

Polidori, M. C., & Mecocci, P. (2022). Modeling The Dynamics Of Energy Imbalance: The Free Radical Theory Of Aging And Frailty Revisited. Free Radical Biology And Medicine, 181(February), 235–240. Https://Doi.Org/10.1016/J.Freeradbiomed.2022.02.009

Septiana, W. I. (2018). Terapi Preventif Ekstrak Etanol Daun Teh Hijau (Terapi Preventif Ekstrak Etanol Daun Teh Hijau (Cameliasinensis Folium) Terhadap Jumlah Sel Radang Dan Tnf-A Pulmo Tikus (Rattusnorvegicus) Hasil Paparan Asap Rokok. Aritkel Univ.Brawijaya.

Sozen, E., Demirel, T., & Ozer, N. K. (2019). Vitamin E: Regulatory Role In The Cardiovascular System. Iubmb Life, 71(4), 507–515. Https://Doi.Org/10.1002/Iub.2020

Strzelak, A., Ratajczak, A., Adamiec, A., & Feleszko, W. (2018). Tobacco Smoke Induces And Alters Immune Responses In The Lung Triggering Inflammation, Allergy, Asthma And Other Lung Diseases: A Mechanistic Review. International Journal Of Environmental Research And Public Health, 15(5). Https://Doi.Org/10.3390/Ijerph15051033

Sun, J., Dong, S., Li, J., & Zhao, H. (2022). A Comprehensive Review On The Effects Of Green Tea And Its Components On The Immune Function. Food Science And Human Wellness, 11(5), 1143–1155. Https://Doi.Org/10.1016/J.Fshw.2022.04.008

Surai, P. F., Kochish, I. I., Fisinin, V. I., & Kidd, M. T. (2019). Antioxidant Defence Systems And Oxidative Stress In Poultry Biology: An Update. Antioxidants, 8(7), 1–36. Https://Doi.Org/10.3390/Antiox8070235