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Research Paper

Green Tea And Coffee Extract Has A Cardioprotective Effect On Rats Caused By Doxorubicin.

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ABSTRACT

Green tea and Coffee extract's (GTE) in vivo antioxidant capabilities were examined in relation to doxorubicin's (DOX) cardiotoxicity in rats. 48 Swiss albino rats weighing 200–250 g were split up into eight groups (n = 6) for this experiment. For thirty days, the control group was given regular saline. On the 29th day of the trial, DOX (20 mg/kg i.p.) was used to cause cardiotoxicity. GTE (100, 200, and 400 mg/kg, p.o.) was administered for 30 days to treat the cardiotoxicity. Along with histopathological examinations, the following biomarkers were assessed: blood glutathione, tissue glutathione, cytochrome P450 (CYP), creatinine kinase (CK), lactate dehydrogenase (LDH), lipid peroxidation (LPO), enzymatic and non-enzymatic antioxidants. Rats treated with DOX exhibited markedly elevated levels of AST, CK, LDH, LPO, and CYP; these were alleviated by oral GTE treatment at 100, 200, and 400 mg/kg for a duration of 30 days. Additionally, GTE treatment dramatically boosted the heart's activities of DOX-treated glutathione peroxidase (GPX), glutathione reductase (GR), glutathione s-transferase (GST), superoxide dismutase (SOD), and catalase (CAT). Our research indicates that the oral administration of GTE inhibited the cardiotoxicity caused by DOX by promoting the heart's antioxidant defense systems and bringing the levels of LPO down to normal.

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I. INTRODUCTION

Doxorubicin pegylated liposomal (marketed as Caelyx) is approved for the treatment of ovarian cancer, breast cancer, and Kaposi's sarcoma associated with AIDS in the EU. It is recommended to use it in conjunction with bortezomib to treat multiple myeloma. Hair loss, inhibition of the bone marrow, vomiting, rash, and oral irritation are common adverse effects. Additional dangerous side effects include anaphylactic allergic responses, heart damage, injection site tissue damage, radiation recall, and treatment-related leukemia. Red urine staining frequently lasts for a few days in people [1]. Doxorubicin belongs to the class of drugs known as anthracyclines and antitumor antibiotics. It partially functions by interfering with DNA function. Doxorubicin's most harmful adverse effect is dilated cardiomyopathy, which can result in congestive heart failure. The incidence of cardiomyopathy is based on the cumulative dosage of doxorubicin; it is around 4% when the dose is between 500 and 550 mg/m2, 18% when the dose is between 551 and 600 mg/m2, and 36% when the dose is greater than 600 mg/m2. Doxorubicin is thought to induce cardiomyopathy by a number of mechanisms, including oxidative stress, downregulation of genes encoding contractile proteins, and p53-mediated apoptosis [2].

Using plants or plant extracts for therapeutic reasons is known as herbal medicine, botanical medicine, or phytotherapy. Many civilizations have utilized herbal treatments to cure a variety of illnesses throughout history. Herbal extracts are concentrated versions of medicinal plants that are created by utilizing different solvents, such as water, alcohol, or oil, to extract the active ingredients from the plant material [3]. High concentrations of bioactive substances, such as vitamins, minerals, antioxidants, and phytochemicals, are present in these extracts, which may explain some of their possible health advantages .

Herbal extracts with a plethora of health advantages, including cardioprotective properties, include green tea and coffee extracts. The following are some potential ways that these extracts might support heart health:

1) Antioxidant Properties: Polyphenols and flavonoids, two types of antioxidants found in high concentrations in both green tea and coffee extracts. These antioxidants aid in the body's defense against dangerous free

radicals, lowering inflammation and oxidative stress—two factors connected to the onset of cardiovascular illnesses.

2) Blood Pressure Regulation: According to some study, the chemicals in coffee and green tea extracts may help control blood pressure by enhancing blood flow and encouraging vasodilation, or the relaxing of blood vessels. The risk of hypertension and cardiovascular events like heart attacks and strokes can be decreased by lowering blood pressure [4].

3) Reducing Cholesterol: Research indicates that coffee and green tea extracts may help raise HDL (good) cholesterol levels while reducing LDL (bad) cholesterol. These extracts have the potential to lower the risk of atherosclerosis and heart disease by encouraging a better lipid profile.

4) Reducing Oxidative Stress in the Heart: Green tea and coffee extracts' antioxidant qualities help shield the heart muscle from oxidative damage, which lowers the risk of cardiovascular conditions including myocardial infarction and heart failure [5].

II. MATERIAL AND METHOD

Experimental Animals

For the study, 8–12 week old Swiss Albino mice of either sex would be employed. The animals were kept in a controlled environment with a 12-hour light/dark cycle, 22°C ± 2°C temperature, and 50 ± 10% humidity. The committee's guidelines are followed in order to control and oversee the conduct of experiments. Animals (CPCSEA) criteria after the institutional animal ethics committee's (IAEC) approval of the experimental methods [6].

Drugs and chemicals

DOX was procured from Thermocil Chemicals, Pune, India.

Every additional chemical utilized in the investigation was of analytical grade.

Plant material

Standardized powdered, aquous extract of green tea and Coffee extract was a purchased from, Sagar herbal, Jaipur India.

Phytochemical Analysis

After that, the extract would go through several phytochemical assays to identify various phytochemical components. The presence of significant phytochemicals, such as flavonoids and total alkaloids, which may be implicated in the activity of the plant, would be investigated in the plant extract. content of triterpenoids, steroids, etc.

Acute dermal toxicity study

The following procedure was used to assess the acute toxicity of prepared oil in accordance with OECD guideline 402.

Table -1. Treatment protocol

Sr.no.	Group	No. of Animals	Treatment and Doses	Route of Administration
1.	I	6	Negative control	Oral
2.	II	6	Positive control (doxorubicin)	Subcutaneous
3.	III	6	doxorubicin (1mg/kg) + Minoxidil (2%) standard drug	Subcutaneous and Topical
4.	IV	6	doxorubicin (1mg/kg) + Green tea coffee extract extract oil (100 ml/kg)	Subcutaneous Topical
5.	V	6	doxorubicin (1mglkg) + Green tea coffee extract extract oil (200 ml/kg)	Subcutaneous Topical

Experimental procedure

The mice were given subcutaneous injections of doxorubicin after being split up into five groups of six animals each. Animals in groups III, IV, and V received different doses of minoxidil hemp oil. Over the course of 29 days, topical applications of between 100 and 200 milliliters per kilogram of the solution were made to the back skin. Mice from each group were chosen at random and put to death after 30 days. From each group of mice's balding spot, a skin sample was collected [7].

Histopathological examination

Shortly after the animal was sacrificed, the heart was removed and preserved in 10% buffered neutral formalin solution. It was then cleaned with ice-cold normal saline. The cardiac tissue was treated by embedding it in paraffin after fixation. Hematoxylin and eosin (H.E.) staining of the sectioned cardiac tissue was followed by a histological analysis.

Statistical analysis

The information was presented as the average ± standard error (SE). Group means were compared using one-way analysis of variance (ANOVA) and post-hoc analysis for a statistical study of the data. To determine group significance, the Tukey-Kramer post-hoc test was used; $p < 0.05$ was regarded as statistically significant [8].

III. RESULTS

Impact of GTE on AST, CK, and LDH levels in serum

Figures 1-3 describe the effect of GTE on serum levels of AST, CK, and LDH, respectively. Rats treated with GTE (groups V, VI, and VII) and vitamin E (group VIII) exhibited a substantial ($p < 0.001$) reduction in blood marker enzyme levels in comparison to rats treated with DOX alone (group II). Comparing the control groups (groups III and IV) to the normal control rats (group I), no discernible difference was seen.

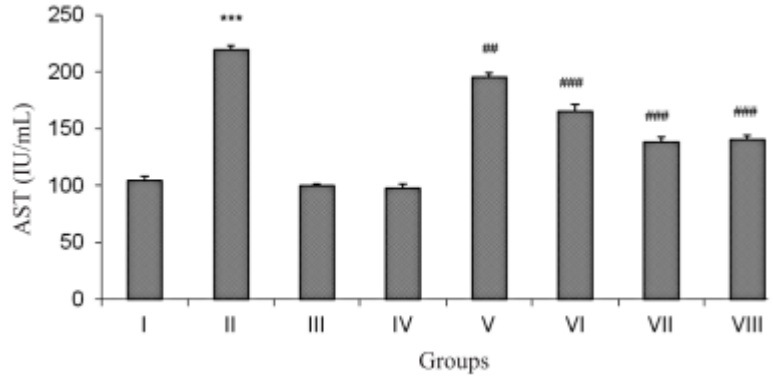

Figure 1: GTE's effect on rats given DOX in terms of serum AST level. The mean ± SEM is used to express the results, and there are six rats in each group. The values for group I (normal control rats) and group II (DOX-treated rats) are compared with "$p < 0.001$ and "$p < 0.01$, respectively.

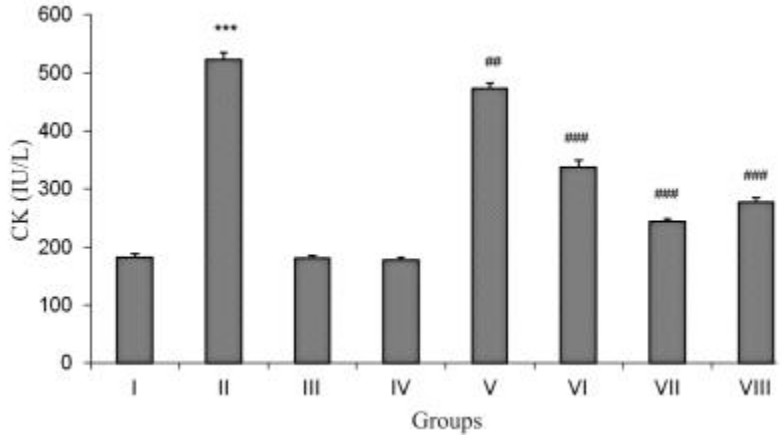

Figure 2: GTE's impact on the amount of serum CK in rats given DOX. The mean ± SEM is used to express the results, and there are six rats in each group. The values for group I (normal control rats) and group II (DOX-treated rats) are compared with "$p < 0.001$ and "$p < 0.01$, respectively.

GTE's impact on CYP contents

Figure 4 shows how GTE affects CYP contents across different categories. Rats treated with DOX alone had a substantial ($p < 0.001$) reduction in CYP. contents in contrast to standard control rats When comparing the CYP contents of groups V, VI, VII treated with GTE and group VIII treated with vitamin E, the differences were considerable ($p < 0.001$) from group II rats. No Rats in control groups III and IV showed a significant difference from rats in group I.

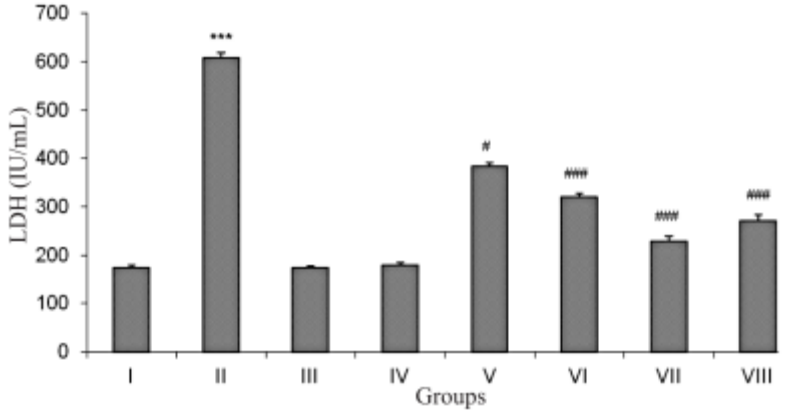

Figure 3: GTE's impact on rats given DOX and their blood LDH levels. The mean ± SEM is used to express the results, and there are six rats in each group. The values for group I (normal control rats) and group II (DOX-treated rats) are compared with "p < 0.001 and "p < 0.01, respectively.

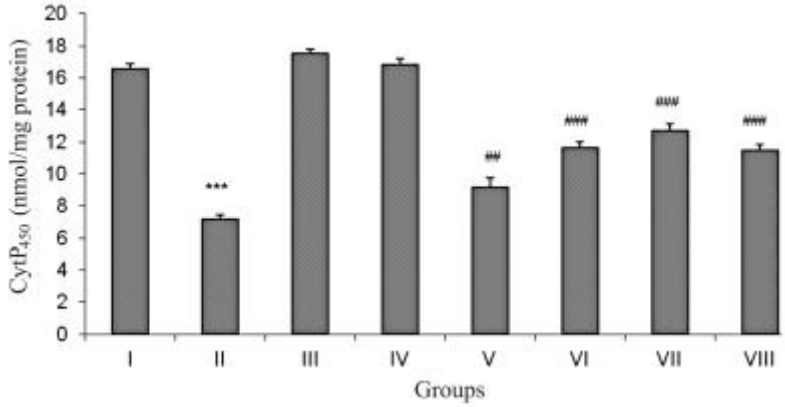

Figure 4: GTE's impact on cytochrome P450 levels in rats given DOX. The mean ± SEM is used to express the results, and there are six rats in each group. The values for group I (normal control rats) and group II (DOX-treated rats) are compared with "p < 0.001 and "p < 0.01, respectively.

GTE's impact on LPO levels

Table 1 shows how different groups' levels of malondialdehyde (MDA), an LPO secondary product, are affected. The DOX therapy produced a noteworthy (p < 0.001) MDA levels were higher in rats treated with DOX alone than in group I, the normal control rats. When compared to rats in group II, the MDA levels in GTE-treated groups V, VI, and VII as well as the vitamin E-treated group were considerably (p < 0.001) lower. When comparing group I rats to control groups (groups III and IV), no discernible change was seen.

GTE's effects on tissue and blood glutathione levels

The activities of tissue and blood glutathione in different groups are also shown in Table 1. Rats given DOX alone had significantly lower blood and tissue glutathione levels (p < 0.001). GTE When compared to group II rats, treated groups V, VI, VII, and vitamin E treated group VIII considerably (p < 0.001) raised the levels of glutathione in the blood and tissue. When comparing group I rats to control groups (groups III and IV), no discernible change was seen.

GTE's effect on the levels of antioxidant enzymes (GPX, GR, GST, SOD, and CAT)

The impact on these antioxidant enzymes in different groups is seen in Table 2. When compared to the normal control rats, the activity of these enzymatic antioxidants in the heart was significantly (p < 0.001) lower in rats treated with DOX alone. When compared to rats treated with DOX alone (group II), the activity of these enzymatic antioxidants was significantly (p < 0.001) higher in GTE-treated groups V, VI, VII, and vitamin E-treated group VIII. When comparing group I rats to control groups (groups III and IV), no discernible change was seen.

Impact of GTE on cardiac histological alterations

The rat heart from the normal control group had normal cardiac fibers, as seen in Figure 5. The group II rats treated with DOX alone displayed a huge and irregularly shaped hypertrophic cardiac fiber with additional fibers nearby with tiny and large vacuoles (Fig. 5B). The group I rats showed no vacuolation, necrosis, or

inflammation. The cardiac muscle fibers' size, form, and configuration were all normal in the histopathology of the heart in the GTE-treated groups V, VI, VII, and vitamin E-treated group VIII (Figs. 5C, 5D, 5E, and 5F, respectively).

IV. DISCUSSION

Reactive oxygen species (ROS) produced by DOX appear to be a major contributor to cardiomyopathy (9, 23). The enzymes AST, CK, and LDH are the diagnostic blood marker enzymes of cardiotoxicity. (24). According to reports, the strongest indicator of cardiotoxicity is the enzymes (AST, CK, and LDH) that leak from tissue injury because of their tissue specificity and serum catalytic activity[9].

Additionally, our research shows that rats treated with DOX alone have higher activity of these marker enzymes. When DOX is administered, the cardiac cell membrane may be harmed or become permeable, which might cause AST, CK, and LDH in the blood. The rise in these marker enzyme levels in the serum is most likely explained by this. GTE pretreatment (100, 200, and 400 mg/kg p.o.) reduced the levels of these marker enzymes in serum to normal levels, which restored the activity. This might be because GTE protects the heart by lowering myocardial damage and preventing the release of these enzymes into the serum [10].

The mitochondria, where DOX accumulates, are the target organelle of DOX-induced cardiotoxicity [11]. The manner in which mitochondrial enzymes, such as NADH dehydrogenase, interact with DOX that redox cycling occurs between the quinone and semiquinone states in the quinone ring [12].

In the course of this process, oxidizing substances, such as oxygen, produce and absorb electrons, which set off a series of events that result in the production of reactive oxygen species (ROS) [13]. It has also been discovered that xanthine oxidase and cytochrome P450 reductase catalyze the reduction. converting anthraquinone to a free radical of semiquinone [14].By inhibiting cytochrome P450, DOX can boost its own metabolism, which could hasten its removal and raise the synthesis of hazardous reactive metabolites. The cytochrome P450 molecule is not quickly arranged inside the microsomal membrane and exhibits lateral mobility, which is mostly contingent upon the membrane's fluidity [15]. When rats receiving DOX were given varying dosages of exogenous GTE supplementation, the amount of cytochrome P450 increased. This might be linked to the decline in Hemoxygenase activity, which therefore raised the cytochrome content. Furthermore, a decrease in the production of lipid peroxides may be linked to an acceleration of the detoxification process [16].

Increased lipid peroxidation (LPO) and modified enzymatic and non-enzymatic antioxidant mechanisms are hallmarks of oxidative stress [17]. In the current investigation, a noteworthy rise in the amount of LPO, It was seen in the heart tissue of rats given DOX alone. Biological membrane LPO is triggered by free radicals. In cell-free settings, GTE has been demonstrated to lessen damage to lipid membranes, proteins, and nucleic acids by neutralizing reactive oxygen species including superoxide radical, single oxygen, hydroxyl radical, peroxyl radical, nitric oxide, nitrogen dioxide, and peroxynitrite. An essential tool for understanding the toxicological processes of these agents and implementing effective treatment strategies to reduce their toxicological effects is the evaluation of the relative contributions of the various routes of free radical generation triggered by DOX.

The glutathione antioxidant system plays a major role in cellular defense against free radicals and other oxidant species (26). GSH plays a part in essential role in both scavenging reactive oxygen species and detoxifying the medications. GSH is vital for H2O2 detoxification and catalyzes disulfide exchange activities because of its αSH group. A GSH deficit results in tissue damage and weakened cell defense. Following the introduction of DOX, there is a considerable reduction in heart and blood GSH levels, indicating an impaired glutathione status. The results of this investigation, which showed that rats given GTE had increased GSH and decreased MDA levels, suggest that the therapy scavenges free radicals generated during oxidative stress [18].

All of the antioxidant enzymes in our investigation, including catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, and glutathione s-transferase, had considerably reduced activity. Rats were treated with DOX alone (27-30). This outcome demonstrated that DOX causes the heart to produce free radicals and reduces its capacity to detoxify ROS. Nonetheless, in the DOX-treated groups, GTE markedly raised the concentration of these antioxidant enzymes. Its ability to scavenge ROS and act as an antioxidant may be the reason for this protection.

The following are the histological alterations associated with DOX-induced cardiotoxicity in increasing order of severity: sarcoplasmic reticulum enlargement, cytoplasmic vacuolization, myofibrillar degeneration, Fibrosis and myocyte disruption [19-22]. In line with the aforementioned finding, our study also revealed hypertropic fiber, disruption of myocyte structure, including damage to microtubules, vacuolization, sarcoplasmic reticulum dilation, loss of myofibrils, and altered mitochondrial functions like decreased mitochondrial enzyme activities. Heart muscle fibers in rats given GTE treatment had normal size, shape, and arrangement. In one of the cardiac fibers, there is just one vacuole visible. The histopathological alterations that show GTE's protection may be caused by its antioxidant capacity to fend off free radicals [23].

GTE's capacity to restore tissue normality under oxidative stress is likely attributed to its antioxidant nature, which counteracts free radicals and has a protective impact overall. But the specific chemical mechanism by which GTE works It is yet unknown how protective it is against oxidative damage. As an adjuvant treatment with DOX, GTE may have significant therapeutic importance if its protective activity in cancer patients is validated [24-25].

V. CONCLUSION

The results and implications of a study examining the cardioprotective effect of green tea extract on doxorubicin-induced cardiotoxicity in rats are usually summarized in the study's conclusion. This is an example of a conclusion drawn using fictitious data:

To sum up, our research indicates that green tea extract has noteworthy cardioprotective qualities that protect rats from the cardiotoxicity caused by doxorubicin. Green tea extract administration successfully reduced the negative effects of doxorubicin on cardiac tissue histology and function. According to these results, green tea extract shows promise as an adjuvant treatment for doxorubicin-induced cardiotoxicity in cancer patients receiving chemotherapy. To clarify the underlying mechanisms of green tea's cardioprotective properties and assess its potential therapeutic uses in humans, further research is necessary."

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