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Green tea supplementation prevented oxidative stress, fibrosis, and myocardial damage in isoproterenol-induced Swiss albino mice

Shampa Akter [a,1], Shatil Rafia [a,1], Raiyana Huda [a], Rashedul Haque [b], Sajib Paul [c], Md.Tipu Sultan [b], Md. Kawser [b], Faizul Islam Chowdhury [b,*]

[a] Department of Pharmaceutical Sciences, North South University, Bangladesh
[b] Department of Pharmacy, Dhaka International University, Bangladesh
[c] Department of Pharmacy, Jagannath University, Bangladesh

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ABSTRACT

Introduction: One of the main causes of death and morbidity in people with cardiovascular disorders is myocardial infarction. This study investigated the impact of green tea (GT) leaf powder on isoproterenol (ISO)-induced myocardial infarction in Swiss albino mice.

Methods and materials: 4 to 5 weeks male Swiss albino mice were divided into four groups with 6 mice in every group: Control, ISO, Control + GT and ISO+ GT leaves powder. All of the mice were sacrificed at the end of the investigation. Heart organ and blood plasma samples were taken, and they were tested for oxidative stress indicators and several biochemical parameters. To investigate the formation of mononuclear cells and fibrosis in the heart histopathological staining of tissue sections was also carried out.

Results: In this experiment, supplementation with green tea leaf powder in ISO-administered mice prevented the increased activity of the AST, ALT, and ALP enzymes. Additionally, three more tests were performed such as CK-MB, Creatinine, and Uric acid whose levels were increased by isoproterenol, and treatment of green tea reduced these elevated levels. Due to the supplementation of green tea leaf powder, ISO-administered mice had lower levels of lipid peroxidation product, nitric oxide, advanced protein oxidation product, and myeloperoxidase. Mice exposed to ISO had considerably higher levels of oxidative stress markers and lower levels of cellular antioxidants including catalase activity, SOD activity, and reduced glutathione concentration. Additionally, the heart of ISO-induced mice revealed substantial fibrosis and inflammatory cell infiltration. Furthermore, green tea leaf powder supplementation prevented inflammatory cell infiltration and fibrosis in ISO-administered mice.

Conclusion: In conclusion, the findings imply that supplementing with green tea leaf powder might lower the risk of myocardial infarction in mice given ISO, potentially by reducing oxidative stress, inflammation, and fibrosis.

1. Introduction

In the United States, about 5.7 million people are affected by heart failure (HF) which is considered a major health issue (Mozaffarian et al., 2015). More than 40 % and 60 % of about 5 years and 10 years mortality rates show a very lethal condition HF, although therapy is very advanced (Levy et al., 2002). There is little information available about HF progression which is related to genetic variants but also there are some other important kinds of stuff involved such as genetic mutation, chemotherapy, alcohol, valvular disease, hypertension, and coronary artery disease which are some etiological factors. HF is progressed by irreversible myocardial injury, maladaptive cellular growth, organ function preservation, cardiac output maintenance to fluid retention, and heart rate (HR) augmentation because of renin-angiotensin

Abbreviations: ALP, Alkaline Phosphatase; ALT, Alanine Aminotransferase; APOP, Advanced Protein Oxidation Product; AST, Aspartate Aminotransferase; CK-MB, Creatinine Kinase Muscle Brain; GSH, Reduced Glutathione; GT, Green tea; H&E staining, Hematoxylin and Eosin staining; ISO, Isoproterenol; MDA, Malondialdehyde; MPO, Myeloperoxidase; NO, Nitric Oxide; SOD, Superoxide Dismutase; SR staining, Sirius Red staining.

* Corresponding author at: Department of Pharmacy, Dhaka International University, House no-772, Lane No-1, Purbachal road, North Badda, Dhaka, 1212, Bangladesh.
E-mail address: fislamrahat@gmail.com (F.I. Chowdhury).

[1] Equal contribution.

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activation and compensatory adrenergic (Lymperopoulos et al., 2013).

In the case of HF progression clinical determinants are one of the most important and it is also known as cardiac remodeling where the function, shape, and size of the heart are changed clinically which includes extracellular, cellular, and molecular change (Cohn et al., 2000). HF therapeutic targets such as those that are related to clinical cardiac remodeling, systolic dysfunction, reverse ventricular dysfunction, mortality, and morbidity benefits provided by therapeutic agents HF, angiotensin-converting enzyme (ACE) inhibitors and beta-adrenergic receptor blockers (Koitabashi and Kass, 2012).

In the matter of experimental and clinical HF progression and development are involved by oxidative stress (Karimi Galougahi et al., 2015; Vincenzi et al., 2017). 'Redox state' is an important mechanism and where endogenous antioxidant defense mechanism and reactive oxygen species (ROS) balance is dysregulated is defined by oxidative stress. In the matter of cell homeostasis critical function is played by ROS when the concentration is low. Irreversible cell death and damage, DNA damage, lipid and protein peroxidation, and cellular dysfunction are caused by excess reactive oxygen species. In cardiomyocytes, gradual loss can be suggested by heart failure progression where troponin release is increased which is shown as high sensitive troponin assay (Sato et al., 2012).

Heart failure and maladaptive myocardial remodeling progression and development can lead to an overabundance of ROS in the heart. Sodium-calcium exchanger, potassium channel, sodium channels, l-type calcium channels, excitation-contraction coupling, a protein central modified by cardiomyocytes with contractile machinery and electrophysiology which is impaired directly by ROS (Takimoto and Kass, 2007). Myofilament calcium sensitivity can be reduced by the activity of ROS (Takimoto and Kass, 2007). Energy metabolism which is related to the function of protein can be affected by energy deficit induced by ROS (Takimoto and Kass, 2007). In extracellular remodeling matrix metalloproteinases and cardiac fibroblast proliferation can be induced by ROS which shows pro-fibrotic function (Takimoto and Kass, 2007).

In developing countries mortality and morbidity is developed which is the leading cause of myocardial infarction (MI) globally (Steffens et al., 2009). When coronary blood delivery and myocardial blood demand get imbalanced they cause myocardial necrosis which is an important acute disease of MI (Liu et al., 2016). Cardiomyocyte degeneration and cardiac ischemia lead to this imbalance (Temesszentandrási et al., 2016). Death or irreversible cardiac injury is caused eventually by ischemia which causes heart tissue damage (Buja and Entman, 1998). Cardiac ischemia is caused by lipid peroxidation, and mitochondrial impairment which is associated with alteration of biochemical and cardiac tissue histopathology changes (Basha and Priscilla, 2013; Zou et al., 2016).

Structurally synthetic catecholamine is known as Isoproterenol. In the heart histological, functional, and biochemical alteration can extensively be produced by a potent beta-adrenergic agonist which is known as called Isoproterenol (Prabhu et al., 2006). With the help of the auto-oxidation process lipid peroxidation and free radicals which are toxic and produced in large doses in experimental animals induce MI applied by Isoproterenol. Endogenous antioxidant activity is reduced because of oxidative stress production which generates free radicals shown in animals by MI induced by isoproterenol (Rathore et al., 1998). In membrane carbohydrates, lipids, and proteins where oxidative injury is caused in ischemia tissue with overprotection of reactive oxygen species (superoxide anions and hydroxyl radicals) (McMichael and Moore, 2004). Myocardial infarction is developed by the influence of reactive oxygen species which is enhanced significantly increased level of oxidative stress which is happed due to myocardial ischemia suggested by several preclinical studies (Hill et al., 2005; Zhou et al., 2009).

In subtropical and heat and humidity *Camellia sinensis* tree principally develops. On the planet perhaps the most consumed refreshment is *Camellia sinensis* which is produced using leaves. Based on the level of maturation tea can be ordered, for example, pu-erh tea, dark tea, oolong tea, yellow tea, white tea, and green tea. Oolong is consumed by about <2 %, green tea is consumed by around 20 %, and dark tea is consumed by around 78 % which is for the most part delivered (Naveed et al., 2018). From leaves of *Camellia sinensis* a few fundamental bioactive mixtures, for example, thearubigins, teaflavins, glycosyl subordinates, for example, rutin, quercetin, apigenin, flavonls, and flavanols, for example, epigallocatechin gallate and essential catechin compounds. With the assistance of the level of aging of leaves sum and kind of these not entirely settled. Trademark kind of dark tea is given when they are handled and when theaflavins are delivered and in green tea principal compound is epigallocatechin-3-gallate (Konieczynski et al., 2017; Tang et al., 2019; Valduga et al., 2019). Cardiovascular assurance properties, cholesterol bringing down an enemy of disease, mitigating and hostile to oxidant properties are remembered for the medical advantage of *Camellia sinensis* (Bedrood et al., 2018; Naveed et al., 2018).

For the treatment of heart failure and myocardial ischemia, *Camellia sinensis* could be an alternative treatment based on the previous publications. So, based on the effect of *Camellia sinensis* on different types of problems related to cardiovascular disease, we hypothesized that cardiac failure and myocardial ischemia which is created by isoproterenol can be countered by the treatment of *Camellia sinensis*. So, our aim will be also in mice' fibrosis and oxidative stress which is induced by isoproterenol can be protected by the administration of *Camellia sinensis*.

2. Materials and methods

2.1. Chemical and reagents

Alanine aminotransferase (ALT) (Ref.: 30253), Aspartate aminotransferase (AST) (Ref.: 30243), Alkaline phosphatase (ALP) (Ref.: 30133), Uric acid (Ref.: 30393), Creatinine (Ref.: 30120), and Creatinine kinase muscle brain (CKMB) (Ref.: 30203) assay kit was purchased from LABKIT, Barcelona, Spain. There were some other chemicals also for assay of antioxidant enzyme activity and oxidative stress which were obtained from the Department of Pharmacy of Dhaka International University, Bangladesh.

2.2. Plant materials

Green tea was collected from the local market of Dhaka which was packeted from Kazi and Kazi tea which was in the form of green tea. It was mentioned in the packet that it was 100 % organic.

2.3. Experimental animals

4 groups were created and in them, 6 mice were involved. A total of 24 mice were taken which were Swiss albino male mice and they were 4 to 5 weeks old. All the activities involving experiments with Swiss albino mice comply with the ARRIVE guidelines and the guidelines approved by the institutional ethical committee. The environment was controlled where the light and day cycle were about 12 h with humidity was about 55 % where the temperature was 22 ± 3 °C. All mice were provided with ad libitum water and laboratory standard chow food.

2.4. Protocols of treatment

At first, there were 4 groups available for this study and 6 mice were available in each group. 4 groups are the Control group, ISO group, Control + GT group, and finally ISO + GT group. Control group mice received standard laboratory chow food and normal water. ISO group received laboratory chow food and normal water with isoproterenol subcutaneously at a dose of 50 mg/kg twice a week for two weeks for 14 days (Flori et al., 2024; Pan et al., 2022). Control + GT group received standard laboratory chow food and normal water with green tea treatment of supplementation 2.5 % with standard laboratory chow food every day for 14 days (Chandra and De, 2010; Misaka et al., 2014). The

final, group is the ISO + GT group which was given standard laboratory chow food and normal water where isoproterenol was given subcutaneously at a dose of 50 mg/kg twice a week for two weeks for 14 days with the treatment of green tea supplementation 2.5 % with standard laboratory chow food every day for 14 days. On the 15th day, all mice were sacrificed by using ketamine hydrochloride at a dose of 250 mg/mL, and for biochemical analysis, blood was withdrawn. For microscopic and biochemical evaluations heart tissues were surgically removed.

2.5. Assessment of biochemical parameters, creatinine, uric acid, and CKMB

Plasma was separated from blood by following some parameters such as revolution per minute was 4500, time was 15 min and the temperature was 4 °C. After separation of plasma, it was kept in a 1.5 mL microcentrifuge tube which was stored at -20 °C for biochemical analysis. Biochemical parameters such as ALT, AST, ALP, and other tests such as creatinine, uric acid, and CKMB all were done by following the manufacturer protocol of LABKIT, Spain, Barcelona.

2.6. Antioxidant enzyme activity and oxidative stress markers analysis

Heart tissue was homogenized by using phosphate buffer with a pH is 7.4 these tissues were centrifuged at 8000 rpm, time duration was 15 min and temperature was 4 °C and this was done for antioxidant enzyme activity and oxidative stress markers analysis. For biochemical studies of heart supernatant was collected and stored at -20 °C which will be used to perform the assay of antioxidant enzyme activity and oxidative stress markers.

2.6.1. Lipid peroxidation assessment

By the estimation of thiobarbituric acid reactive substances (TBRAS) plasma, cardiac tissue lipid peroxidation was measured. Trichloroacetic acid (15 % v/v), HCl (0.25 N), and thiobarbituric acid (0.37 % v/v) were mixed in the same amount for the preparation of TBA-TCA-HCl reagent with total volume was 2 mL and from it, 0.1 mL tissue homogenate was processed. After that, for 15 min it was kept in the hot water bath and after that, it was cooled at room temperature. Against a reference blank, the absorbance was taken at 535 nm (Stocks and Dormandy, 1971).

2.6.2. Nitric oxide (NO) assessment

Instead of 1-napthylamine (5 %) napthylethylene diamine dihydrochloride (0.1% w/v) was used which was the modification of Griess-Illosvoy reagent to quantify nitric oxide. At 25 °C temperature phosphate buffer (0.5 mL) was combined with plasma and heart tissue homogenate (2 mL) which was incubated for 2.5 h. Absorbance was taken at 540 nm against a blank solution. The unit of nitric oxide was nmol/g tissue (Tracey et al., 1995).

2.6.3. Advanced protein oxidation products (APOP) assessment

0.2 mL of acetic acid was mixed after 2 min with 1.16 M 0.1 mL of potassium iodide where two mL of plasma, supernatant of heart tissue was diluted with PBS at the ratio of 1:5. Absorbance was taken at 340 nm against a blank solution only supernatant or plasma was not added. The unit of APOP was chloramine T equivalent of nmol mL^{-1} (Witko-Sarsat et al., 1996).

2.6.4. Myeloperoxidase (MPO) activity assessment

By following method which is a previously described protocol *o*-dianisidine-H_2O_2 method was selected to determine myeloperoxidase activity (Bradley et al., 1982).

2.6.5. Assay of catalase (CAT) activity

Hydrogen peroxide was 5.9 mM and the volume was 0.4 mL which was mixed with phosphate buffer where concentration was 50 mM and volume was 2.5 mL and also 0.1 mL of plasma or heart tissue homogenates were mixed for the determination of catalase activity. To take absorbance UV–visible spectrophotometer was used and the absorbance was 240 nm after one minute of reaction mixture. One unit of catalase activity was considered an absorbance change of 0.01 units/minute (Chance and Maehly, 1955).

2.6.6. Assay of superoxide dismutase (SOD) activity

By the previously described method plasma, heart, and tissue homogenates were performed for SOD activity assay (Khan et al., 2020). The total volume of the reaction mixture is 3 mL and in that reaction mixture PBS is about 2.94 mL and the rest of them is enzyme preparation. The amount of epinephrine was 0.06 mL and the concentration was 15 mM. For 1 min at the interval of 15 s, absorbance was taken at 480 nm. In the sample solution where epinephrine was considered as auto-oxidation of 50 % inhibition which is evidenced as one unit enzyme activity.

2.6.7. Reduced glutathione (GSH) assessment

By the protocols which were described previously reduced glutathione (GSH) level was determined (Jollow et al., 1974). The absorbance of the reaction mixture was taken instantly and the wavelength for the absorbance was 405 nm. The unit of reduced glutathione is expressed as ng/mg protein.

2.7. Examination of histopathology

At first heart tissue was kept in neutral buffered formalin for fixation. After fixation heart tissues were embedded in paraffin. After that these tissues were sectioned at 5 micrometers with the help of microtome and these sliced sections were kept for staining such as hematoxylin and eosin (H & E) staining and Sirius red (SR) staining. Hematoxylin and eosin staining was done for the detection of inflammatory cells in the heart. On the other hand, Sirius red staining was done to determine collagen deposition of the heart. After staining light microscope (Optika) was used to take pictures at 40X magnification.

2.8. Statistical analysis

All tests were measured with mean ± SEM (standard error mean). For analysis of statistics one way ANOVA Dunnett test was followed with the help of GraphPad Prism Software, version 9. In all cases of statistical significance, the p-value was less than 0.05 ($p < 0.05$).

3. Results

3.1. Effect of green tea on heart specific marker (ALT, AST, and ALP) in isoproterenol administered Swiss albino mice

In ALT, AST and ALP isoproterenol increased ALT, AST, and ALP plasma concentration significantly **($p \leq 0.01$)** compared to the Control group (Fig. 1**A, B, C**). Similarly, the ISO + GT group decreased plasma concentration of ALT, AST, and ALP significantly **($p \leq 0.01$)** compared to the ISO group (Fig. 1**A, B, C**). Mice that received control food and treatment of green tea showed declined plasma levels of ALT, AST, and ALP significantly **($p \leq 0.01$)** compared to mice that received isoproterenol (Fig. 1**A, B, C**).

3.2. Effect of green tea on CK-MB, creatinine, and uric acid in isoproterenol administered Swiss albino mice

Isoproterenol administered to mice elevated CKMB, Creatinine, and Uric acid plasma levels significantly **($p \leq 0.01$)** compared to mice belonging to the control situation (Fig. 2**A, B, C**). Mice that received isoproterenol and green treatment reduced CKMB, creatinine, and uric acid plasma levels significantly **($p \leq 0.01$)** compared to mice that were

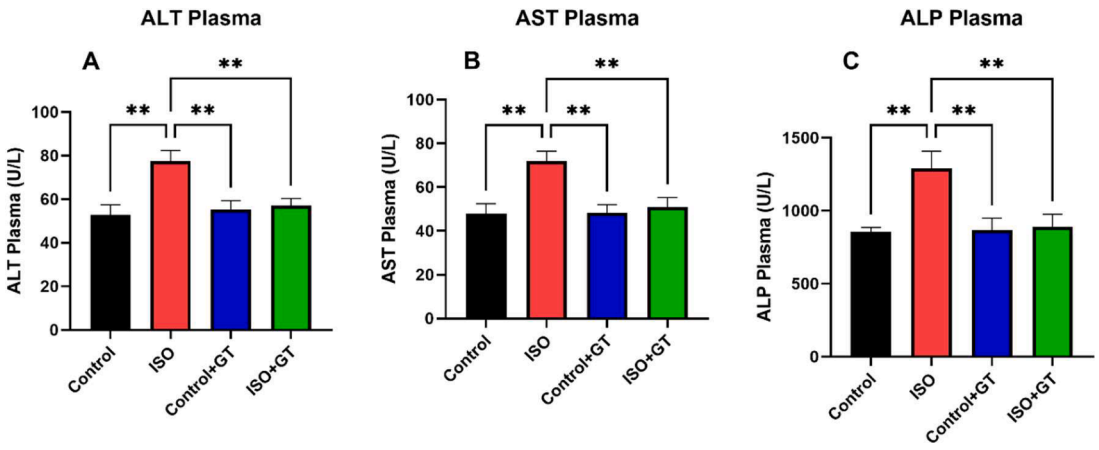

Fig. 1. Effect of green tea on Heart specific marker (ALT, AST, and ALP) plasma in isoproterenol administered mice. A. ALT Plasma; B. AST Plasma and C. ALP Plasma. For statistical analysis one way ANOVA Dunnett test was performed. In all cases p value was $p < 0.05$. Here, ns is $p > 0.05$, * is $p \leq 0.05$, ** is $p \leq 0.01$, *** is $p \leq 0.001$ and **** is $p \leq 0.0001$.

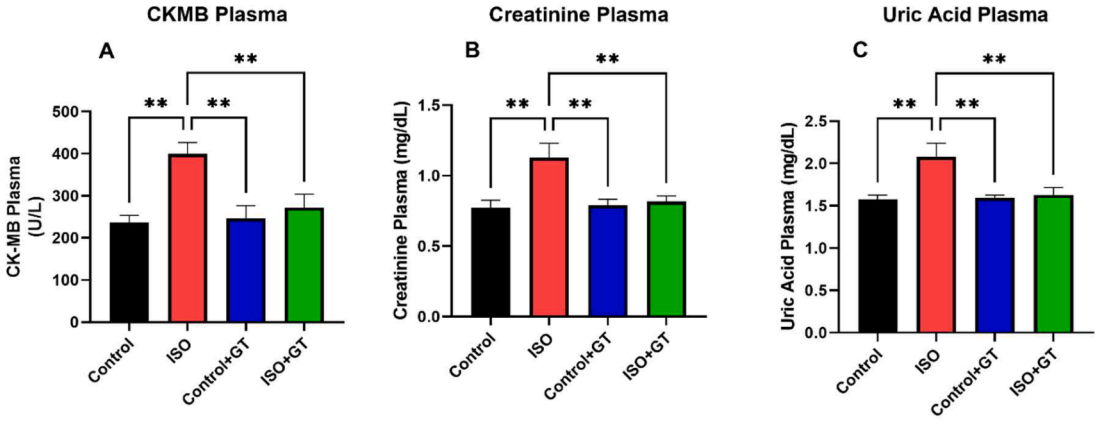

Fig. 2. Effect of green tea on CK-MB, Creatinine, and Uric acid plasma in isoproterenol administered mice. A. CK-MB Plasma; B. Creatinine Plasma and C. Uric acid Plasma. For statistical analysis one way ANOVA Dunnett test was performed. In all cases p value was $p < 0.05$. Here, ns is $p > 0.05$, * is $p \leq 0.05$, ** is $p \leq 0.01$, *** is $p \leq 0.001$ and **** is $p \leq 0.0001$.

administered with isoproterenol (Fig. 2**A, B, C**). Control + GT significantly (**$p \leq 0.01$**) decreased CKMB, Creatinine, and Uric acid plasma levels compared to the ISO group (Fig. 2**A, B, C**).

3.3. Effect of green tea on oxidative stress parameters in isoproterenol administered Swiss albino mice

In MDA plasma and heart isoproterenol increased MDA concentration significantly (**$p \leq 0.01$**) compared to control mice (Fig. 3**A and B**). Mice that received isoproterenol and green tea treatment declined MDA plasma and heart concentration significantly (**$p \leq 0.01$**) compared to mice that actually received only isoproterenol which proves green tea decreases MDA plasma and heart concentration levels (Fig. 3**A and B**). Control + GT did not show any kind of abnormality level (**$p \leq 0.01$**) of MDA plasma and heart level concentration compared to the ISO group which also means no toxicity was found (Fig. 3**A and B**).

ISO group increased NO plasma and Heart concentration levels significantly (**$p \leq 0.01$**) compared to the Control group (Fig. 3**C and D**). ISO + GT group lowered the concentration of NO plasma and heart significantly (**$p \leq 0.01$**) compared to the ISO group (Fig. 3**C and D**). The Control + GT group also decreased NO plasma and heart concentration significantly (**$p \leq 0.01$**) compared to the ISO group (Fig. 3**C and D**).

In APOP plasma and heart isoproterenol increased APOP concentration significantly (**$p \leq 0.01$**) compared to control mice (Fig. 3**E and F**). Mice that received isoproterenol and green tea treatment declined APOP plasma and heart concentration significantly (**$p \leq 0.01$**) compared to mice which actually received only isoproterenol which proves green tea decreases APOP plasma and heart concentration levels (Fig. 3**E and F**). Control + GT did not show any kind of abnormality level (**$p \leq 0.01$**) of APOP plasma and heart level concentration compared to ISO group which also means no toxicity was found (Fig. 3**E and F**).

ISO group increased MPO plasma and Heart activity levels significantly (**$p \leq 0.01$**) compared to the Control group (Fig. 3**G and H**). ISO + GT group lowered the activity of MPO plasma and heart significantly (**$p \leq 0.01$**) compared to the ISO group (Fig. 3**G and H**). The Control + GT group also decreased MPO plasma and heart activity significantly (**$p \leq 0.01$**) compared to the ISO group (Fig. 3**G and H**).

3.4. Effect of green tea on antioxidant enzyme activity parameters in isoproterenol administered Swiss albino mice

The group of ISO where mice were administered with isoproterenol showed a declined amount of catalase plasma and heart activity (**$p \leq 0.01$**) compared to mice that belong to the control condition (Fig. 4**A and B**). Mice that were treated with green tea and was administered with isoproterenol elevated plasma and heart activity of catalase significantly (**$p \leq 0.01$**) which was declined by administration of isoproterenol (Fig. 4**A and B**). The Control + GT group also kept the activity of catalase plasma and heart normal (**$p \leq 0.01$**) compared to the ISO group (Fig. 4**A and B**).

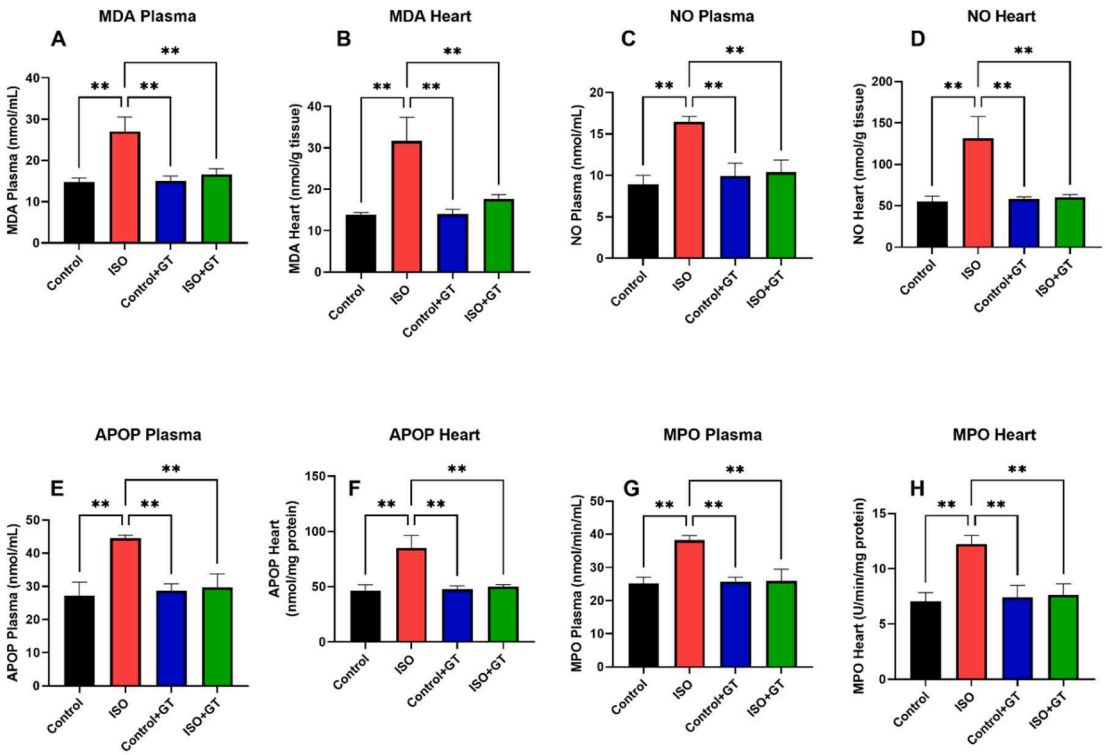

Fig. 3. Effect of green tea on oxidative stress parameters in isoproterenol administered mice. A. MDA Plasma; B. MDA Heart; C. NO Plasma; D. NO Heart; E. APOP Plasma; F. APOP Heart; G. MPO Plasma and H. MPO Heart. For statistical analysis one way ANOVA Dunnett test was performed. In all cases p value was $p < 0.05$. Here, ns is $p > 0.05$, * is $p \leq 0.05$, ** is $p \leq 0.01$, *** is $p \leq 0.001$ and **** is $p \leq 0.0001$.

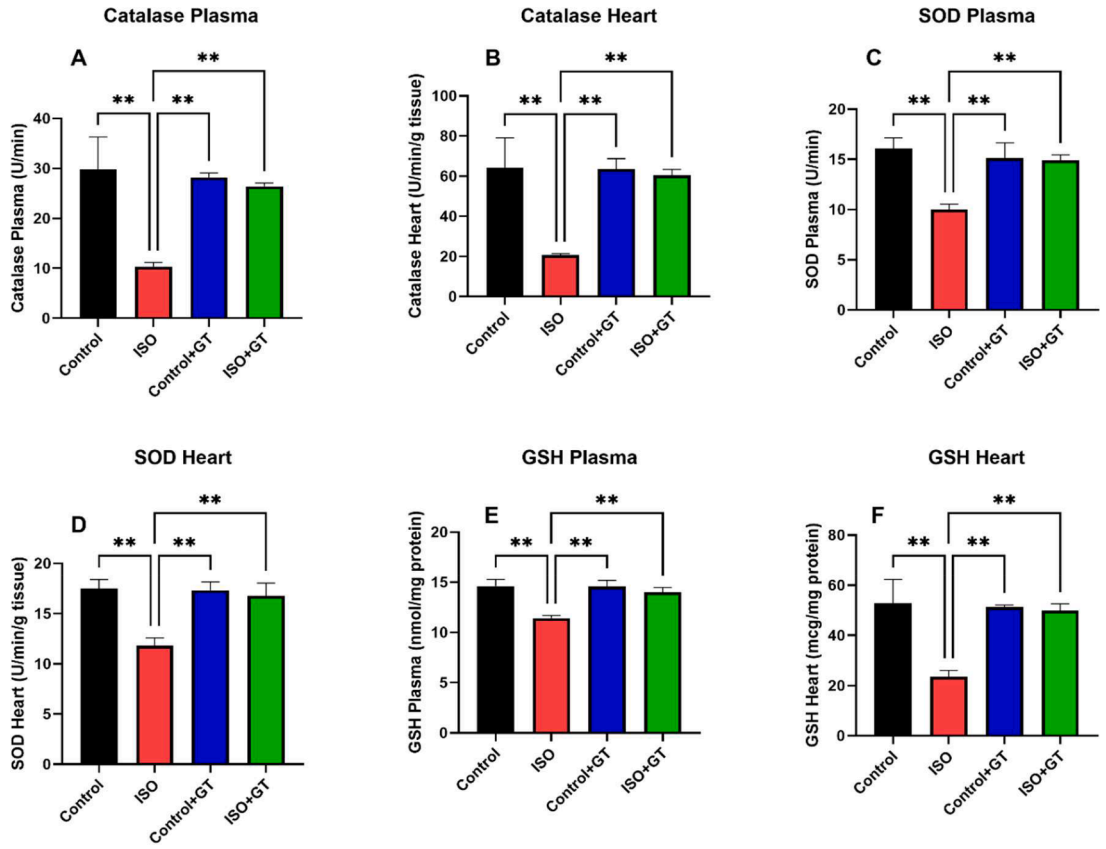

Fig. 4. Effect of green tea on antioxidant enzyme activity parameters in isoproterenol administered mice. A. Catalase Plasma; B. Catalase Heart; C. SOD Plasma; D. SOD Heart; E. GSH Plasma and F. GSH Heart. For statistical analysis one way ANOVA Dunnett test was performed. In all cases p value was $p < 0.05$. Here, ns is $p > 0.05$, * is $p \leq 0.05$, ** is $p \leq 0.01$, *** is $p \leq 0.001$ and **** is $p \leq 0.0001$.

ISO group decreased SOD plasma and heart activity significantly **($p \leq 0.01$)** compared to the Control group (Fig. 4**C and D)**. ISO + GT group regained the activity of SOD plasma and heart significantly **($p \leq 0.01$)** which was declined by the ISO group which also means that green tree retains antioxidant activity (Fig. 4**C and D)**. The Control + GT group also showed the normal amount of SOD activity **($p \leq 0.01$)** compared to the ISO group (Fig. 4**C and D)**.

The group of ISO where mice were administered with isoproterenol showed a decreased amount of GSH plasma and heart concentration **($p \leq 0.01$)** compared to mice that belonged to the control condition (Fig. 4**E and F)**. Mice that were treated with green tea and was administered with isoproterenol elevated plasma and heart concentration of GSH significantly **($p \leq 0.01$)** which was declined by administration of isoproterenol (Fig. 4**E and F)**. The Control + GT group also kept the concentration of GSH plasma and heart normal **($p \leq 0.01$)** compared to the ISO group (Fig. 4**E and F)**.

3.5. Effect of green tea on histology of heart in isoproterenol administered Swiss albino mice

In histology, the upper part is H & E staining, and in lower part is SR staining. H & E staining is done to find out cell infiltration and fibrosis. Here, in the Control group fibrosis and cell infiltration are not found (Fig. 5**A)**. The next picture is about ISO where cell fibrosis is found (Fig. 5**B)**. In the heart of Control + GT H&E staining there are no fibrosis or cell infiltration is visible (Fig. 5**C)**. In the group, ISO + GT less amount of fibrosis and cell infiltration is found compared to ISO group (Fig. 5**D)**.

The lower part is SR staining. SR staining is done to find collagen deposition in the cell. Here, in the Control group collagen deposition is not found (Fig. 5**E)**. The next picture is about ISO where a massive amount of collagen deposition is found (Fig. 5**F)**. In the heart of Control + GT group SR staining, there are no collagen deposition is visible (Fig. 5**G)**. In the group, ISO + GT less amount of collagen deposition is found compared to the ISO group (Fig. 5**H)**.

4. Discussion

In this study, there are four groups were available among them Control + GT and ISO + GT groups were treated with green tea (GT). The other two groups were Control and ISO groups. All group's body weight, food intake, and water intake were daily noted. After the sacrifice of all mice organs wet weight was taken. Three organs wet weight was recorded such as the heart where the heart was divided into two parts left ventricle and right ventricle. There are two parts such as Plasma and heart which bioassay was done. From plasma ALT, AST, ALP, CKMB, Creatinine, and Uric acid were done. For oxidative stress related such as MDA (lipid peroxidation), NO, APOP, and MPO were done. In the matter of antioxidant enzyme activity Catalase, SOD, and GSH. In the matter of heart oxidative stress like MDA (lipid peroxidation), NO, APOP, and MPO were performed, and also for antioxidant enzyme activity Catalase, SOD, and GSH were done. Molecular work was also done on the heart where anti-inflammatory and antioxidant gene expression were performed. Finally, histology was done such as for the heart where hematoxylin and eosin, and Sirius red were also done.

At first heart specific markers were tested. Among them, ALT, AST, and ALP tests were done where isoproterenol increased these levels. When treatments were done with green tea in our study it reduced the levels of ALT, AST, and ALP. So, oxidative stress which was created by isoproterenol by increasing the level of ALT, AST, and ALP was decreased by green tea treatment. In another study, green tea showed a protective effect on non-alcoholic fatty liver disease where the level of ALT, AST, and ALP was decreased which was increased by oxidative stress (Pezeshki et al., 2016).

The next tests are about CKMB, creatinine, and uric acid. Isoproterenol causes an increased amount of free radicals which creates reactive oxygen species which is harmful to the heart and it also increases the concentration of CKMB, creatinine, and uric acid. In our experiment, all the concentration of CKMB, creatinine, and uric acid was reduced by green tea treatment. In another study, where Doxorubicin was induced it created nephrotoxicity and cardiotoxicity in rats and that's why oxidative stress was created. It is prevented by the fraction which is named Methylxanthine extracted from leaves of tea and protects and heart by decreasing CKMB, creatinine, and uric acid levels (Radeva-Ilieva et al., 2020). In another study, it was clearly mentioned that taking green tea reduces the level serum creatinine level in obese women (Bijeh et al., 2018). In another study, it was stated that green tea can play an important role in the metabolism of uric acid (Kawakami et al., 2021). In the case of CKMB cisplatin increased the level of CKMB which was ameliorated by the combination treatment of vitamin E and green tea (Ibrahim et al., 2019).

Oxidative stress tests include MDA (lipid peroxidation), NO, APOP, and MPO. In our study lipid peroxidation was increased by the administration of isoproterenol which was downregulated by green tea treatment. In another study, it was said that the matter of MDA which is created by oxidative stress was beneficiated by the administration of Epigallocatechin-3-Gallate which is a chemical constituent of green tea (Gumay et al., 2018). Another test of oxidative stress is the nitric oxide or NO test where isoproterenol also increased NO level. In our study,

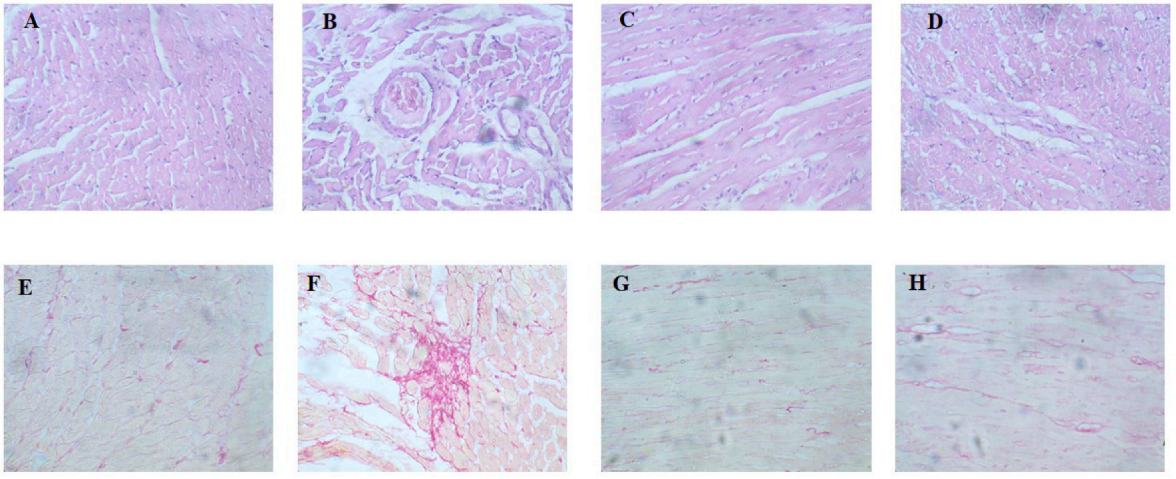

Fig. 5. Effect of green tea on histology of heart in isoproterenol administered mice. A. Control (H&E staining); B. ISO (H&E staining); C. Control + GT (H&E staining); D. ISO + GT (H&E staining); E. Control (SR staining) F. ISO (SR staining); G. Control + GT (SR staining) and H. ISO + GT (SR staining).

because of the administration of green tea, NO level was reduced. In a previous study, nitric oxide was attenuated by the action of Epigallocatechin-3-Gallate which modulates oxidative stress (Ding et al., 2018). The other oxidative stress form is the advanced protein oxidation product which is also called APOP. As usual, isoproterenol increased the concentration level of APOP which was decreased by green tea. The final test for oxidative stress is MPO whose full form is myeloperoxidase. MPO activity is increased by isoproterenol because of oxidative stress and in our experiment, green tea reduced MPO activity. In the previous study, MPO activity was increased because of the administration of ethanol in rats which is caused because of oxidative stress and it was reduced by the action of Pu-erh tea extract which contains antioxidant activity and shows free radical scavenging activity (Yang et al., 2018).

From oxidative stress now we will move on to antioxidant enzyme activity. There were three tests were available for antioxidant enzyme activity such as Catalase, SOD, and GSH. In our study antioxidant level was reduced by the administration of isoproterenol which was restored by the administration of green tea. In another study, green tea extract showed a protective effect against subacute toxicity and retained the antioxidant enzyme level of Catalase, SOD, and GSH (Khan and Kour, 2007).

In histology, isoproterenol created cell infiltration in heart tissue in hematoxylin and eosin staining whereas in Sirius red staining collagen deposition was created by isoproterenol in the heart. To prevent and reduce those problems as treatment green tea was used and cell infiltration and collagen deposition were reduced in heart tissue. In a study, nicotine-induced testicular damage and oxidative stress were improved by the treatment of green tea which was expressed in histology (Mosbah et al., 2015).

5. Conclusion

Our study suggests that green tea contains some important polyphenolic compounds and among those compounds, they provide antioxidants that provide free radical scavenging activity which is why isoproterenol creates oxidative stress and causes reactive oxygen species countered by antioxidant activity. However, it is true that for clinical trials more necessary exams should be carried out on humans because clinical trials are very low for the proof of antioxidant and beneficiary activity of green tea. So, further investigations are required to understand the correct mechanism of green tea on the human body.

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CRediT authorship contribution statement

Shampa Akter: Writing – original draft, Data curation, Conceptualization. **Shatil Rafia:** Writing – original draft, Conceptualization. **Raiyana Huda:** Writing – original draft, Formal analysis, Conceptualization. **Rashedul Haque:** Formal analysis, Data curation. **Sajib Paul:** Methodology, Formal analysis. **Md.Tipu Sultan:** Methodology, Investigation, Formal analysis. **Md. Kawser:** Supervision, Project administration. **Faizul Islam Chowdhury:** Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Basha, R.H., Priscilla, D.H., 2013. An in vivo and in vitro study on the protective effects of N-acetylcysteine on mitochondrial dysfunction in isoproterenol treated myocardial infarcted rats. Experim. Toxicol. Pathol. 65, 7–14.
Bedrood, Z., Rameshrad, M., Hosseinzadeh, H., 2018. Toxicological effects of Camellia sinensis (green tea): a review. Phytother. Res. 32, 1163–1180.
Bijeh, N., Jamali, F.S., Nejati, F., Lotfalizade, M., 2018. The effect of aerobic exercise in water with and without green tea consumption on kidney function in sedentary postmenopausal women. J. Sabzevar Univer. Med. Sci. 25, 706–714.
Bradley, P.P., Priebat, D.A., Christensen, R.D., Rothstein, G., 1982. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J. Invest. Dermatol. 78, 206–209.
Buja, L.M., Entman, M.L., 1998. Modes of myocardial cell injury and cell death in ischemic heart disease. Am. Heart Assoc. 1355–1357.
Chance, B., Maehly, A., 1955. [136]Assay of catalases and peroxidases.
Chandra, A.K., De, N., 2010. Goitrogenic/antithyroidal potential of green tea extract in relation to catechin in rats. Food Chem. Toxicol. 48, 2304–2311.
Cohn, J.N., Ferrari, R., Sharpe, N., Remodeling, a.I.F.o.C., 2000. Cardiac remodeling—Concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. J. Am. Coll. Cardiol. 35, 569–582.
Ding, L., Gao, X., Hu, J., Yu, S., 2018. (-)Epigallocatechin-3-gallate attenuates anesthesia-induced memory deficit in young mice via modulation of nitric oxide expression. Mol. Med. Rep. 18, 4813–4820.
Flori, L., Lazzarini, G., Spezzini, J., Pirone, A., Calderone, V., Testai, L., Miragliotta, V., 2024. The isoproterenol-induced myocardial fibrosis: a biochemical and histological investigation. Biomed. Pharmacother. 174, 116534.
Gumay, A.R., Bakri, S., Pudjonarko, D., 2018. The effect of green tea epigallocatechin-3-gallate on spatial memory function, malondialdehyde and TNF-α level in D-galactose-induced BALB/C mice. Hiroshima J. Med. Sci. 41–48.
Hill, M.F., Palace, V.P., Kaur, K., Kumar, D., Khaper, N., Singal, P.K., 2005. Reduction in oxidative stress and modulation of heart failure subsequent to myocardial infarction in rats. Experim. Clin. Cardiol. 10, 146.
Ibrahim, M.A., Bakhaat, G.A., Tammam, H.G., Mohamed, R.M., El-Naggar, S.A., 2019. Cardioprotective effect of green tea extract and vitamin E on Cisplatin-induced cardiotoxicity in mice: toxicological, histological and immunohistochemical studies. Biomed. Pharmacother. 113, 108731.
Jollow, D., Mitchell, J., Zampaglione, N., Gillette, J., 1974. Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3, 4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology 11, 151–169.
Karimi Galougahi, K., Antoniades, C., Nicholls, S.J., Channon, K.M., Figtree, G.A., 2015. Redox biomarkers in cardiovascular medicine. Eur. Heart J. 36, 1576–1582.
Kawakami, Y., Yasuda, A., Hayashi, M., Akiyama, M., Asai, T., Hosaka, T., Arai, H., 2021. Acute effect of green tea catechins on uric acid metabolism after alcohol ingestion in Japanese men. Clin. Rheumatol. 40, 2881–2888.
Khan, S., Rahman, M.M., Kabir, F., Nahar, K., Mamun, F., Lasker, S., Subhan, N., Hossain, M.H., Nahar, L., Sarker, S.D., 2020. Trichosanthes dioica Roxb. prevents hepatic inflammation and fibrosis in CCl4-induced ovariectomized rats. Clin. Nutr. Exp. 33, 1–17.
Khan, S.M., Kour, G., 2007. Subacute oral toxicity of chlorpyriphos and protective effect of green tea extract. Pestic Biochem. Physiol. 89, 118–123.
Koitabashi, N., Kass, D.A., 2012. Reverse remodeling in heart failure—mechanisms and therapeutic opportunities. Nat. Rev. Cardiol. 9, 147–157.
Konieczynski, P., Viapiana, A., Wesolowski, M., 2017. Comparison of infusions from black and green teas (Camellia sinensis L. Kuntze) and erva-mate (Ilex paraguariensis A. St.-Hil.) based on the content of essential elements, secondary metabolites, and antioxidant activity. Food Anal. Methods 10, 3063–3070.
Levy, D., Kenchaiah, S., Larson, M.G., Benjamin, E.J., Kupka, M.J., Ho, K.K., Murabito, J. M., Vasan, R.S., 2002. Long-term trends in the incidence of and survival with heart failure. N. Engl. J. Med. 347, 1397–1402.
Liu, H., Li, G., Zhao, W., Hu, Y., 2016. Inhibition of MiR-92a may protect endothelial cells after acute myocardial infarction in rats: role of KLF2/4. Med. Sci. Monitor: Int. Med. J. Experim. Clin. Res. 22, 2451.
Lymperopoulos, A., Rengo, G., Koch, W.J., 2013. Adrenergic nervous system in heart failure: pathophysiology and therapy. Circ. Res. 113, 739–753.
McMichael, M., Moore, R.M., 2004. Ischemia–reperfusion injury pathophysiology, part I. J. Vet. Emerg. Crit. Care 14, 231–241.
Misaka, S., Yatabe, J., Müller, F., Takano, K., Kawabe, K., Glaeser, H., Yatabe, M.S., Onoue, S., Werba, J.P., Watanabe, H., Yamada, S., Fromm, M.F., Kimura, J., 2014. Green tea ingestion greatly reduces plasma concentrations of nadolol in healthy subjects. Clin. Pharmacol. Therap. 95, 432–438.
Mosbah, R., Yousef, M.I., Mantovani, A., 2015. Nicotine-induced reproductive toxicity, oxidative damage, histological changes and haematotoxicity in male rats: the protective effects of green tea extract. Experim. Toxicol. Pathol. 67, 253–259.
Mozaffarian, D., Benjamin, E.J., Go, A.S., Arnett, D.K., Blaha, M.J., Cushman, M., De Ferranti, S., Després, J.-P., Fullerton, H.J., Howard, V.J., 2015. Heart disease and

stroke statistics—2015 update: a report from the American Heart Association. Circulation 131, e29–e322.
Naveed, M., BiBi, J., Kamboh, A.A., Suheryani, I., Kakar, I., Fazlani, S.A., FangFang, X., Yunjuan, L., Kakar, M.U., Abd El-Hack, M.E., 2018. Pharmacological values and therapeutic properties of black tea (Camellia sinensis): a comprehensive overview. Biomed. Pharmacother. 100, 521–531.
Pan, Y., Gao, J., Gu, R., Song, W., Li, H., Wang, J., Gu, Y., Chen, H., Zhang, H., 2022. Effect of injection of different doses of isoproterenol on the hearts of mice. BMC Cardiovasc. Disord. 22, 409.
Pezeshki, A., Safi, S., Feizi, A., Askari, G., Karami, F., 2016. The effect of green tea extract supplementation on liver enzymes in patients with nonalcoholic fatty liver disease. Int. J. Prev. Med. 7.
Prabhu, S., Jainu, M., Sabitha, K., Devi, C., 2006. Cardioprotective effect of mangiferin on isoproterenol induced myocardial infarction in rats.
Radeva-Ilieva, M.P., Georgiev, K.D., Hvarchanova, N.R., Stoeva, S.S., Slavov, I.J., Dzhenkov, D.L., Georgieva, M.P., 2020. Protective effect of methylxanthine fractions isolated from bancha tea leaves against doxorubicin-induced cardio-and nephrotoxicities in rats. Biomed. Res. Int. 2020.
Rathore, N., John, S., Kale, M., Bhatnagar, D., 1998. Lipid peroxidation and antioxidant enzymes in isoproterenol induced oxidative stress in rat tissues. Pharmacol. Res. 38, 297–303.
Sato, Y., Fujiwara, H., Takatsu, Y., 2012. Cardiac troponin and heart failure in the era of high-sensitivity assays. J. Cardiol. 60, 160–167.
Steffens, S., Montecucco, F., Mach, F., 2009. The inflammatory response as a target to reduce myocardial ischaemia and reperfusion injury. Thromb. Haemost. 102, 240–247.
Stocks, J., Dormandy, T., 1971. The autoxidation of human red cell lipids induced by hydrogen peroxide. Br. J. Haematol. 20, 95–111.
Takimoto, E., Kass, D.A., 2007. Role of oxidative stress in cardiac hypertrophy and remodeling. Hypertension 49, 241–248.
Tang, G.-Y., Zhao, C.-N., Xu, X.-Y., Gan, R.-Y., Cao, S.-Y., Liu, Q., Shang, A., Mao, Q.-Q., Li, H.-B., 2019. Phytochemical composition and antioxidant capacity of 30 Chinese teas. Antioxidants 8, 180.
Temesszentandrási, G., Vörös, K., Márkus, B., Böröcz, Z., Kaszás, E., Prohászka, Z., Falus, A., Cseh, K., Kalabay, L., 2016. Human fetuin-A Rs4918 polymorphism and its association with obesity in healthy persons and in patients with myocardial infarction in two Hungarian Cohorts. Med. Sci. Monitor Int.Med. J. Experim. Clin. Res. 22, 2742.
Tracey, W.R., Tse, J., Carter, G., 1995. Lipopolysaccharide-induced changes in plasma nitrite and nitrate concentrations in rats and mice: pharmacological evaluation of nitric oxide synthase inhibitors. J. Pharmacol. Experim. Therap. 272, 1011–1015.
Valduga, A.T., Gonçalves, I.L., Magri, E., Finzer, J.R.D., 2019. Chemistry, pharmacology and new trends in traditional functional and medicinal beverages. Food Res. Int. 120, 478–503.
Vincenzi, A., Cesana, F., Ciro, A., Garatti, L., Achilli, F., 2017. Sacubitril/valsartan in “field practice” patients with advanced heart failure: a monocentric Italian experience. Cardiology 138, 13–16.
Witko-Sarsat, V., Friedlander, M., Capeillère-Blandin, C., Nguyen-Khoa, T., Nguyen, A.T., Zingraff, J., Jungers, P., Descamps-Latscha, B., 1996. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney Int. 49, 1304–1313.
Yang, J., Zhou, W., Gu, Y., Dai, J., Li, X., Tai, P., Li, Y., Ma, X., Zhang, Y., 2018. Protective effect of Pu-erh tea extracts against ethanol-induced gastric mucosal damage in rats. Biomed. Rep. 8, 335–342.
Zhou, S.-x., Zhou, Y., Zhang, Y.-l., Lei, J., Wang, J.-f., 2009. Antioxidant probucol attenuates myocardial oxidative stress and collagen expressions in post-myocardial infarction rats. J. Cardiovasc. Pharmacol. 54, 154–162.
Zou, Y., Lin, L., Xiao, H., Xiang, D., 2016. A rare case of toxic myocarditis caused by bacterial liver abscess mimicking acute myocardial infarction. Am. J. Case Rep. 17, 1.