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An animal study of the histo-physiological influence of green tea extract on the lead nitrate-treated heart

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Abstract

Background: Green tea extracts have shown an interesting influence on human body health. The lead from higher ecological pollution and industrialized usage has become a danger to community health. The existing work aims to scrutinize the histo-physiological changes of green tea extract on the rats' hearts after treatment with lead nitrates. Methods: The female rats were erratically divided into four groups of six rats. The first control group received distilled drinking water. The second group received treatment with an extract of green tea solution. The third group received a lead nitrates treatment, while the fourth received an 8-week treatment of a green tea extract, lead nitrates, and distilled water mixture. After the animals were killed, blood samples were taken to quantify troponin-I and calcium. All the rats then had their hearts histologically examined. Results: The primary finding of this study was that rats treated with green tea and lead nitrate had higher troponin-I levels in their blood than rats treated with lead nitrate alone. The heart's histopathological sections also showed that rats treated with lead suffered greater damage and adverse morphological changes than rats treated with green tea extract and lead nitrates. The study demonstrated how well green tea extract protects the heart from harm caused by lead nitrates. Conclusion|: In terms of troponin-I serum levels and histological changes in the rats' heart tissues, the green tea extract revealed good protective effects against cardiac damage caused by the harmful effects of lead nitrates.

Keywords

Green tea extract, lead nitrates, troponin-I, calcium, heart, rats.

Green tea "(Camellia sinensis)" is a non-fermented tea leaf that preserves the normal ingredient in its fresh leaves extensively. It is the 2nd most common worldwide drink in addition to water (1). Green tea consumption every day may decrease the risk of death from cardiovascular disease (2), hypertension, and stroke (3, 4). Such beneficial influences are attained by protecting the cellular organelles from damage and reducing the body's formation of free radicals (5), which have a role in aging and several types of diseases (6). Currently, multiple pharmacologic active substances have been extracted and recognized from extracted green tea, like poly phenols (flavonoids), volatile components, alkaloids, polysaccharides, amino acids, and Epigallocatechin Gallate (EGCG) (1, 7).

Lead is a poisonous weighty metal, that has extensive environmental spread. The main lead environmental sources are lead shots, leaded gasoline, batteries, cosmetics, dust, and paints (8). Several chemical and physiological behavior dysfunctions are triggered by prolonged exposure to low doses of lead (9). Lead may cause oxidative stress by reactive oxygen species generation, which

has been proven as an important pathway underlying lead intoxication (10). There is an association between inflammation, cardiovascular disease, inflammation, satiety, and oxidative stress (11-15).

Lead impedes glutathione production and activity directly or indirectly, and suppresses superoxide dismutase enzyme activity, which increases the thrombogenic free radicals. Pro-atheromatous changes due to lead intoxication have also been related to the deactivation of paraoxonase function, which reduces the antioxidant activity of high-density lipoproteins (16).

Troponin is a complex protein of three regulatory subunits (C, I, and T). Troponin is fundamental to muscular contraction in cardiac and skeletal muscles. It has well-known as an analytic biomarker for different cardiac disorders (highly specific) cardiac myocytes death or infarction (15, 17-19). Several academics have published well-designed peer-reviewed clinical trials that documented troponin-I as a highly-sensitive and specific biomarker of myocardial injuries (20-22).

Calcium ion (Ca^{2+}) binds troponin-C and acts as a vital regulator of myocyte activity. Ca^{2+} is the connection between the electrical signals, which enhances the cardiac contraction to force blood. Furthermore, Ca^{2+} controls several other myocytes' activities, like gene transcription (23).

Objectives: The goal of the current study was to examine the physiological and histological effects that green tea extract had on the hearts of female rats that had been given lead nitrate treatment.

Materials and methods

A total of 16 healthy female "Sprague−Dawley rats" (weighted around 180−200 grams) were obtained from the animal house at the College of Medicine. All the rats were accustomed to a natural photoperiod at ordinary room temperature for 14 days before trial initiation. Well-adjusted nourishment and tap water were supplied. The total period of the trial was 4 weeks. The experimental rats were crudely separated into four groups (6 animals each): the control rats' group had received distilled water (DW) only. The 2nd group had given a green tea solution. The 3rd group had administered 0.5% of lead nitrate in DW (24). The 4th group had fed a combination of lead nitrate with green tea as the only drink.

The extract of green tea was prepared by saturation of 15 grams of powder of instant green tea in one liter of boiling DW for five minutes (25), then the mixture was filtered to produce (1.5% green tea). All the rats were sacrificed before being anesthetized using diethyl-ether a day after the fourth week. The hematogenous samples from every rat were drained from the heart in heparinized test tubes for chemical investigations. Troponin-I was measured using a specific "rat ELIZA kit (Elabscience®)". While, the serum Ca^{+2} was measured by Ca^{2+} "colorimetric assay, MAK022, Sigma-Aldrich (Merk®)". Tissue sections from the hearts of the experimental rats were obtained for a further histopathology examination. The data were collected and transferred to a Windows excel sheet analyzed.

Results

When compared to rats only receiving lead nitrate treatment, rats who were given green tea plus lead treatment had higher blood levels of troponin-I. While Troponin-I levels were not elevated and comparable among the two groups of rats (figure 1).

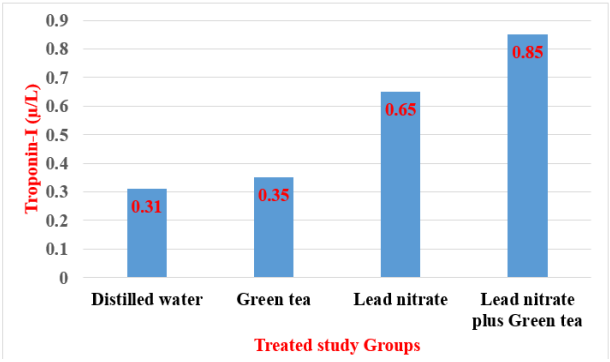
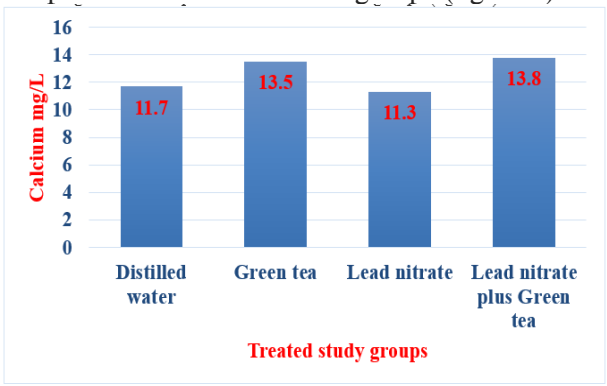


Figure 1: Distribution of serum troponin-I levels (µ/L) among the four groups of the studied rats

The concentration of plasma Ca^{2+} was higher among the female rats fed with green tea extracts and female rats fed with lead and green tea compared with the other two groups (figure 2)

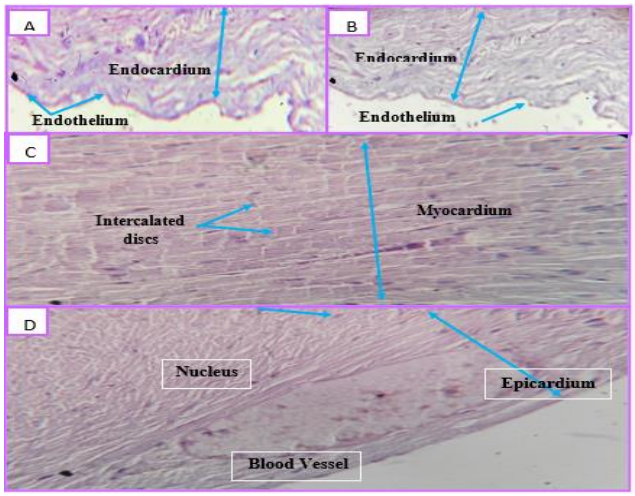


Histopathology Results

Amongst the control rats, the myocardium muscular layer is striated and normally arranged in a linear outline that branched and interconnected in a definite architecture giving the impression of sheets.

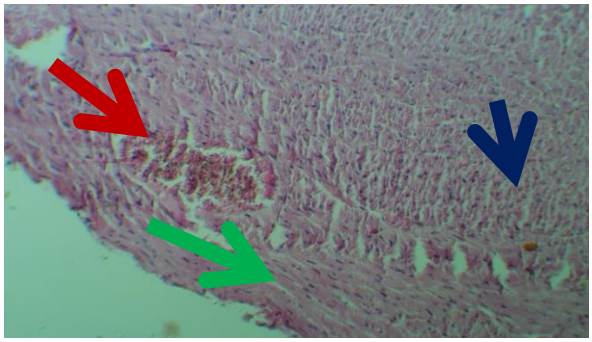
The myocytic fibrils are linked by frequent "intercalated discs". Myocytes revealed acidophilic cytosol with oval central nuclei. The cardiac muscular fibers are enclosed by a thin coat of connective tissues with well-defined vessels (Figure 1C).

The endocardium also appeared, comprising endothelium (simple squamous layer), a middle layer of connective tissue intermeshed with muscular fibers, and the outer layer of the endocardium is the subendocardium (Figure-1A). The outermost (epicardium) layer of the heart was also illustrated (Figure-1D) and contains nerves and vessels supplying the heart. The epicardium included abundant adipose tissue.

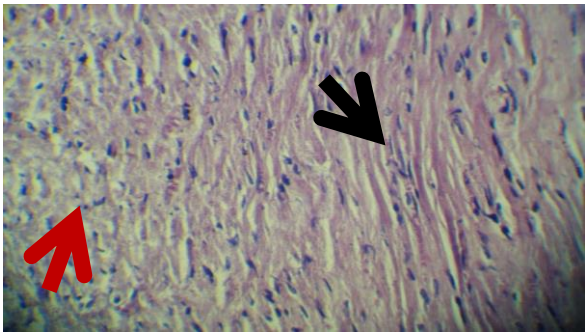


Picture-1: (A, B, C, D): Light micrograph of a cardiac section, of the control albino rats. H & E, 40X, 10X respectively.

Picture 2 showed the histological section of the heart of the rat group treated with lead. The normal muscular tissue disappears, with necrosis and loss of normal striation of muscle fibers associated with massive hemorrhages between the muscle fibers. These changes can be attributed to the deposition of lead molecules between the muscle fibers and its cause damaging potential on cells might have led to hemorrhages and lost striations (42).

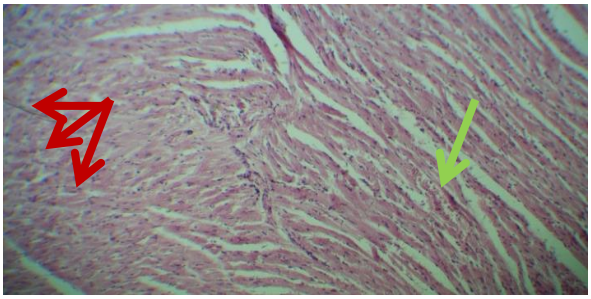


Picture 2: Light micrograph of the heart section of an albino rat treated with lead, shows hemorrhage (red arrow), necrosis (blue arrow), and loss of striation (green arrow) H & E 40X.



Picture 3: Light photomicrograph of a histological section of the heart of an albino rat treated with green tea, shows an aggregation of inflammatory cells (black arrow), with diffusely deformed cells (red arrow) H & E, 10X.

Numerous myocytes revealed nuclei, which were distorted and heterogeneous. Few myocytes were bizarrely extended and thin. There are groups of atypical myocytes that revealed an atypical appearance instead of the ordinary branched interconnected pattern of the muscular fiber like that seen in the control rat (26).



Picture-4: Light photomicrograph of a histological section of the heart of an albino rat treated with lead and green tea. It reveals the appearance of an intercalated disc (red arrow) and mild hemorrhage H & E, 10X.

Among female experimental rats treated with green tea extract and lead nitrates, the histological sections reveal areas of minor hemorrhage and loss of striation of cardiac muscle and lack of intercalated discs.

Discussion

A large number of scholars have reported that green tea has chemical elements that are closely linked with human well-being. Tea polyphenols, EGCG, and other constituents, which are isolated from green tea leaves have various pharmacologic effects against tumors, oxidation, hyperlipidemia, hyperglycemia, hypertension, ischemic heart disease, and DM.

The objective of the current study is to evaluate the histo-physiological effects of green tea extract on the lead nitrates-treated rat heart. The primary

finding of this study was a reduction in troponin-I levels in the blood of rats given lead nitrates and green tea compared to rats given lead nitrates alone. The rats treated with lead also had more damage and toxic morphological changes in their hearts than the rats treated with green tea extract and lead nitrates, who had fewer changes. Together, this study revealed to some extent a potential protective role of green tea extract against the toxic effects of lead on the cardiac tissues.

Plentiful revisions have inspected relations between the intake of green tea and a wide variety of health consequences. Green tea flavonoids may amend body enzymes included in oxidative or inflammatory stresses, enhance endothelial activity and improve nitric oxide (NO) function, which may employ in part potential assistance to cardiovascular health (7). The outcomes of experimental trials propose that the cardiac protective effects of catechins could be facilitated via various pathways, such as depressed oxidative effects, vessel inflammation, or atherogenesis (27). In experimental animal trials, catechins affect nitric oxide synthesis (coronary vasodilator) and hence enhance endothelium dysfunction and systemic hypertension (28).

Numerous pharmacological actions of tea catechins enable their antihypertensive properties. These actions comprise a raise in the release of plasma NO, which can suppress inflammatory mediators and platelet aggregation and enhance endothelial functions (29). Moreover, tea catechins may possess anti-inflammatory activities by the suppression of inflammatory modulators, like cellular adhesion molecules and cytokines (30). They may as well exert vasodilation via reduced contractile responses owing to inhibited mRNA expression of NO- synthase and endothelin-1 (31). Recent evidence about tea catechins has been shown to enhance the "cholesterol 7α-hydroxylase gene" expression in human hepatoma cells, which may enhance bile acid synthesis and decrease hepatic cholesterol. Green tea constituents have been shown to reduce the absorption of GIT lipids and up-regulation of hepatic LDL receptors, which alter lipidemia status (7).

Few arguments can be advanced to support the advantageous properties of tea polyphenols on calcium metabolism and bone mineralization. The earlier reports have found higher bony densities in old females who consume tea, compared to those who do not, signifying a positive impact of tea (32). Furthermore, studies have exposed that green tea drinking may trigger a positive effect on bones through other ingredients, which possess antioxidant, estrogenic, and anti-inflammatory effects (33). Other scholars showed that if the consumed tea exceeded 5 cups daily (each 1 cup equal to 300cc), the positive effect was not observed consequently (34).

However, there is no study convincingly indicating the link between green tea intake with bone metabolism, and even the sex variations are still debatable. As a rebuttal to this point, other studies assumed that the caffeine in the tea can reduce intestinal Ca^{+2} absorption, stimulate Ca^{+2} excretion, and result in the loss of bone Ca^{+2} (35).

Histologically, the anti-inflammatory properties of green tea could be attributed to their decreasing effect on leukocyte adhesion to the endothelial layer and consequent transmigration via suppression of cytokine production, adhesion molecules, and inflammatory cells in the endothelium (5) which may explain our findings (figures 1, 2).

In the existing study, there were lower troponin-I levels in the rat treated with green tea alone compared with the control group. While the serum troponin-I concentrations were higher in the lead-treated rats compared with the control and the green tea extract groups. Undoubtedly, these high troponin-I concentrations were related to myocardial damage caused by lead nitrate that perhaps recovered by the administration of green tea. Green tea catechins, EGCG, and probably some other active components contain antioxidant properties of scavenging reactive oxygen species, which certainly play a protective and repairing role in myocardial changes or damage induced by lead nitrate.

Conclusion

In terms of troponin-I serum levels and histological changes in the rats' heart tissues, the green tea extract revealed good protective effects against cardiac damage caused by the harmful effects of lead nitrates. Hence, additional evaluation of the biological effects of green tea in controlled large clinical cohorts would be mandatory to understand the precise influences of green tea extracts on the health of the cardiovascular system.

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