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# INVESTIGATING PHARMACEUTICAL POTENTIAL OF GREEN TEA ON BIOCHEMICAL ALTERATIONS IN PLASMA AND BLOOD CAUSED BY CIGARETTE SMOKING

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**Abstract**
Several harmful biochemical changes in plasma and blood are attributed to cigarette smoking, and these alterations are the target of treatment efforts. The current research looked at how regularly drinking green tea affects hard-core smokers. The blood of 120 healthy male volunteers was divided into four groups: nonsmokers, smokers, nonsmokers who also drank green tea, and smokers who also drank green tea. In addition to measuring glucose, HbA1c, hemoglobin, hematocrit, total cholesterol, lipoprotein patterns (HDL, LDL, VLDL), lipid peroxidation, vitamin D, vitamin B12, vitamin C, iron, total iron binding capacity, calcium, sodium, potassium, phosphorus, chloride, and aminotransferases (alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transfer (ALP) were used as biochemical markers. In addition, phenols, flavonoids, and tannins were found in green tea in a phytochemical study. Both 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS+) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH+) were used to measure the antioxidant and free radical scavenging capacities of green tea, respectively. The phytoconstituents in green tea were shown to be responsible for the reversal of the negative alterations seen in the aforementioned biochemical parameters in smokers, as shown by the results of this study. Green tea's phytocompounds have been shown to scavenge free radicals and protect against the smoking-induced metabolic changes in both in vivo and in vitro experiments.

**Keywords**: pharmaceutical aspects, green tea, biochemical changes, plasma, cigarette smoking

## Introduction:

Millions of lives are lost every year because of the alterations in signal transduction and metabolic imbalance brought on by the high concentrations of oxidative stress and inflammation caused by ROS and RNS in smokers' lungs. The 8% tar and 92% gaseous elements or components (carbon monoxide, hydrogen cyanide, ammonia etc.) in regular cigarette smoke are all absorbed into the bloodstream and have negative consequences on the health of smokers (Forester and Lambert, 2011; Padmavathi et al., 2020). The plasma, RBCs, platelets, and white blood cells in the blood are all exposed to the toxins in the smoke. More than 1014 carbon and oxygen-centered radicals with low molecular weight are released into the lungs with each cigarette, along with around 500 ppm of nitric oxide and other reactive nitrogen oxides (Mosbah et al., 2015). The antioxidant status is lowered as a result of these free radicals, which increases the negative health impacts. Several different biochemical changes in the blood profile are brought on by increased oxidative stress and nitrosative stress, both of which are brought on by a lowered antioxidant status. These biochemical changes cause a wide range of cardiovascular and respiratory disorders, for which treatments are actively being researched (Horinouchi et al., 2016).

Many enzymes and proteins' catalytic activity are regulated, in part, by vitamins and minerals. Electrolyte imbalances can cause illnesses of the endocrine system, reproductive system, and cardiovascular system, as well as other significant metabolic problems (Chowdhury et al., 2016). Tobacco smoke contains very unstable free radicals that may react with DNA, proteins, lipids, and amino acids, rendering them inactive and altering intracellular signaling systems by creating DNA, protein, and lipid adducts. Peroxynitrite (ONOO), a more hazardous radical formed when cigarette smoke's nitrogen radicals combine with superoxide, is responsible for extensive damage to all tissues and organs in the body (Choi et al., 2017).

Herbal drugs have no known adverse effects, therefore many who smoke are seeking for ways to mitigate the negative health impacts of their habit. People from various walks of life drink tea in its many forms (Delwing-Dal Magro et al., 2016). Green tea's active phytoconstituents are what give it their health advantages, which include the treatment of a wide range of ailments and conditions (Ferreira et al., 2016; Yalcin et al., 2016). Green tea has been linked to a variety of health benefits, including its ability to inhibit platelet aggregation as well as its antioxidant, anti-inflammatory, anti-diabetic, and anti-hyperlipidemic properties (Hayakawa et al., 2016; Naumovski et al., 2015). Both the negative health impacts and the underlying molecular processes of smoking are well documented, and novel therapy techniques are constantly being developed as a result. The use of blood in pathological studies is widespread. We set out to see if green tea and its main components might counteract the metabolic alterations in plasma and blood that smoking causes (Cohn et al., 2000).

## Materials and methods.

### Preparation of green tea

Green tea was prepared in a microwave-oven at 60°C for 5-7 minutes, using 1 gram of green tea leaves per 100 ml of water. The research made use of the filtrate after the liquid extract was filtered.

### Work design

A questionnaire was used to choose 120 males from the general population of Faisalabad, Pakistan aged 35 to 55 for participation in the study. Everyone who participated in the study provided written informed consent. There was a total of 120 participants; 30 each were assigned to the control, smoking, green tea, and smoker + green tea groups. Equal amounts of warm water were given to both the controls and the smokers. The participants were healthy adults without a history of significant illness or hospitalization who did not use alcohol or hypertension medications. Participants who disclosed using any prescription medicine, including antibiotics, within the previous six months were disqualified. Cigarette smokers have averaged between fifteen and twenty filter cigarettes a day over the previous seven years. All smokers and volunteers in the green tea group were given green tea

bottles. All of the study's subjects were asked to keep their diets and tea consumption consistent throughout the experiment. Subjects were asked to fast overnight before having blood collected.

**HPLC verification of a green tea sample**.
The HPLC system was fitted with a UV-VIS detector (LC, Phenomenex), and the wavelength was adjusted to 254 nm before 20 l of green tea sample was injected. The phytocompounds were separated using a Luna 5 m C18 (100oA, LC Column 250 4.6 mm) reverse phase chromatography column. The mobile phase was a 70:30 molar ratio of 20 mM $KH_2PO_4$ buffer and acetonitrile. The flow rate was 1 ml/min. Retention time analysis was used to determine the identities of the various phytocompounds. Before use, the sample and HPLC-grade solvents were degassed in an ultrasonic bath and filtered over a 0.45 m membrane.

**In vitro tests**
**Analysis of Green Tea's Phytochemicals**
Quantitative phytochemical screening was used to determine the presence of flavonoids, phenols, tannins, saponins, glycosides, and terpenoids in green tea, as previously described. Using the technique, we were able to precisely quantify the total phenolic, flavonoid, and tannin content (Zhao et al., 2013; Lee et al., 2016).

**Nitric oxide radical scavenging activities**, (ABTS+ and DPPH+)
Absorbance at 734 nm and 517 nm was used to evaluate green tea's ability to scavenge free radicals produced by the addition of the stable free radicals ABTS+ and DPPH+. The quantity of nitric oxide scavenged was expressed as a function of the amount of NOx radicals created because of a Griess reaction using sodium nitroprusside. We used the absorbance at 546 nm of the chromophore that was generated by diazotization of nitrite with sulphanilamide, followed by coupling with naphthalene diamine (Jain et al., 2011).

**HbA1c, plasma glucose, and other biomarkers**
All the hematological parameters were measured as before, including HbA1c, plasma glucose, hemoglobin, iron, lymphocyte, total leucocyte, total iron binding capacity and total RBC (Venkataraman et al., 2012).

**Evaluation of Lipid and Enzyme Markers in Plasma**
The levels of gamma glutamyl transferase, alkaline phosphatase, alanine transaminase, and aspartate transaminase in the plasma were determined. As detailed before, total cholesterol (TC), phospholipids (PL), triglycerides (TG) and high-density lipoprotein (HDL) were measured. The formula for determining LDL-C and VLDL-C was previously described (Jain et al., 2011).

**Vitamin and mineral content analysis**
Calcium, phosphorus, potassium, sodium, and chloride concentrations in the plasma were measured, as were vitamin D, C levels and B12 (Padmavathi et al., 2009).

**Statistical analysis**
Values are presented as a mean SD due to the statistical treatment of the data. The significance of differences between groups was determined using Duncan's Multiple Range (DMR) and Students' t-tests. The p-value was set at p 0.05.

**Results**
All the study subjects ate a regular, healthy diet. Participants smoked an average of 20 or more filter cigarettes per day for at least seven years and drank three cups of green tea per day (in the morning, afternoon, and night) for a total of sixty days. Green tea in the quantity of 100 ml each cup. Subjects

in this study were all healthy adults who reported no history of substance abuse or psychiatric disorders. Alcoholics and those who were on legal or illegal drugs were also not allowed to take part in this study.

Quantitative and qualitative green tea results are presented in Table 1. Phenolics, proteins, tannins, flavonoids, carbohydrates, amino acids, steroids, glycosides, and alkaloids were all found in the samples.

**Table 1: Phyto-constitute of the green tea**

| **Phytoconstituents** | |
|---|---|
| **Lead acetate** | + |
| **Flavonoids** | ++ |
| **Ninhydrin** | ++ |
| **Proteins** | ++ |
| **Ferric chloride** | ++ |
| **Glycosides** | +++ |
| **Liebermann-Burchard** | – |
| **Carbohydrates** | + |
| **Saponins** | – |
| **Alkaloids** | + |

The selected participants' basic characteristics like BMI, Age, and consumption of cigarettes are listed in table 2.

**Table 2: Summary information on the study's subjects**

| **Parameter** | **Control (s)** | **Smoker (s)** | **GT** | **Smoker(s) + GT** |
|---|---|---|---|---|
| **Volunteer (s)** | 30.00 | 30.00 | 30.00 | 30.00 |
| **BMI** | 22.52 | 23.12 | 22.19 | 22.81 |
| **Age** | 35–55 | 35–55 | 35–55 | 35–55 |
| **Green tea** | - | - | 300 ml/day | 300 ml/day |
| **Cigarettes/day** | - | 15–20 | - | 15–20 |

A reduction in hematocrit was associated with an increase in total white blood cell, red blood cell lymphocyte, neutrophil, basophil,monocyte, eosinophil, and platelet counts, platelet crit, and mean c orpuscular hemoglobin levels in smokers' samples. Hematological abnormalities in smokers who dr ank green tea were considerably decreased compared to those in the control and experimental group s (Figure 1).

**Figure 1: The influence of green tea on the white blood cell counts and red blood cell count**

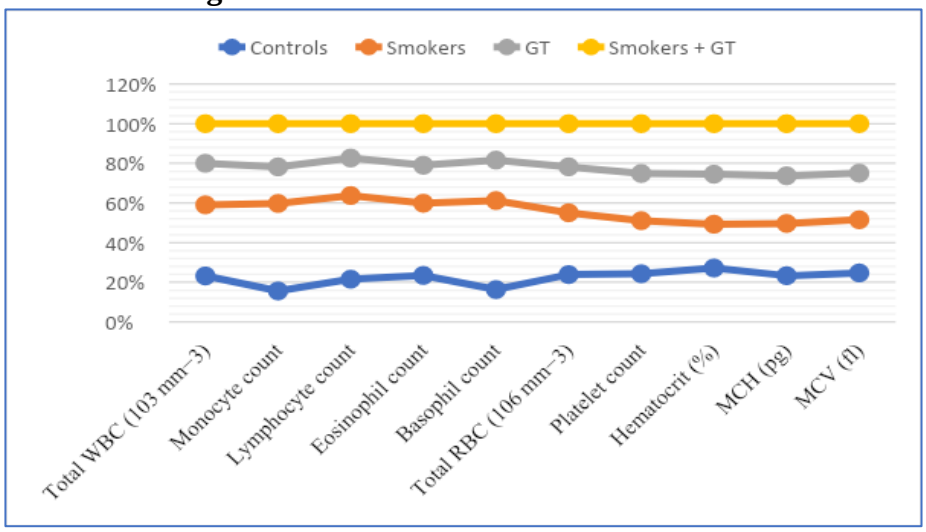

Figure 2 displays the results of a series of measurements taken from cigarette smokers to determine the effect of green tea on their oxidative stress state, hemoglobin, plasma glucose, TIBC, and iron. Cigarette smokers had higher levels of hemoglobin, glucose, HbA1c, iron, LPO and TIBC, but their transferrin levels were unaffected. Nevertheless, changes in the parameters were normalized in the green tea-drinking group of former smokers.

**Figure 2: Green tea's impact on cigarette users' blood sugar, lipids, iron, transferrin saturation, and liver peroxide levels**

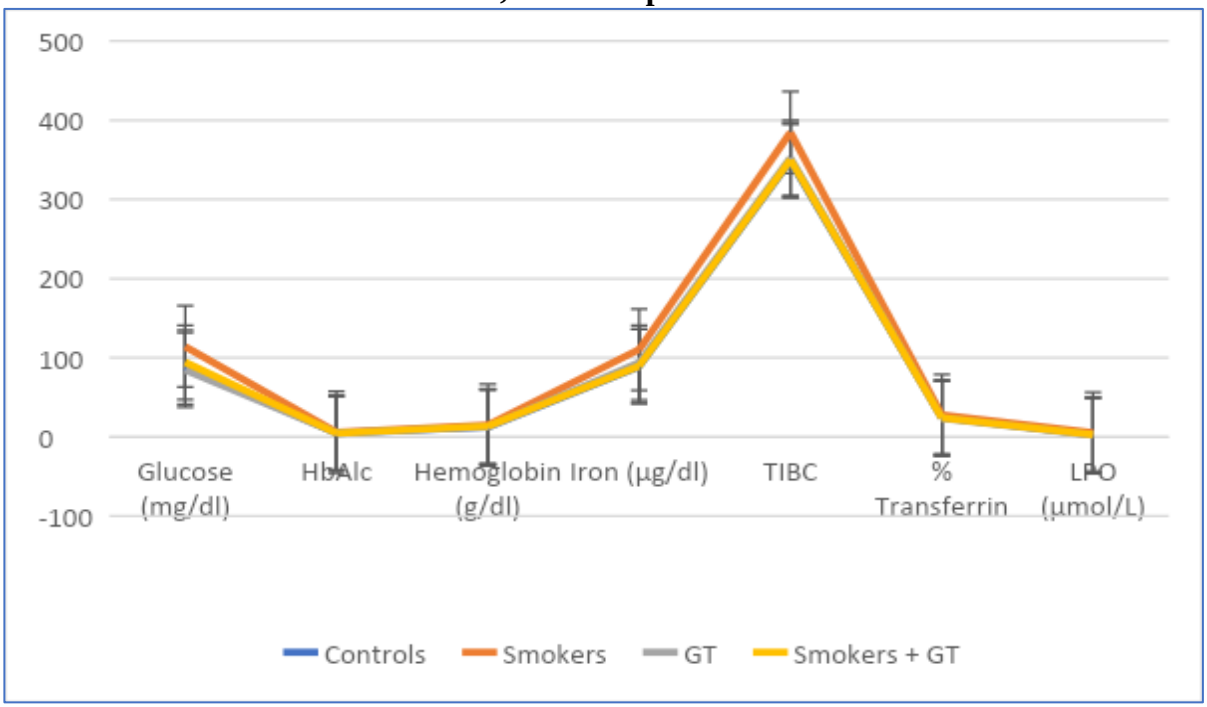


Table 3 shows how regularly consuming green tea counteracts the changes that take place in the plasma marker enzymes as well as the plasma lipids and lipoproteins as a direct result of smoking. HDL-C levels decreased, but there was no statistically significant difference in GGT levels between smokers and nonsmokers. Total cholesterol, phospholipids, LDL-C, VLDL-C, triglycerides, and the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were all higher in smokers' plasma than in nonsmokers' plasma. Among smokers who ingested green tea, there was a normalization of their lipids, lipoprotein abnormalities, and plasma enzymes in table 3.

**Table 3: Lipoproteins, plasma lipids and enzymes**

| Parameter | Control (s) | Smoker (s) | GT | Smoker (s) + GT |
|---|---|---|---|---|
| **Cholesterol (mg/dl)** | 165.45±1.12 | 219.21±1.04 | 154.32±1.11 | 161.45±0.88 |
| **Triglycerides (mg/dl)** | 134.56±0.45 | 169.45±2.14 | 118.45±1.05 | 138.32±0.45 |
| **Phospholipids (mg/dl)** | 209.12±1.21 | 257.56±2.44 | 189.45±0.45 | 198.45±0.78 |
| **LDL (mg/dl)** | 112.34±1.44 | 174.56±1.08 | 121.24±0.05 | 117.28±1.08 |
| **TC/HDL ratio** | 3.98±0.21 | 8.48±1.01 | 3.48±1.78 | 3.89±1.67 |
| **VLDL (mg/dl)** | 26.45±0.54 | 34.28±1.56 | 21.45±1.45 | 26.48±1.58 |
| **HDL (mg/dl)** | 39.4±1.88 | 23.45±3.04 | 43.45±1.67 | 34.56±0.002 |
| **GGT (U/l)** | 6.78±0.98 | 7.45±2.078 | 5.89±1.23 | 6.89±1.99 |
| **ALP (U/l)** | 61.56±0.78 | 89.45±0.87 | 54.78±204 | 69.45±2.14 |
| **LDL/HDL ratio** | 2.48±0.49 | 6.89±1.45 | 2.89±1.49 | 2.78±0.05 |
| **ALT (U/l)** | 21.65±0.07 | 62.47±4.5 | 17.45±0.09 | 28.45±0.023 |
| **AST (U/l)** | 34.56±1.47 | 59.45±0.241 | 31.56±0.347 | 36.89±0.14 |

Table 4 shows that green tea lowers cigarette users' levels of vitamin D, B12, and C. When compared to the control and green tea groups, smokers had significantly lower levels of vitamin D and B12, and only a little drop in vitamin C. In addition, we found that smokers had higher levels of calcium, phosphorus, potassium, salt, and chloride than nonsmokers or non-active controls. In addition, drinking green tea normalized vitamin and mineral deficiencies.

**Table 4: Minerals and plasma vitamins**

| Parameter | Controls | GT | Smokers | GT + Smokers |
|---|---|---|---|---|
| **Vitamin-C (mg/dl)** | 1.32 ±1.21 | 1.34 ± 0.54 | 1.21 ± 1.78 | 1.42 ± 1.01 |
| **Vitamin-B12 (pg/ml)** | 432 ± 0.54 | 498.45 ± 0.34 | 198.45± 1.34 | 478.45± 1.24 |
| **P (mg/dl)** | 2.89± 1.01 | 3.98 ± 0.02 | 2.4 ± 1.02 | 4.78 ± 2.08 |
| **Ca (mg/dl)** | 6.59 ± 1.45 | 7.45 ± 1.09 | 8.45± 0.88 | 8.11 ± 0.06 |
| **Cl (mmol/L)** | 101.21± 2.01 | 104.24±0.65 | 113.24±0.45 | 104.21±1.45 |
| **Na (mmol/L)** | 132.12± 0.65 | 124.23 ±1.11 | 174.25±0.65 | 138.45±0.98 |

## Discussion

Therapeutic strategies are being researched to combat the effects of smoking, which have been linked to the development of cardiovascular disease, respiratory illness, and various types of cancer (Padmavathi et al., 2010). Ammonia, acetaldehyde, formaldehyde, nitrogen oxides, hydrogen cyanide, and certain nitrosamines are just some of the oxidants, toxic metabolites, and carcinogenic compounds that are introduced by cigarette smoke. When these oxidants enter the body, they interact with healthy cells and tissues, amplifying the damaging effects of oxidative stress (Kamceva et al., 2016).

Malondialdehyde levels were found to be significantly higher in smokers than in the control group, suggesting that oxidants in cigarette smoke are having a negative effect by causing oxidative stress and membrane damage (Lymperaki et al., 2016). Green tea drinkers who smoke cigarettes have malondialdehyde levels similar to nonsmokers, suggesting that green tea consumption may have an effect. Researchers concluded that the Increased lipid peroxidation was seen in smokers due to the presence of oxygen-centered, carbon-centered, and nitrogen-centered free radicals and non-radical oxidants present in the tar phase and gas phase constituents of cigarette smoke. Smokers who consumed green tea did not show the same increase (Al-Malki and Moselhy, 2013). The catechins in green tea and the byproducts of their degradation are able to enter the bloodstream via the hepatic portal vein and scavenge free radicals. These chemicals prevent essential steps in the lipid peroxidation process from occurring. Our in vitro research also showed green tea's potent free radical scavenging action (Ounjaijean et al., 2008). Cigarette smoke increases peroxidation, which in turn destroys erythrocytes and other oxidation-vulnerable components. Heavy metal ions in cigarette smoke speed up lipid peroxidation in smokers. Perhaps due to the metal chelating action of green tea phytoconstituents, this study demonstrated that administering green tea to smokers decreased lipid peroxidation. Cigarette smoke's oxidants and green tea's antioxidants may interact in ways that affect therapeutic efficacy.

White blood cell (WBC) count changes in smokers provide evidence that smoking affects both the humoral and cellular immunological systems. This study found that cigarette smoking negatively affected hematological parameters putting smokers at increased risk for atherosclerosis, polycythemia, chronic obstructive pulmonary disease, pulmonary disease, and an immune system imbalance. Green tea's protective effects are shown by the fact that it normalizes abnormal alterations in the body. We found that the calming impact of numerous green tea ingredients is responsible for green tea's ability to reverse cigarette smoke's effects on the body's hematological profile (Bhardwaj and Khanna, 2013).

Cigarette smoking has intricate effects on the control of glucose metabolism. Disturbance in glucose homeostasis was suggested by the higher plasma glucose and HbA1c concentrations among smokers in this research. The plasma glucose and HbAlc levels of smokers were successfully reduced by supplementation with green tea. About 88-122 mg EGCG per gram of catechins may be found in a

single cup of green tea. Catechins and their derivatives may have helped maintain glucose homeostasis by re-entering circulation from the digestive tract to the liver following their initial phase of responses. It has also been found that the colonic microflora aid in the breakdown of catechins into smaller, more absorbable forms. In addition, Smoking and the decrease of insulin secretion in smokers have been connected to the effects of nicotine on beta cells and its effects on insulin secretion via nicotinic acetylcholine receptors on beta cells. Researchers found that giving rats green tea extract reduced insulin resistance and enhanced glucose metabolism. This study demonstrates that catechins in green tea provide protection against glucose metabolism abnormalities via different pathways (Watanabe et al., 2011).

Cigarette smoking is linked to cardiovascular illnesses, and lipid measurements are thought to be good predictors of this. Smokers were shown to have higher total cholesterol, LDL-C, VLDL-C, and triglycerides, as well as lower HDL-C, which is consistent with previous research. Smokers who drank green tea had significantly lower levels of cholesterol, triglycerides, and other lipoprotein patterns than those who didn't, indicating that green tea may be a useful tool in the fight against cardiovascular disease. Green tea's high polyphenol and catechin content has been shown to reduce cholesterol levels in hypercholesterolemia mice. Overall, the results of this study showed that drinking green tea is beneficial for your health because of its high catechin content, which works to counteract the negative effects of smoking in various ways (Huang et al., 2016).

Smokers may have damaged their liver cells, leading to a leakage of the enzymes ALT, AST, GGT, and ALP into the plasma. Increased lipid peroxidation and resulting changes in membrane characteristics may be to blame. Researchers observed that smokers who drank green tea had enzyme activity that were equivalent to controls. The catechins in green tea may have prevented the release of these enzymes by scavenging free radicals that had mediated damage to the membranes (Toolsee et al., 2013).

Vitamins are chemical molecules that are needed in extremely small amounts to maintain healthy cell function. Several enzyme-catalyzed processes rely on vitamins as their primary catalyst. Cells are shielded from oxidative stress because of vitamin C's antioxidant properties. Vitamin levels are impacted by long-term cigarette smoking (Wannamethee and Shaper, 2010). The levels of vitamin D, B12, and C were found to be lower in people who smoked. Possible cause: more radicals being created. Green tea's active phytocompounds have been shown to reduce this in smokers by neutralizing harmful free radicals. Most cellular enzyme catalyzed reactions rely on electrolytes and minerals as cofactors, giving them a pivotal role in metabolic processes (Padmavathi et al., 2009). They help regulate acid-base balance, neuron transmission, blood coagulation, and muscle contractions, among many other bodily processes. Results showed that cigarette smokers had higher levels of certain plasma elements compared to nonsmokers. Calcium channel modulation might include high-density lipoprotein cholesterol. Calcium levels may have risen because of changes in intracellular calcium release, decreased HDL-C, or enhanced activity of membrane-bound calcium ATPase. Increased electrolytes in smokers may result from a dysfunctional sodium potassium pump. Smoking may cause hypoxia, leading to elevated plasma iron and hemoglobin levels. There was a rise in red blood cell count because hypoxia stimulates erythropoiesis. The number of dead RBCs floating around in the bloodstream rises in tandem with the overall red cell mass, leading to an iron overload. Overall, the results of this investigation showed that the number of cigarettes smoked significantly affected the biochemical profile. According to the findings of this study, the catechins found in green tea not only chelate multiple ions, including calcium, very well but also have the ability to interfere with signaling processes via unidentified mechanisms, which in turn helps to restore normal mineral homeostasis (Kim et al., 2014).

## Conclusion

In conclusion, our research showed that green tea drinking among smokers reduces the negative biochemical alterations in plasma and blood caused by their habit. Green tea's phytocomponents give

protection through many methods, including interactions with biomolecules at the membrane and sub-cellular levels and modifications to signaling pathways.

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