

## LJMU Research Online

Alimullah, M, Jahan, I, Rahman, MM, Khan, F, Shahin Ahmed, K, Hossain, MH, Arabnozari, H, Alam, MA, Sarker, SD, Nahar, L and Subhan, N

Camellia sinensis powder rich in epicatechin and polyphenols attenuates isoprenaline induced cardiac injury by activating the Nrf2 HO1 antioxidant pathway in rats

https://researchonline.ljmu.ac.uk/id/eprint/26737/

Article

**Citation** (please note it is advisable to refer to the publisher's version if you intend to cite from this work)

Alimullah, M, Jahan, I, Rahman, MM, Khan, F, Shahin Ahmed, K, Hossain, MH, Arabnozari, H, Alam, MA, Sarker, SD ORCID logoORCID: https://orcid.org/0000-0003-4038-0514, Nahar, L and Subhan, N (2025) Camellia sinensis powder rich in epicatechin and polyphenols attenuates

LJMU has developed LJMU Research Online for users to access the research output of the University more effectively. Copyright © and Moral Rights for the papers on this site are retained by the individual authors and/or other copyright owners. Users may download and/or print one copy of any article(s) in LJMU Research Online to facilitate their private study or for non-commercial research. You may not engage in further distribution of the material or use it for any profit-making activities or any commercial gain.

The version presented here may differ from the published version or from the version of the record. Please see the repository URL above for details on accessing the published version and note that access may require a subscription.

For more information please contact <a href="mailto:researchonline@ljmu.ac.uk">researchonline@ljmu.ac.uk</a>

http://researchonline.ljmu.ac.uk/

# scientific reports

### OPEN



# *Camellia sinensis* powder rich in epicatechin and polyphenols attenuates isoprenaline induced cardiac injury by activating the Nrf2 HO1 antioxidant pathway in rats

Mirza Alimullah<sup>1</sup>, Ishrat Jahan<sup>1</sup>, Md. Mizanur Rahman<sup>1</sup>, Ferdous Khan<sup>1</sup>, Khondoker Shahin Ahmed<sup>2</sup>, Md. Hemayet Hossain<sup>2</sup>, Hesamoddin Arabnozari<sup>3⊠</sup>, Md. Ashraful Alam<sup>1</sup>, Satyajit D. Sarker<sup>4</sup>, Lutfun Nahar<sup>5⊠</sup> & Nusrat Subhan<sup>1</sup>

Myocardial infarction is a leading cause of death and morbidity in individuals with cardiovascular diseases. Natural antioxidants, such as those found in green tea leaves, are beneficial in preventing these diseases. This study evaluated the protective effects of green tea leaves powder against isoprenaline (ISO)-induced myocardial infarction in rats. Four groups of male Long Evans rats were used: Control, Control + green tea leaves powder, ISO, and ISO + green tea leaves powder. Organ and blood plasma samples were collected to measure oxidative stress biomarkers, biochemical parameters, and gene expressions. Furthermore, tissue sections were prepared and stained histologically. ISOinduced rats showed decreased cellular antioxidants (catalase activity and glutathione concentration) and elevated oxidative stress markers. Notable inflammatory cell infiltration and fibrosis were observed in the heart and kidneys of ISO-induced rats. Supplementation with green tea leaves powder significantly restored catalase activity and glutathione concentration (p < 0.05) in plasma and tissues. It also considerably reduced lipid peroxidation, nitric oxide, and advanced oxidation protein products (p < 0.05) in ISO-administered rats. Furthermore, green tea leaves powder supplementation halted inflammatory gene expression (p < 0.05), restored antioxidant genes (p < 0.05) such as Nrf-2-HO-1, and prevented cardiac fibrosis in ISO-administered rats. Green tea leaves powder supplementation may reduce oxidative stress, inflammation, and fibrosis in ISO-administered rats, potentially through the Nrf-2-HO-1-mediated restoration of antioxidant enzymes and prevention of heart inflammation.

Keywords Isoprenaline, Myocardial infarction, Fibrosis, Inflammation, Camellia sinensis

One of the deadliest non-communicable diseases that contributes significantly to global mortality rates is cardiovascular disease  $(CVD)^1$ . In countries with low or middle incomes, CVDs are very severe<sup>2,3</sup>. Hypertension, atherosclerosis, dyslipidemia, coronary heart disease, myocardial infarction (MI), cerebrovascular diseases, cardiac hypertrophy and remodeling, and congestive heart failure (CHF) are considered diseases associated with the heart and vascular system. Among them, future research points out that myocardial infarction (MI), one of the world's leading causes of death, is going to get more severe<sup>4,5</sup>. It occurs due to ischemia in the heart as the coronary blood supply becomes insufficient to meet the demand of the cardiomyocytes. Prolonged ischemia

<sup>1</sup>Department of Pharmaceutical Sciences, North South University, Dhaka, Bangladesh. <sup>2</sup>BCSIR Laboratories, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh. <sup>3</sup>Medicinal Plants Research Centre, Institute of Herbal Medicines and Metabolic Disorders, Mazandaran University of Medical Sciences, Sari, Iran. <sup>4</sup>Centre for Natural Products Discovery, School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, James Parsons Building, Byrom Street, Liverpool L3 3AF, UK. <sup>5</sup>Laboratory of Growth Regulators, Palacký University and Institute of Experimental Botany, The Czech Academy of Sciences, Šlechtitelů 27, 78371 Olomouc, Czech Republic. <sup>Sz</sup>email: hesamarabnozari@yahoo.com; profnahar@outlook.com leads to necrosis in cardiomyocytes, which is irreversible myocardial damage. Oxygen-derived free radicals are produced in the cardiac cells due to myocardial ischemia<sup>6</sup>. The sources of oxygen-free radicals in the heart are the mitochondrial electron transport chain, nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase), infiltrating neutrophils, myeloperoxidase (MPO), etc.<sup>7</sup>.

A previous report suggests that a failing heart produces enormous excess free radicals and lowers the tissue antioxidant enzymes such as superoxide dismutase and catalase<sup>6,8</sup>. The oxygen-free radicals are generally superoxide anion ( $O^{2-}$ ), hydrogen peroxide ( $H_2O_2$ ), hydroxyl radical (HO), and peroxynitrite (ONOO–), which may react with the DNA, proteins, and lipids in cell membranes, triggering damage to the cardiac tissues and developing MI<sup>9</sup>. Therefore, therapeutic strategies should focus on lessening reactive oxygen species (ROS) generation or increasing the endogenous antioxidant enzymes, which can contribute towards reducing the damage in MI. Several techniques are now available to produce experimental MI in animals, such as genetically engineered spontaneously hypertensive rats, coronary artery ligation, and isoprenaline (ISO) administration<sup>10,11</sup>. The ISO is a beta-adrenergic receptor agonist, which may produce free radicals in the heart and develop MI-like symptoms in experimental animals<sup>12</sup>.

The ISO model has an advantage over other animal models in that myocardial infarction can be studied independently of hypertension. It has been observed that several natural compounds can protect rats from ISO-induced MI by scavenging and neutralizing ROS<sup>13</sup>. Previous reports showed that epicatechin prevented hypertension, restored vascular dysfunction, and prevented oxidative stress in hypertensive rats<sup>14</sup>. The epigallocatechin gallate (EGCG) may prevent cardiac oxidative stress by decreasing the expression of nitric oxide synthase 2, Toll-like receptor 4, and Sirt1 in MnSOD-deficient mice<sup>15</sup>. The Nrf-2 is another target for preventing cardiac oxidative stress and lipid peroxidation and activate the Nrf-2-mediated pathways in phthalates and bisphenol A-induced cardiac injury<sup>17</sup>.

Tea leaves (*Camellia sinensis*) have strong antioxidant components that can scavenge free radicals; several substances have been shown to have a good effect on reducing the risk of cardiovascular disease<sup>18</sup>. A popular beverage in many nations, green tea has numerous health advantages. Prior research demonstrated that normolipidemic diet-fed mice positively affected fat metabolism during rest and activity, whereas obese adult humans obtained only little weight loss<sup>19–21</sup>. Green tea also showed beneficial effects in diabetes, inflammatory diseases, cancer, and neurodegenerative diseases<sup>22–25</sup>. The bioactivities in green tea, such as flavanols and flavonoids, principally contribute to the health benefits. Tea leaves contain 40–50% of their dry weight in flavanols, called catechins<sup>26</sup>.

*Camellia sinensis* is rich in polyphenolic compounds, especially catechins like epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG). Research has extensively highlighted these catechins' antioxidant and anti-inflammatory effects<sup>27</sup>. For instance, epicatechin shows notable cardioprotective advantages by inhibiting myocardial apoptosis, reducing cardiac fibrosis, and attenuating hypertrophy<sup>28</sup>.

While numerous studies have concentrated on the effects of specific green tea catechins or purified extracts, whole green tea powder offers a greater concentration of bioactive compounds, including catechins, amino acids, vitamins, and minerals, which may work together to enhance health benefits<sup>27</sup>.

Previous research demonstrated that the addition of green tea and vitamin E reduced lipid peroxidation and ventricular hypertrophy in the hearts of rats given ISO<sup>29</sup>. Moreover, green tea also prevented the oxidation of LDL and lowered the atherogenic index in cholesterol-fed rats<sup>30</sup>. A new research investigation concluded that by altering the function of genes associated with obesity in rats, green tea polyphenols could lower body weight<sup>31</sup>. However, it is unknown how the tea leaves powder affects antioxidant function and myocardial oxidative stress in the heart of ISO-administered rats. Considering the protective role of green tea polyphenols, this investigation was conducted to evaluate the preventive effect against ISO-administered MI in rats. The present study was also extended to assess the impact of green tea powder on biochemical and histological changes, lipid peroxidation, and antioxidant enzyme activities, along with determining the antioxidant gene expression in the heart of ISO-induced rats.

#### Results

#### HPLC–DAD analysis of phenolic compounds in green tea leaf extract

The distinct phenolic components in the ethanol extract of tea leaves were identified and quantified using HPLC. The chromatographic separations of polyphenols in the ethanol extract are shown in Fig. 1. The mean of five measurements was used to compute the level of each phenolic component, displayed in Table 1, along with the relevant calibration curve. Epicatechin and catechin hydrate were highly concentrated in the ethanol extract of green tea leaves (Table 1). The ethanol extract also included significant concentrations of rutin hydrate, syringic acid, and rosmarinic acid (Table 1).

#### Effect of green tea treatment on body weight and organ wet weight in iso-induced rats

The body weights of all rats were noted during the experiment, and the percentage shift was established for each group. In the ISO-administered rat group, body weight was significantly reduced compared to the control. Rat weight increased in the isoprenaline group when treated with green tea leaf powder supplementation (1% supplement with 100 g of crushed meal) (Table 2). Rats treated with ISO also showed substantially higher heart and kidney wet weights than control rats (p < 0.05). Green tea leaf powder supplementation significantly decreased the wet weights of the kidneys and heart in the ISO-administered rats (p < 0.05) (Table 2).



**Fig. 1**. HPLC chromatogram of 16 phenolic compounds (**A**). HPLC chromatogram of tea leaf extract (**B**). The peaks detected are: 1, (+)-catechin hydrate (CH); 2, (–)-epicatechin (ECA); 3, syringic acid (SA); 4, rutin hydrate (RH); 5, rosemarinic acid; 6, quercetin; 7, kaempferol.

Name of the compounds	Green tea (mg/100 g dry extract)
Gallic acid	ND
3,4-Dihydroxy benzoic acid	ND
Catechin hydrate	83.21±0.17
Catechol	ND
(-)-Epicatechin	601.32±1.39
Caffeic acid	ND
Vanillic acid	ND
Syringic acid	22.33±0.27
Rutin hydrate	$45.49 \pm 0.46$
<i>p</i> -Coumaric acid	ND
Trans-Ferulic acid	ND
Rosmarinic acid	17.61±0.38
Myricetin	ND
Quercetin	2.43±0.19
Trans-Cinnamic acid	ND
Kaempferol	0.68±0.02

**Table 1**. Contents of polyphenolic compounds in the ethanol extract of tea leaves (n = 5). ND, not detected;RSD, relative standard deviation.

,

# Effects of green tea leaf powder supplementation on oxidative markers MDA and NO in the plasma, heart, and kidneys

Levels of numerous indicators, including nitric oxide and malondialdehyde (MDA), were investigated to assess oxidative stress in rats given isoprenaline. The MDA, a byproduct of lipid peroxidation and a key component

Parameters	Control	Control + Green Tea leaves	ISO	ISO + Green Tea leaves
Initial Body weight (g)	186.16±1.9a	183.33±1.54a	$185.00 \pm 1.12a$	$184.00 \pm 1.00a$
Final Body weight (g)	$275.50\pm2.94a$	275.5±3.30a	$242.5\pm1.76b$	257.33±3.94a, c
Kidney wet weight (g/100 g of body weight)	$0.76 \pm 0.04a$	$0.78\pm0.04$	$0.72\pm0.02b$	0.68±0.02a,c
Total Heart wet weight (g/100 g of body weight)	$0.32\pm0.01a$	$0.33 \pm 0.02$	$0.38\pm0.02b$	0.35±0.01a,c
LV of Heart wet weight (g/100 g of body weight)	$0.23 \pm 0.01a$	$0.23\pm0.01$	$0.29\pm0.01b$	0.27±0.01c
RV of Heart wet weight (g/100 g of body weight)	$0.08 \pm 0.01a$	$0.09 \pm 0.01$	$0.10\pm0.01b$	0.08±0.01a,b

**Table 2.** Effects of green tea leaf (*Camellia sinensis*) powder supplementation on body weight and organ wet weight in ISO-treated rats. Values are presented as mean  $\pm$  SEM. N = 6 in each group or otherwise specified. One-way ANOVA followed by Tukey tests was conducted as a post hoc analysis. Values are considered significant at *p* < 0.05. The significant differences are indicated by a versus b, representing control versus ISO; b versus c denotes ISO versus green tea leaves treatment.



**Fig. 2.** Effect of green tea leaf powder supplementation on oxidative stress parameters MDA and NO in the plasma, heart, and kidney tissue homogenates of ISO-administered rats. Data are expressed as mean  $\pm$  SEM, n = 6. Statistical analysis was done using one-way ANOVA, followed by a Tukey post hoc test. Statistical significance was considered as *p* < 0.05 and marked as an asterisk mark.

of oxidative stress, was considerably higher in the plasma of ISO-administered rats compared to control rats  $(p \le 0.01)$  (Fig. 2A). Green tea leaf powder supplementation (100 g/kg) decreased the concentration of MDA in the plasma (Fig. 2B). ISO-administered rats also showed higher levels of MDA in their hearts than the control group  $(p \le 0.01)$  (Fig. 2B). Green tea supplementation halted the increase in MDA levels in the hearts of ISO-administered rats (p < 0.01) (Fig. 2B). The MDA concentration in the kidney was significantly higher in ISO-induced rats than in control rats  $(p \le 0.01)$  (Fig. 2C). Green tea supplementation lowered the MDA level in kidney tissues of ISO-administered rats. Treatment with green tea did not alter the MDA level in the plasma, heart, and kidneys of the control + green tea group rats compared to control rats (Fig. 2).

Nitric oxide, which has a vital physiological role within human cells, can lead to nitrosative stress when combined with superoxide free radicals. ISO-administered rats had noticeably higher plasma levels of nitric oxide compared to control rats ( $p \le 0.01$ ) (Fig. 2D). Green tea leaf powder supplementation decreased the plasma nitric oxide concentration to almost normal levels in ISO-administered rats ( $p \le 0.01$ ) (Fig. 2D). The ISO-administered rats also showed higher levels of nitric oxide in their hearts and kidneys ( $p \le 0.01$ ) compared to control rats (Fig. 2E). Green tea leaf powder supplementation normalized nitric oxide levels in the heart and kidneys of ISO-treated rats ( $p \le 0.01$ ), indicating a reduction in the overproduction of nitric oxide (Fig. 2E,F).

### Effects of green tea leaves powder supplementation on oxidative stress parameter AOPP in the plasma, heart, and kidney tissue

Proteins in the plasma, heart, and kidneys can be oxidized due to nitrosative and oxidative stress. The amount of advanced protein oxidation products (AOPP) development in tissue samples was ascertained. ISO administration significantly ( $p \le 0.01$ ) raised the plasma level of AOPP compared to control rats (Fig. 3A). Green tea supplementation decreased the levels of AOPP in the plasma of ISO-administered rats ( $p \le 0.01$ ) (Fig. 3A). ISO-administered rats also showed higher levels of AOPP in the heart compared to control rats ( $p \le 0.01$ ) (Fig. 3B). Green tea supplementation reduced AOPP levels in the hearts of ISO-administered rats (Fig. 3B). The ISO administration increased the AOPP level in kidney tissues significantly (p < 0.001) compared to control rats, which was lowered by green tea supplementation in ISO-administered rats (Fig. 3C).

### Effect of green tea powder supplementation on catalase and SOD activity in the plasma, heart, and kidneys

Catalase activity in the plasma, heart, and kidneys was investigated. Plasma catalase activities in ISO-administered rats were significantly decreased compared to control rats (p < 0.05) (Fig. 4A). Green tea administration in ISO-administered rats significantly increased plasma catalase activity ( $p \le 0.01$ ) compared to ISO-administered rats (Fig. 4A). Green tea therapy showed higher catalase activity in cardiac tissue of ISO-administered rats compared to ISO-administered animals only ( $p \le 0.01$ ) (Fig. 4B). The ISO-administered animals showed a lower level of catalase activity in the kidney ( $p \le 0.01$ ) compared to control rats (Fig. 4B). Green tea supplementation adequately recovered catalase activity in ISO-administered rats ( $p \le 0.01$ ) (Fig. 4C).

SOD activity was also measured in the plasma, heart, and kidneys. ISO-administered rats showed decreased SOD activity significantly (p<0.05) in the plasma which was restored to near normal by green tea powder supplementation (Fig. 4D). ISO administration in rats also showed a significant (p<0.05) decline in SOD activity in the heart compared to the control rats (Fig. 4E). Green tea powder supplementation prevented the loss of SOD activity in the heart of ISO-administered rats (Fig. 4E). ISO-induced sympathetic stimulation also caused a significant (p<0.05) decline in SOD activity in the kidney tissues compared to the control rats (Fig. 4F). Green tea powder supplementation prevented the decrease in SOD activity in the kidneys of ISO-administered rats (Fig. 4F).

## Effect of green tea powder supplementation on reduced glutathione level in the plasma, heart, and kidneys

Reduced glutathione (GSH) is another parameter of antioxidant enzyme activity. GSH concentration was significantly lowered in the plasma of ISO-administered rats ( $p \le 0.01$ ) compared to the control group (Fig. 5A). ISO-administered rats that received green tea supplementation showed significantly increased GSH concentration in the plasma ( $p \le 0.01$ ) compared to the ISO group (Fig. 5A). ISO administration also lowered



**Fig. 3**. Effect of green tea powder supplementation on oxidative stress parameters APOP in plasma, heart, and kidney tissue homogenates of ISO-administered rats. Data are expressed as mean  $\pm$  SEM, n = 6. Statistical analysis was done using one-way ANOVA, followed by a Tukey post hoc test. Statistical significance was considered as p < 0.05 and marked as an asterisk mark.



**Fig. 4**. Effect of green tea powder supplementation on catalase activity in the plasma, heart, and kidneys of ISO-administered rats. Data are expressed as mean  $\pm$  SEM, n = 6. Statistical analysis was done by One-way ANOVA, followed by a Tukey post hoc test. Statistical significance was considered as *p* < 0.05 and marked with an asterisk.



**Fig. 5**. Effect of green tea powder supplementation on reduced glutathione level in the plasma, heart, and kidneys of ISO-administered rats. Data are expressed as mean  $\pm$  SEM, n = 6. Statistical analysis was done using one-way ANOVA, followed by a Tukey post hoc test. Statistical significance was considered as p < 0.05 and marked with an asterisk.

the GSH level in the heart, which was restored significantly ( $p \le 0.01$ ) by green tea supplementation (Fig. 5B). The ISO administration significantly lowered the GSH level in kidneys (p < 0.05). Green tea supplementation restored the GSH level in the kidneys of ISO-administered rats (Fig. 5C). The control + green tea group showed no changes in GSH levels in the plasma, kidneys, and heart compared to control rats (Fig. 5A–C).

# Effect of green tea powder supplementation on creatinine kinase- muscle brain (CK-MB) activity in the plasma of ISO-administered rats

CK-MB activity was measured in the plasma to assess cardiac toxicity. ISO-administered rats exhibited a significant increase in CK-MB activity (p < 0.05) in the plasma, which was reduced to nearly normalized by green tea powder supplementation (Fig. 6). Green tea powder supplementation did not alter the CK-MB activity in control rats (Fig. 6).

#### The Principal Component Analysis (PCA) of plasma and cardiac parameters

The PCA results for the plasma and cardiac parameters are shown in Figs. 7 and 8. They indicate that the control group data cluster is on the left side of the graph, while the ISO group data cluster is on the right. The ISO + green tea data also appears on the left side of the graph, demonstrating a strong correlation with the control data sets. The Biplot analysis further reveals that MDA, AOPP, NO, and CK-MB are associated with the ISO groups, whereas catalase, SOD, and GSH associate with the control and treatment data sets.

### Effect of green tea powder supplementation on antioxidant gene expression in the heart of ISO-administered rats

Antioxidant gene expression was evaluated to reveal the modulatory effect of green tea supplementation in ISO-administered rats. ISO-administered rats showed reduced Nrf2 transcript levels in the heart compared to control rats ( $p \le 0.01$ ) (Fig. 9A). Treatment with green tea supplementation restored Nrf2 expression in the heart of ISO-administered rats (Fig. 9A). A significant ( $p \le 0.01$ ) up-regulation of HO-1 and HO-2 transcript levels was detected in ISO-administered rats treated with green tea supplementation (Fig. 9B,C). Gene expression of endogenous antioxidant enzymes such as catalase, SOD, and GPx was also significantly decreased ( $p \le 0.01$ ) in the heart of ISO-administered rats (Fig. 9D–F). Green tea supplementation significantly ( $p \le 0.01$ ) enhanced the expression of these antioxidant enzymes in the heart of ISO-treated rats (Fig. 9D–F).

## Effect of green tea powder supplementation on inflammation gene expression in the heart of ISO-administered rats

The transcription levels of six genes related to inflammation, including interleukin-1 (IL-1), interleukin-6 (IL-6), nuclear factor kappa B (NF- $\kappa$ B), transforming growth factor beta-1 (TGF- $\beta$ ), tumor necrosis factor-alpha (TNF- $\alpha$ ), and inducible nitric oxide synthase (iNOS), were assessed in the left ventricle of the heart (Fig. 10). ISO-administered rats showed elevated levels of IL-1, IL-6, and TNF- $\alpha$  gene expression in the heart ( $p \le 0.01$ ) (Fig. 10).

The ISO-administered rats also showed substantially higher levels of TGF- $\beta$ , iNOS, and NF- $\kappa$ B expression in the heart compared to control rats (Fig. 10). Green tea treatment effectively reduced the expression of these inflammatory and pro-inflammatory genes in the hearts of ISO-administered rats (Fig. 10). Green tea supplementation considerably lowered the gene expression of important fibrosis-associated proteins, including TGF- $\beta$  and IL-1, in the heart of ISO-administered rats ( $p \le 0.01$ ) (Fig. 10).

### Effect of green tea powder supplementation on uric acid and creatinine levels in the plasma of iso-administered rats

The plasma of ISO-administered rats showed considerably higher concentrations of uric acid (p < 0.05) than control rats (Fig. 11A). Green tea leaves supplementation inhibited the increased uric acid concentration in the plasma of ISO-administered rats (Fig. 11A). Furthermore, the plasma of ISO-administered rats showed a significantly higher concentration of creatinine (p < 0.05) compared to control rats (Fig. 11B). Green tea leaf supplementation significantly inhibited the increase in creatinine concentration in the plasma of ISO-administered rats (Fig. 11B).

### Effect of green tea treatment on histological assessments of the heart and kidney structure in ISO-induced rats

To evaluate the pathological changes in the tissues, the heart and kidney tissues were used to prepare histological slides and stained with several staining methods. Hematoxylin and eosin staining revealed mononuclear inflammatory cell infiltration in the heart of ISO-administered rats compared to control rats (Fig. 12). The infiltration of inflammatory cells into the heart of ISO-administered rats was inhibited by green tea leaves supplementation (Fig. 12). Compared to control rats, ISO-induced rats also displayed inflammation, fibrosis, and hypertrophy of cardiomyocytes (Fig. 12). Green tea supplementation in ISO-administered rats prevented inflammatory cell infiltration, cardiomyocyte hypertrophy, and fibrosis (Fig. 12).

The histological architecture of proximal and distal tubules was found to be normal in the histological sections of control rats (Fig. 13). The histological characteristics of the renal sections from control rats also showed no pathological necrosis (Fig. 13). In ISO-administered rats, renal sections showed hyaline deposits, tubular brush boundary loss, and mononuclear cell infiltration in the kidney cortex (Fig. 13C). Furthermore, renal sections from the ISO-treated group demonstrated significant cortical fibrosis and extracellular matrix accumulation (Fig. 13G). Infiltration of inflammatory cells and tubular congestion were reduced by green tea leaf powder supplementation in ISO-administered rats (Fig. 13C). In addition, green tea leaves powder supplementation in ISO-administered rats showed reduced renal cortical fibrosis (Fig. 13H).

**CK-MB** Plasma



**Fig. 6.** The effect of green tea powder supplementation on CK-MB levels in ISO-administered rats is shown. The values are expressed as mean  $\pm$  SEM, n = 6. GraphPad Prism Software, version 9, was used for analysis, applying a one-way ANOVA and a Tukey multiple comparisons test. A significance level of *p* < 0.05 is applied to the results.

The Prussian blue staining also showed the blue marking deposition in the heart and kidney sections. This signifies the free iron accumulation in the heart and kidney tissue of ISO-administered rats (Fig. 14). Green tea leaf powder supplementation reduced iron pigment deposition in the heart and kidney sections of ISO-administered rats (Fig. 14).

#### Discussion

Aging is a degenerative process that leads to the progressive decline of various physiological functions, including neurological, endocrine, renal, and cardiovascular systems. It particularly affects heart function by reducing



**Fig.** 7. Score plot (**A**) and biplot (**B**) for the Principal Component Analysis (PCA) of biochemical parameters in the plasma across different groups. The PAST software, version 4.03, was used to perform the PCA analysis.



**Fig. 8**. Score plot (**A**) and biplot (**B**) graph for the Principal Component Analysis (PCA) of biochemical parameters for the heart in various groups. The PAST software, version 4.03, was used to analyze the PCA.

elasticity and the ability to respond to changes in arterial pressure. Myocardial infarction (MI) is one of the most severe causes of heart failure, and its prevalence is increasing, especially among older people in developing countries<sup>32</sup>. In this study, a rat model of MI, similar to that observed in humans, was created using subcutaneous injections of isoprenaline (ISO)<sup>33,34</sup>. Rats administered ISO exhibited elevated oxidative stress markers and reduced cellular antioxidants in the plasma and heart. Additionally, ISO administration increased fibrosis, inflammatory cell infiltration, and left ventricular hypertrophy. Therefore, antioxidant supplementation could be a viable strategy to mitigate oxidative stress. Green tea, rich in polyphenolic antioxidants, has shown beneficial effects on various diseases, including cardiovascular disorders. The green tea leaves extract used in this study contained several polyphenolic compounds, such as epicatechin, catechin hydrate, syringic acid, rosmarinic acid, and rutin hydrate (RH), demonstrating potent anti-inflammatory and antioxidant activities. Supplementation



**Fig. 9**. Effect of green tea powder supplementation on antioxidant gene expression in the heart of ISOadministered rats. All data were presented as mean ± SEM. For statistical analysis, One-Way ANOVA followed by the Tukey multiple comparisons test was done, where significance was indicated as ns means p < 0.05; \* means  $p \le 0.05$ ; \*\* means  $p \le 0.01$ .

with green tea leaves powder also prevented oxidative stress and inflammatory cell infiltration in the hearts of ISO-administered rats.

The Nrf2-HO-1 pathway is a central mechanism for cellular protection against oxidative stress by regulating the expression of antioxidant and cytoprotective genes<sup>35</sup>. Excessive production of reactive oxygen species (ROS)



**Fig. 10**. Effect of green tea powder supplementation on anti-inflammatory gene expression in the heart of ISO-administered rats. All data were presented as mean  $\pm$  SEM. For statistical analysis, One-Way ANOVA followed by the Tukey multiple comparisons test was done, where significance was indicated as ns means p < 0.05; \* means  $p \le 0.05$ ; \*\* means  $p \le 0.01$ .

leads to oxidative damage, inflammation, and apoptosis in models of cardiac injury induced by isoprenaline (ISO). While various studies have proven that activation of the Nrf2-HO-1 pathway can prevent ISO-induced myocardial injury<sup>36,37</sup>, the specific mechanisms and potential of natural substances in modulating this pathway remain an ongoing area of research. For instance, compounds like umbelliferone and taxifolin have been reported



**Fig. 11**. The effect of green tea powder supplementation on uric acid and creatinine levels in the plasma and urine of ISO-administered rats. Data are expressed as mean  $\pm$  SEM, n = 6. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Statistical significance was considered as *p* < 0.05 and marked with an asterisk.

to show cardioprotective effects by inducing Nrf2 and HO-1 expression, thereby preventing oxidative stress and inflammation in ISO-treated rats. However, the efficacy of powdered green tea leaves with high polyphenol content, such as epicatechin, in triggering the Nrf2-HO-1 pathway and cardiac protective effects against ISO-induced models is not well investigated.

Elevation in CK-MB levels is a specific marker for myocardial damage. ISO-administered rats showed increased CK-MB activity in the plasma, consistent with previous reports<sup>38,39</sup>. Green tea leaf powder supplementation prevented the rise in CK-MB activity in the plasma of ISO-administered rats. This finding aligns with earlier studies that reported a combination of green tea and vitamin E reduced CK-MB activity, lipid peroxidation, and heart weight in ISO-induced myocardial infarction in rats<sup>29</sup>. The beneficial effects observed in this study can be attributed to the high-density catechins present in green tea leaf powder<sup>40</sup>.

Oxidative stress is a major contributor to cardiomyocyte damage in ISO-treated hearts. ISO administration increases calcium influx, generates highly cytotoxic free radicals, and induces mitochondrial damage in the heart. These processes, along with other ROS forms produced by ISO auto-oxidation, lead to excessive free radical production and lipid peroxidation, ultimately resulting in myocardial infarction and irreversible cardiac damage<sup>39,41</sup>. Peroxidation of the myocardial phospholipid membrane causes permeability changes, intracellular calcium overload, and permanent damage<sup>42</sup>. One should anticipate that ISO administration in rats increased the plasma and cardiac MDA content, indicating lipid peroxidation. Another oxidative stress marker, AOPP, was also elevated in ISO-administered rats. Green tea leaf powder supplementation reduced or normalized MDA and AOPP levels. ISO-administered rats also exhibited higher nitric oxide levels, which were associated with increased NO generation and iNOS expression during the acute phase of MI<sup>41</sup>. Additionally, beta-adrenergic stimulation further enhanced NO production and up-regulated iNOS<sup>43,44</sup>.

An increase in nitric oxide content brings on a nitrosative stress and generates the toxic, unstable structural isomer of nitrate peroxynitrite (·ONOO-), triggering apoptosis and eventually causing myocardial injury<sup>45</sup>. Controlling the generation of NO may help to avoid the formation of peroxynitrite. Moreover, ISO administration resulted in an increased heart weight and reduced overall body weight compared to the control, suggesting hypertrophic action. Previous studies have shown that epigallocatechin gallate (EGCG), a major catechin in green tea, reduces heart hypertrophy by inhibiting ROS-dependent p38 and JNK-signaling pathways<sup>46–48</sup>. Similar results were observed in studies where tea leaves inhibited ROS production and the Src/EGFR/Akt signaling pathway activated by Ang-II, reducing heart hypertrophy<sup>49</sup>. It was also observed that dialysis patients treated with tea leaves notably decreased the expression of oxidative stress-related proteins which are closely associated with increased LV mass<sup>50</sup>.

Maintaining redox equilibrium is a prerequisite for retaining tissues' ability to execute their functions effectively. By amplifying ROS and starting other harmful reactions, hypoxia throws off this equilibrium and damages tissue. Antioxidants can lessen the tissue damage resulting from free radical generation. As the initial line of defense for cells against nitrosative and oxidative stress, free radical scavenging enzymes such as SOD, CAT, and GSH are standing in the frontline<sup>51</sup>. ISO administration increased lipid peroxidation selectively in the heart and reduced the antioxidant enzymes at the tissue level<sup>52,53</sup>. Catalase and SOD are prominent enzymes that



**Fig. 12**. Effect of green tea leaves powder supplementation on inflammation and fibrosis in left ventricular myocardium of ISO-treated rats. The upper panel shows H and E staining, (A) Control; (B) Control + tea leaves; (C) ISO; and (D) ISO + tea leaves. The lower panel shows Sirius red staining, (E) Control; (F) Control + tea leaves; (G) ISO; and (H) ISO + tea leaves. (Magnification 40X).

prevent the formation of hydroxyl radicals, whereas one of the body's most prevalent non-enzymatic antioxidants is GSH<sup>54</sup>. In this investigation, rats given ISO showed considerably reduced levels of glutathione (GSH) in the plasma and decreased catalase activity in the heart and kidney tissues as compared to control rats. Green tea leaf supplementation restored the antioxidant level in the heart of ISO-administered rats. The antioxidant enzyme activities are regulated by Nrf-2-mediated HO-1 and HO-2-dependent pathways in the heart<sup>55</sup>. These are the genes family activated in response to oxidative stress in the cells and enhance the expression of SOD, catalase, GPx, etc.<sup>55</sup>. ISO administration showed a declining state of Nrf-2 expression which could be a reason for the decline in antioxidant enzyme activities in the heart of rats. Nonetheless, rats given green tea supplements in the diet were able to raise the levels of Nrf-2-HO-1 expression as well as increased the levels of SOD, catalase, and GPx expression in the heart of ISO-administered rats.

The aging process initiates progressive myocardial degeneration and fibrosis in most heart failure patients<sup>56</sup>. The experimental animal model used aged animals also demonstrated increased collagen deposition in the aging heart<sup>57,58</sup>. In this study, fibrosis and mononuclear cell infiltration were observed in the left ventricle of the heart upon histological evaluation of the myocardium of ISO-administered rats. MI leads to ventricular enlargement and progressive collagen deposition as compensation for cardiomyocyte loss. These post-MI changes may thus contribute to the development of congestive heart failure (CHF). Myocardial and arterial compliance are decreased in the heart as a result of interstitial and perivascular collagen formation<sup>59</sup>. An increased collagen deposition also increases cardiac stiffness constant which leads to diastolic dysfunction of the heart<sup>60</sup>. TGF- $\beta$  mediated signaling controls the amount of collagen and fibrosis in the heart<sup>61</sup> and is a direct consequence of inflammation and oxidative stress<sup>62</sup>. ISO-administered rats showed increased inflammatory gene expression as well as the TGF- $\beta$  expression in the heart. In this study, green tea leaf powder supplementation attenuates





Fig. 13. Effect of green tea leaves powder supplementation on the inflammatory scar and fibrosis in the kidneys of ISO-treated rats. The upper panel shows H and E staining, (A) Control; (B) Control + tea leaves; (C) ISO; and (D) ISO + tea leaves. The lower panel shows Sirius red staining, (E) Control; (F) Control + tea leaves; (G) ISO; and (H) ISO + tea leaves. (Magnification 40X).

.....

infiltration of inflammatory cells, and inflammatory gene expression in the left ventricle. Moreover, green tea leaf powder supplementation lowers TGF- $\beta$  expression, fibrosis, and necrosis of cardiomyocytes in ISO-administered rats. The large quantity of polyphenolic substances in the powdered tea leaves may be responsible for this protective function. The anti-fibrotic activity of green tea leaf powder is also supported by other researchers<sup>63</sup>.

This study provides valuable evidence related to the cardioprotective benefits of green tea leaves powder in ISO-induced myocardial damage in rats. However, this study did not explore the effects of this supplementation on other organs and tissues. Therefore, further research is needed to understand the safety of a green teasupplemented diet on vital organs, especially the brain, lungs, liver, and kidneys. In addition, human studies are necessary to validate these findings and determine the relevance and applicability of green tea supplementation in clinical settings. The study has some limitations, such as the short duration (two weeks), which may not adequately capture the long-term effects of green tea leaf powder supplementation. Additionally, a single dose (1% w/w) was studied, which limits the information on potential dose-dependent effects. Furthermore, the study did not assess changes in the lipid profile, which are clinically significant in isoprenaline-induced myocardial damage, and this omission may have hindered a better understanding of the cardioprotective effects of the intervention. The present study did not include a reference antioxidant or quantitative histological scoring due to a focus on evaluating the standalone effects of green tea powder and limited resources for advanced comparative



G

H



Fig. 14. Effect of green tea leaf powder supplementation on iron pigment deposition in the heart and kidneys of ISO-treated rats. The upper panel shows the heart section, (A) Control; (B) ISO; and (C) ISO + tea leaves. The lower panel shows the kidney section, (D) Control; (E) ISO; and (F) ISO + tea leaves—magnification 40X.

analyses. Additionally, this study is limited by the lack of Western blotting to validate gene expression at the protein level.

In summary, the current study suggests that green tea leaf powder supplementation attenuates ISO-induced myocardial and kidney damage in rats. The cardioprotective actions of green tea leaf powder supplementation may be mediated by restoring antioxidant enzyme functions. The precise cardiac protection provided by tea leaves during ISO-induced MI has yet to be delineated. A hypothetical mechanism of action of tea polyphenol has been proposed in Fig. 15. Further research is needed to precisely understand the mechanism by which green tea prevents cardiovascular diseases.

#### Conclusion

This study demonstrates that green tea leaf powder supplementation protects against isoprenaline (ISO)induced oxidative stress, inflammation, and tissue damage in rats. It improves antioxidant status, modulates gene expression related to oxidative and inflammatory pathways, and mitigates histological damage in the heart



Fig. 15. Hypothetical role of green tea polyphenol in cardiac remodeling and fibrosis.

.....

and kidneys. These findings suggest that green tea leaf powder may serve as a natural therapeutic strategy for preventing cardiovascular and renal complications associated with oxidative stress. Importantly, the results provide a foundation for future translational research to evaluate green tea supplementation as an adjunctive dietary intervention in managing cardiovascular diseases in humans.

### **Materials and methods**

### Chemical compounds used in this study

The reagents and compounds used in this work are provided below. Arbutin (AR), hydroquinone (HQ), benzoic acid (BA), gallic acid (GA), vanillic acid (VA), ellagic acid (EA), vanillin (VL), rosmarinic acid (RA), caffeic acid (CA), syringic acid (SA), (+)-catechin hydrate (CH), (-)-epicatechin (EC), quercetin (QU), *p*-coumaric acid (PCA), *trans*-cinnamic acid (TCA), *trans*-ferulic acid (FA), rutin hydrate (RH), myricetin (MC), and kaempferol (KF) were bought from Sigma-Aldrich (St. Louis, MO, USA). Merck (Darmstadt, Germany) provided methanol (HPLC grade), acetonitrile (HPLC grade), acetic acid (HPLC grade), and ethanol. The manufacturer of the isoprenaline was Sigma-Aldrich (3050 Spruce St., 63103 St. Louis, USA). For the biochemical markers and staining tests, standards and all other reagents were bought from Merck (Darmstadt, Germany) and Sigma-Aldrich (3050 Spruce St. 63103 St. Louis, USA). For the biochemical markers and staining tests, standards and all other reagents were bought from Merck (Darmstadt, Germany) and Sigma-Aldrich (3050 Spruce St. 63103 St. Louis, USA). For the biochemical markers and staining tests, standards and all other reagents were bought from Merck (Darmstadt, Germany) and Sigma-Aldrich (3050 Spruce St. 63103 St. Louis, USA). For the biochemical markers and staining tests, standards and all other reagents were bought from Merck (Darmstadt, Germany) and Sigma-Aldrich (3050 Spruce St. 63103 St. Louis, USA). From SR Group (Delhi, India), SOD standards and other test components were purchased. DCI Diagnostics (Budapest, Hungary) provided the test kits for uric acid, creatinine, and creatinine kinase-muscle brain (CK-MB). Thermo Fisher Scientific Inc. (Waltham, Massachusetts, United States) supplied the SYBRTM Green PCR Master Mix, RevertAid First Strand cDNA Synthesis Kit (Catalog number: K1621), and GeneJET RNA Purification Kit.

### **Plant materials**

Green tea was procured from the local marketplace of the manufacturing company Kazi and Kazi Tea, located in Mirzapur, Shalbahan, Tetulia, Panchagar 5000, Bangladesh. No specific collection permits were required because the plant material was obtained from a licensed supplier, and no wild collection was involved. The tea leaves were authenticated by Khandoker Kamrul Islam, an expert scientific officer at the National Herbarium in Mirpur, Dhaka. A voucher specimen was also deposited for future reference (DACB No. 66757). Green tea leaves were ground in an electric grinder machine to get a coarse powder. This powder of green tea was used as a supplement

to the chow diet in this study. Tea leaf powder (20 g) was soaked in 70% ethanol (200 mL) in a sealed container for a week. After that, the extracted substance was decanted and put through a filter paper for filtration. After that, the ethanol was eliminated by employing a rotary evaporator, yielding 3.4 g of sticky semisolid extract. The HPLC–DAD technique was then utilized to investigate the phenolic content of the tea extract.

#### HPLC detection and quantification of polyphenolic compounds

As previously mentioned, the polyphenolic constituents of the extract were identified and measured by implementing an HPLC–DAD system<sup>64</sup>. A Dionex Ultimate 3000 system (Shimadzu Corporation, Kyoto, Japan) equipped with a photodiode array detector (DAD-3000RS) and quaternary rapid separation pump (LPG-3400RS) (Shimadzu Corporation, Kyoto, Japan) was added to carry out the experiment. Green tea leaves extract in ethanol at a concentration of 10 mg/ml was produced. All working solutions (mixed standards, sample, and spiked solutions) were degassed in an ultrasonic bath (Hwashin, Korea) for 15 min and filtered through a 0.20 µm syringe filter (Sartorius, Germany) before HPLC analysis.

Separation was performed using a Luna C18 (5  $\mu$ m) Phenomenex column (4.6 mm × 250 mm) at 33°C. The mobile phase composed of A (1% acetic acid in acetonitrile) and B (1% acetic acid in water) with gradient elution: 0.01–20 min (5–25% A), 20–30 min (25–40% A), 30–35 min (40–60% A), 35–40 min (60–30% A), 40–45 min (30–5% A), and 45–50 min (5% A) was used in this study. The sample injection volume was 20 $\mu$ L, and the flow rate was 0.5 mL/min. The UV detector was set at 270 nm and applied to validate the method and analysis. Data collection, peak integration, and analytical calibration sessions took place employing Dionex Chromeleon software (Version 6.80 RS 10).

#### **Dosage rationale**

The 1% (w/w) dietary addition of green tea leaf powder was selected based on earlier reports indicating its effectiveness and safety in rodent models. For example, a safety study on green tea polyphenols in middle-aged ovariectomized rats showed that daily administration at levels up to 1.5% (w/w) in drinking water (approximately 633 mg/kg body weight/day) for six months was safe without signs of toxicity<sup>65</sup>. Additionally, broiler chicks fed up to 1% (w/w) green tea powder positively affected growth performance and lipid metabolism without any adverse side effects<sup>66</sup>. Similarly, the chosen ISO dosage regimen of 50 mg/kg, administered intraperitoneally three times a week, was grounded in its established efficacy for inducing myocardial damage in rodent models. Research has indicated that ISO doses ranging from 25 to 100 mg/kg effectively induce cardiac stress and replicate myocardial infarction-like situations in rats. For instance, a study investigating the development of a stress cardiomyopathy mouse model found that daily administrations of ISO doses of 25 and 50 mg/kg for 14 consecutive days resulted in significant cardiac changes with minimal mortality<sup>67</sup>.

#### Animals and treatment

Long Evans rats (24 male) weighing between 180 g and 200 g were obtained from the Animal House's animal breeding unit at the Department of Pharmaceutical Sciences at North South University. Long Evans rats were selected due to their well-defined physiological and behavioral characteristics, which make them excellent candidates for studies on cardiovascular health and oxidative stress. This outbred strain exhibits genetic diversity similar to human populations, thereby increasing the applicability of the research outcomes. Notably, Long Evans rats have been reported to exhibit increased basal levels of oxidative stress compared with Sprague Dawley rats and other strains and therefore may be more sensitive to oxidative damage. This heightened sensitivity makes them particularly appropriate for evaluating antioxidant interventions<sup>68,69</sup>. The rats were relocated to individual cages in an enclosed area with controlled humidity (55%) and ambient temperature ( $22 \pm 3$  °C). Everything in the chamber was designed to mimic a cycle of light and dark for 12 h, and the rats all had access to food and drinking water. All the rats had been housed in this environment for a week to adapt. For this experiment, the rats were randomly allocated into four distinct exploration groups, and each rat was housed in a standard plastic cage. Daily weight increases and food and water intake were recorded by the same individual every day.

The following are the experimental groups:

- (a) Control (Group I, n = 6), For two weeks, the rats in this group were fed chow meal and regular water daily.
- (b) Control + green tea (Group II, n=6), For two weeks, the rats in this group were given regular water and chow meal supplemented with green tea leaves powder (1% w/w with 100 g of chow food).
- (c) Isoprenaline (Group III, n = 6), For two weeks, the ISO group received subcutaneous injections of isoprenaline at a rate of 50 mg/kg every three days. The ISO group was given isoprenaline along with water and chow food.
- (d) Isoprenaline + green tea (Group IV, n = 6), For two weeks, the rats in this group were given 50 mg/kg of isoprenaline subcutaneously every three days, along with chow food and water. This group also received a 1% w/w supplement of green tea leaves powder.

Figure 16 illustrates the overall experimental design. Animals were randomly assigned to the experimental groups using a computer-generated randomization sequence. To minimize bias, the investigators conducting the histopathological and biochemical assessments were blinded to the treatment groups.

#### *Approval for animal experiments*

All experiments involving live vertebrates were approved by the North South University Committee on the Ethics of Animal Experiments (Protocol Number: 2022/OR-NSU/IACUC/0301). All procedures were performed in accordance with the relevant national and institutional guidelines and regulations, and the study was conducted



Fig. 16. Flow chart of the experimental design.

in full compliance with the ARRIVE guidelines. With every effort to lessen the animal's suffering, euthanasia was performed on animals with sodium pentobarbital anesthesia (90 mg/kg).

#### Tissue samples collection

After the knockout of the animal due to euthanasia, the abdomen was opened surgically. The blood sample was collected in an anticoagulant-containing tube, using an 18-gauge needle and syringe (10 mL), from the abdominal aorta of rats. Plasma was isolated from blood samples by centrifuging them at 4000 rpm (4 °C), and the plasma was then frozen at -20 °C for later examination. Other internal organs, notably the kidney and heart, were taken soon after the sacrifice. Each organ tissue was blotted and appropriately weighed. The tissues were separated into three parts: one part was preserved in neutral buffered formalin (NBF) (pH 7.4) for histological examination, and another part was preserved for tissue homogenization and stored in a refrigerator (-20 °C). For additional study, the third part of the tissues was preserved for mRNA isolation in a fridge (-80 °C).

#### Assessment of cardiotoxicity

Creatinine kinase-MB (CK-MB) was measured in plasma using assay kits, collected from Diatec Diagnostic Ltd (Budapest, Hungary), following the procedure supplied by the manufacturer. Creatinine and uric acid levels were also measured using the assay kits purchased from Diatec Diagnostic Ltd (Budapest, Hungary).

#### Preparation of tissue sample for the assessment of oxidative stress markers

The kidney and heart tissues were homogenized in 10 volumes of phosphate buffer (pH 7.4) in order to determine the oxidative stress markers. The samples were then centrifuged at 8,000 rpm for 30 min at 4 °C. As shown below, the supernatant was collected and utilized for enzymatic and protein analysis.

#### Estimation of oxidative stress parameters such as lipid peroxidation assay (malondialdehyde, MDA), nitric oxide (NO) and advanced oxidation protein products (AOPP) assay

Using the previously mentioned technique, spectrophotometric analysis was used for determining the amount of lipid peroxidation in the plasma, heart, as well as in kidney<sup>70</sup>. At 535 nm, the absorbance of the clear supernatant was determined in comparison to a reference blank. A standard curve created using standard MDA concentrations was used to estimate the MDA concentration. Using the procedure outlined by Tracy et al., nitric oxide (NO) was measured as nitrate<sup>71</sup>. Instead of utilizing 1-naphthylamine (5%), naphthyl ethylene diamine

Name of gene	Туре	Sequence	
Nuf 2	Forward	5'-CCC AGCACA TCC AGACAGAC-3'	
INI1-2	Reverse	5'-TATCCAGGGCAAGCGACT C-3'	
Heme oxigenase-1 (HO-1)	Forward	5'-TGCTCGCATGAACACTCTG-3'	
	Reverse	5'-TCCTCTGTCAGCAGTGCCT-3'	
Heme oxigenase-2 (HO-2)	Forward	5'-CACCACTGCACTTTACTTCA-3'	
	Reverse	5'-AGTGCTGGGGAGTTTTAGTG-3'	
MnSOD	Forward	5'-GCTCTAATCACGACCCACT-3'	
	Reverse	5'-CATTCTCCCAGTTGATTACATTC-3	
Catalase	Forward	5'-ATTGCCGTCCGATTCTCC-3'	
	Reverse	5'-CCAGTTACCATCTTCAGTGTAG-3'	
Glutathione peroxidase (GPx)	Forward	5'-GGGCAAAGAAGATTCCAGGTT-3'	
	Reverse	5'-GGACGGCTTCATCTTCAGTGA-3'	
IL-1	Forward	5'-ATGCCTCGTGCTGTCTGACC-3'	
	Reverse	5'-CCATCTTTAGGAAGACACGGGTT-3'	
IL-6	Forward	5'-AGCGATGATGCACTGTCAGA-3'	
	Reverse	5'-GGTTTGCCGAGTAGACCTCA-3'	
TNF-α	Forward	5'-ATGTGGAACTGGCAGAGGAG-3'	
	Reverse	5'-CCACGAGCAGGAATGAGAAGAG-3'	
TGF-β	Forward	5'-AAGAAGTCACCCGCGTGCTA-3'	
	Reverse	5'-TGTGTGATGTCTTTGGTTTTGTC-3'	
iNOS	Forward	5'-TGGTCCAACCTGCAGGTCTTC-3'	
	Reverse	5'-CAGTAATGGCCGACCTGATGTTG-3'	
NF-ĸB	Forward	5'-TGTGAAGAAGCGAGACCTGGAG-3'	
	Reverse	5'-GGCACGGTTATCAAAAATCGGATG-3'	
β-Actin	Forward	5'-GCGAGAAGATGACCCAGATC-3'	
	Reverse	5'-GGATAGCACAGCCTGGATAG-3'	

Table 3. The primer was used in this experiment in both forward and reverse order.

dihydrochloride (0.1% w/v) was applied in this investigation to modify the Griess-Illosvoy reaction. Utilizing a UV spectrophotometer, the absorbance of the final solution was measured at 540 nm in comparison to the matching blank solutions. Using a standard curve, the NO level was calculated and reported as nmol/mL or nmol/g of tissue.

AOPP levels in plasma and tissue were ascertained using a technique described in the literature<sup>39</sup>. Using a UV spectrophotometer, the absorbance of the reaction mixture was measured instantaneously at 340 nm compared to a blank. At 340 nm, the chloramine-T absorbance was found to be linear between 0 and 100 nmol/mL. The amounts of AOPP were represented as nmol/mL equivalents of chloramine-T.

# Analysis of cellular antioxidants catalase activity assay (CAT) and reduced glutathione concentration assay (GSH)

Catalase (CAT) activities were identified using the previously mentioned procedure<sup>72,73</sup>. Alterations to the absorbance of the final reaction solution at 240 nm were noted every minute. A change in absorbance of 0.01 was deemed equivalent to one unit of CAT activity and was reported in units/min.

SOD activity was determined in plasma and tissues according to the methods described previously<sup>39</sup>. The epinephrine auto-oxidation rate was determined due to SOD activity. The 50% inhibition is considered as one unit. The SOD activity was expressed as unit/minute in plasma and unit/min/g tissue in the heart and kidney tissues.

For assessing reduced glutathione (GSH), the previously described procedure was used<sup>74</sup>. The approach that had previously been described was used to estimate reduced glutathione. A 1.0 mL 10% homogenate sample was precipitated using 4.0 mL of sulfosalicylic acid. The samples were centrifuged at 4000 rpm for 20 min after being stored at 4 °C for 1 h. The test combination contained DTNB (5,5-dithiobis-2-nitrobenzoic acid) (100 mM) and phosphate buffer (0.1 M, pH 7.4) in a total volume of 3.0 mL. The resulting mixture instantly produced a yellow hue that was evaluated at 412 nm on a Smart SpecTM plus Spectrophotometer and represented as ng/mg of protein.

#### RT-PCR for antioxidant and inflammation gene expression

The heart's left ventricle was used for RT-PCR, and mRNA was extracted using an RNA purification kit purchased from Thermo-Fisher Scientific (MA, USA). The amount of RNA samples was then measured with a NanoDrop 2000 (Bio-Rad, California, USA). From these samples, approximately 1  $\mu$ g of RNA was taken for the next generation step, known as cDNA, according to the protocol of the RevertAid First Strand cDNA Synthesis Kit (Thermo-Fisher Scientific, USA). The Primer 3 program was used to generate target genes (Table 3).

Quantitative RT–PCR was then analyzed using Maxima SYBR Green qPCR master mix (Thermo-Scientific, USA). Lastly, PCR was conducted using the approach as mentioned earlier<sup>75</sup>, and the instrument employed was the "Bio-Rad, California, USA" CFX96 C1000 Touch real-time PCR detection system. Table 1 displays the primer sequences. Data was recorded and analyzed using CFX ManagerTM software (Bio-Rad, CA, USA). Standard  $\beta$ -actin was used to standardize the mRNA levels of different genes at the transcript level.

#### Histopathological determination

Neutral buffered formalin (NBF) fixed the heart and kidney tissues were cleaned properly and cut into small pieces of 4-5 mm thickness. These tissues were embedded in a block of paraffin after undergoing several treatments with xylene and alcohol. The tissue architecture of the heart and kidneys was then visible by sectioning both tissue blocks at a thickness of 5  $\mu$ m and staining them with hematoxylin and eosin. Hematoxylin and eosin staining also revealed the inflammatory cell accumulation in inflamed tissues.

The second set of staining was done with Sirius Red. Tissue sections were stained with Sirius red to detect collagen accumulation and fibrosis. The percentage of fibrosis was also estimated in each section semiquantitatively, using Image-J free software from the National Institute of Health (NIH) in the United States of America (USA). In addition, a third staining set was also carried out to check free iron deposition. The tissue sections of the heart and kidneys were stained with Prussian blue to show the presence of iron deposits. After that, every stained segment was analyzed and captured on camera using a light microscope (Carl Zeiss, Germany) set to 40X magnification.

#### **Statistical analysis**

All quantitative data are expressed as mean  $\pm$  standard error of the mean (SEM). Statistical analyses were conducted using GraphPad Prism (Version 9). To compare means across multiple groups, we employed one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. A *p* value of less than 0.05 was considered statistically significant. The assumptions of normality and variance homogeneity were assessed before conducting ANOVA. The PAST version 4.03 software was used to analyze the Principal Component Analysis (PCA).

#### Data availability

The data supporting this study's findings are available from the corresponding author, MAA, upon reasonable request.

Received: 17 February 2025; Accepted: 23 June 2025 Published online: 01 July 2025

#### References

- Chong, B. et al. Global burden of cardiovascular diseases: Projections from 2025 to 2050. Eur. J. Prev. Cardiol. https://doi.org/10.1 093/eurjpc/zwae281 (2024).
- Mocumbi, A. O. Cardiovascular health care in low- and middle-income countries. *Circulation* 149, 557–559. https://doi.org/10.11 61/CIRCULATIONAHA.123.065717 (2024).
- Sliwa, K. et al. Cardiovascular disease in low- and middle-income countries associated with environmental factors. *Eur. J. Prev. Cardiol.* 31, 688–697. https://doi.org/10.1093/eurjpc/zwad388 (2024).
- 4. Młynarska, E. et al. From atherosclerotic plaque to myocardial infarction—The leading cause of coronary artery occlusion. *Int. J. Mol. Sci.* 25, 7295 (2024).
- Kumar, A. et al. The canadian cardiovascular society classification of acute atherothrombotic myocardial infarction based on stages of tissue injury severity: An expert consensus statement. *Can. J. Cardiol.* 40, 1–14. https://doi.org/10.1016/j.cjca.2023.09.020 (2024).
- Peoples, J. N., Saraf, A., Ghazal, N., Pham, T. T. & Kwong, J. Q. Mitochondrial dysfunction and oxidative stress in heart disease. *Exp. Mol. Med.* 51, 1–13. https://doi.org/10.1038/s12276-019-0355-7 (2019).
- Jomova, K. et al. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: Chronic diseases and aging. Arch. Toxicol. 97, 2499–2574. https://doi.org/10.1007/s00204-023-03562-9 (2023).
- Romuk, E. et al. Superoxide dismutase activity as a predictor of adverse outcomes in patients with nonischemic dilated cardiomyopathy. *Cell Stress Chaperones* 24, 661–673 (2019).
- Taverne, Y. J. H. J., Bogers, A. J. J. C., Duncker, D. J. & Merkus, D. Reactive oxygen species and the cardiovascular system. Oxid. Med. Cell. Longev. 2013, 862423. https://doi.org/10.1155/2013/862423 (2013).
- Doggrell, S. A. & Brown, L. Rat models of hypertension, cardiac hypertrophy and failure. *Cardiovasc. Res.* 39, 89–105. https://doi. org/10.1016/S0008-6363(98)00076-5 (1998).
- Pham, V. A. et al. Myocardial infarction model induced by isoproterenol in rats and potential cardiovascular protective effect of a nattokinase-containing hard capsule. *Phytomedicine Plus* 3, 100472. https://doi.org/10.1016/j.phyplu.2023.100472 (2023).
- 12. Abdelzaher, W. Y. et al. Dapsone ameliorates isoproterenol-induced myocardial infarction via Nrf2/ HO-1; TLR4/ TNF-α signaling pathways and the suppression of oxidative stress, inflammation, and apoptosis in rats. *Front. Pharmacol.* **12**, 669679. https://doi.org/10.3389/fphar.2021.669679 (2021).
- 13. Upaganlawar, A. & Balaraman, R. Protective effects of Lagenaria siceraria (Molina) fruit juice in isoproterenol induced myocardial infarction. *Int. J. Pharmacol.* **6**, 645–651 (2010).
- Gómez-Guzmán, M. et al. Epicatechin lowers blood pressure, restores endothelial function, and decreases oxidative stress and endothelin-1 and NADPH oxidase activity in DOCA-salt hypertension. *Free Radic. Biol. Med.* 52, 70–79. https://doi.org/10.1016/ j.freeradbiomed.2011.09.015 (2012).
- Oyama, J.-I. et al. EGCG, a green tea catechin, attenuates the progression of heart failure induced by the heart/muscle-specific deletion of MnSOD in mice. J. Cardiol. 69, 417–427. https://doi.org/10.1016/j.jjcc.2016.05.019 (2017).
- 16. Zhang, L. et al. New perspectives on the therapeutic potential of quercetin in non-communicable diseases: Targeting Nrf2 to counteract oxidative stress and inflammation. *J. Pharmaceut. Anal.* https://doi.org/10.1016/j.jpha.2023.12.020 (2024).
- Oluranti, O. I., Alabi, B. A., Michael, O. S., Ojo, A. O. & Fatokun, B. P. Rutin prevents cardiac oxidative stress and inflammation induced by bisphenol A and dibutyl phthalate exposure via NRF-2/NF-κB pathway. *Life Sci.* 284, 119878. https://doi.org/10.1016/ j.lfs.2021.119878 (2021).

- Widowati, W. et al. Green tea extract protects endothelial progenitor cells from oxidative insult through reduction of intracellular reactive oxygen species activity. Iran. J. Basic Med. Sci. 17, 702–709 (2014).
- 19. Jurgens, T. et al. Green tea for weight loss and weight maintenance in overweight or obese adults. Cochrane Database Syst. Rev. (2012).
- 20. Santana, A. et al. Decaffeinated green tea extract rich in epigallocatechin-3-gallate improves insulin resistance and metabolic profiles in normolipidic diet-but not high-fat diet-fed mice. J. Nutr. Biochem. 26, 893–902 (2015).
- Hodgson, A., Randell, R. & Jeukendrup, A. The effect of green tea extract on fat oxidation at rest and during exercise: Evidence of efficacy and proposed mechanisms. Adv. Nutr. (Bethesda, Md.) 4, 129–140 (2013).
- 22. Park, J.-H., Bae, J.-H., Im, S.-S. & Song, D.-K. Green tea and type 2 diabetes. *Integr. Med. Res.* **3**, 4–10. https://doi.org/10.1016/j.im r.2013.12.002 (2014).
- Ohishi, T., Goto, S., Monira, P., Isemura, M. & Nakamura, Y. Anti-inflammatory action of green tea. AntiInflamm. Antiallergy Agents Med. Chem. 15, 74–90. https://doi.org/10.2174/1871523015666160915154443 (2016).
- Fujiki, H., Watanabe, T., Sueoka, E., Rawangkan, A. & Suganuma, M. Cancer prevention with green tea and its principal constituent, EGCG: From early investigations to current focus on human cancer stem cells. *Mol. Cells* 41, 73–82. https://doi.org/10.14348/mol cells.2018.2227 (2018).
- 25. Pervin, M. et al. Beneficial effects of green tea catechins on neurodegenerative diseases. *Molecules (Basel, Switzerland)* 23, 1297. https://doi.org/10.3390/molecules23061297 (2018).
- Reto, M., Figueira, M. E., Filipe, H. M. & Almeida, C. M. M. Chemical composition of green tea (Camellia sinensis) infusions commercialized in Portugal. *Plant Foods Hum. Nutr.* 62, 139–144 (2007).
- Hinojosa-Nogueira, D., Pérez-Burillo, S., de la Cueva, S. P. & Rufián-Henares, J. Á. Green and white teas as health-promoting foods. Food Funct. 12, 3799–3819. https://doi.org/10.1039/D1FO00261A (2021).
- Cao, S.-Y. et al. Effects and mechanisms of tea and its bioactive compounds for the prevention and treatment of cardiovascular diseases: An updated review. Antioxidants 8, 166. https://doi.org/10.3390/antiox8060166 (2019).
- Upaganlawar, A., Gandhi, C. & Balaraman, R. Effect of green tea and vitamin E combination in isoproterenol induced myocardial infarction in rats. *Plant Foods Human Nutr. (Dordrecht, Netherlands)* 64, 75–80. https://doi.org/10.1007/s11130-008-0105-9 (2009).
- Yokozawa, T., Nakagawa, T. & Kitani, K. Antioxidative activity of green tea polyphenol in cholesterol-fed rats. J. Agric. Food Chem. 50, 3549–3552. https://doi.org/10.1021/jf020029h (2002).
- Lu, C., Zhu, W., Shen, C.-L. & Gao, W. Green tea polyphenols reduce body weight in rats by modulating obesity-related genes. *PLoS ONE* 7, e38332. https://doi.org/10.1371/journal.pone.0038332 (2012).
- Salari, N. et al. The global prevalence of myocardial infarction: A systematic review and meta-analysis. BMC Cardiovasc. Disord. 23, 206. https://doi.org/10.1186/s12872-023-03231-w (2023).
- Hosseini, A. et al. Attenuation of isoprenaline-induced myocardial infarction by Rheum turkestanicum. *Biomed. Pharmacother.* 148, 112775. https://doi.org/10.1016/j.biopha.2022.112775 (2022).
- Wu, H. et al. Protective effects and mechanisms of Saikosaponin A against myocardial ischemia based on network pharmacology, molecular docking, and experimental validation. *Naunyn Schmiedebergs Arch. Pharmacol.* https://doi.org/10.1007/s00210-025-04 203-x (2025).
- Yang, X. et al. Targeting epigenetic and post-translational modifications of NRF2: Key regulatory factors in disease treatment. *Cell Death Discov.* 11, 189. https://doi.org/10.1038/s41420-025-02491-z (2025).
- Velusamy, P. et al. Targeting the Nrf2/ARE signalling pathway to mitigate isoproterenol-induced cardiac hypertrophy: Plausible role of hesperetin in redox homeostasis. Oxid. Med. Cell. Longev. 2020, 9568278. https://doi.org/10.1155/2020/9568278 (2020).
- 37. Abdelzaher, W. Y. et al. Dapsone ameliorates isoproterenol-induced myocardial infarction via Nrf2/HO-1; TLR4/TNF-α signaling pathways and the suppression of oxidative stress, inflammation, and apoptosis in rats. *Front. Pharmacol.* 12, 669679. https://doi.org/10.3389/fphar.2021.669679 (2021).
- Hasan, R. et al. Canagliflozin attenuates isoprenaline-induced cardiac oxidative stress by stimulating multiple antioxidant and antiinflammatory signaling pathways. Sci. Rep. 10, 14459. https://doi.org/10.1038/s41598-020-71449-1 (2020).
- Rahman, M. M. et al. Cardioprotective action of apocynin in isoproterenol-induced cardiac damage is mediated through Nrf-2/ HO-1 signaling pathway. Food Sci. Nutr. 12, 9108–9122. https://doi.org/10.1002/fsn3.4465 (2024).
- Sakata, R., Nakamura, T., Torimura, T., Ueno, T. & Sata, M. Green tea with high-density catechins improves liver function and fat infiltration in non-alcoholic fatty liver disease (NAFLD) patients: A double-blind placebo-controlled study. *Int. J. Mol. Med.* 32, 989–994. https://doi.org/10.3892/ijmm.2013.1503 (2013).
- Chisty, T. T. E. et al. Protective effects of l-carnitine on isoprenaline -induced heart and kidney dysfunctions: Modulation of inflammation and oxidative stress-related gene expression in rats. *Heliyon* 10, e25057. https://doi.org/10.1016/j.heliyon.2024.e250 57 (2024).
- 42. Zhang, S. et al. The pathological mechanisms and potential therapeutic drugs for myocardial ischemia reperfusion injury. *Phytomedicine* **129**, 155649. https://doi.org/10.1016/j.phymed.2024.155649 (2024).
- 43. Li, H. et al. Epigallocathechin-3 gallate inhibits cardiac hypertrophy through blocking reactive oxidative species-dependent and -independent signal pathways. *Free Radical Biol. Med.* **40**, 1756–1775 (2006).
- Roy, R., Wilcox, J., Webb, A. J. & O'Gallagher, K. Dysfunctional and dysregulated nitric oxide synthases in cardiovascular disease: Mechanisms and therapeutic potential. *Int. J. Mol. Sci.* 24, 15200 (2023).
- Wróbel-Nowicka, K., Wojciechowska, C., Jacheć, W., Zalewska, M. & Romuk, E. The role of oxidative stress and inflammatory parameters in heart failure. *Medicina* 60, 760 (2024).
- 46. Zheng, Y. et al. Inhibitory effect of epigallocatechin 3-O-gallate on vascular smooth muscle cell hypertrophy induced by angiotensin II. *J. Cardiovasc. Pharmacol.* **43**, 200–208 (2004).
- Li, D. et al. Inhibition of iNOS protects the aging heart against beta-adrenergic receptor stimulation-induced cardiac dysfunction and myocardial ischemic injury. J. Surg. Res. 131, 64–72. https://doi.org/10.1016/j.jss.2005.06.038 (2006).
- Dong, Q., Dai, G., Quan, N. & Tong, Q. Role of natural products in cardiovascular disease. Mol. Cell. Biochem. 480, 733–745. https://doi.org/10.1007/s11010-024-05048-3 (2025).
- Papparella, I. et al. Green tea attenuates angiotensin II-induced cardiac hypertrophy in rats by modulating reactive oxygen species production and the Src/epidermal growth factor receptor/Akt signaling pathway. J. Nutr. 138, 1596–1601. https://doi.org/10.1093 /jn/138.9.1596 (2008).
- Calo, L. A. et al. Molecular biology based assessment of green tea effects on oxidative stress and cardiac remodelling in dialysis patients. *Clin. Nutr.* 33, 437–442. https://doi.org/10.1016/j.clnu.2013.06.010 (2014).
- 51. Jomova, K. et al. Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. *Arch. Toxicol.* **98**, 1323–1367. https://doi.org/10.1007/s002 04-024-03696-4 (2024).
- 52. Verma, P. K. et al. Acute cardiorenal dysfunctions induced by isoprenaline in Wistar rats: Mitigating potential of Juglans regia hull extract. *Food Chem. Adv.* 5, 100811. https://doi.org/10.1016/j.focha.2024.100811 (2024).
- Kerli Fernandes, E. et al. Boldine reduces left ventricle oxidative stress in isoproterenol-induced adrenergic overload experimental model. Arch. Physiol. Biochem. 1, 10. https://doi.org/10.1080/13813455.2024.2441363 (2024).
- Anwar, S. et al. Exploring therapeutic potential of catalase: strategies in disease prevention and management. *Biomolecules* 14, 697 (2024).

- Chen, Q. M. & Maltagliati, A. J. Nrf2 at the heart of oxidative stress and cardiac protection. *Physiol. Genomics* 50, 77–97. https://doi.org/10.1152/physiolgenomics.00041.2017 (2018).
- Fang, Z. et al. Systemic aging fuels heart failure: Molecular mechanisms and therapeutic avenues. ESC Heart Fail. 12, 1059–1080. https://doi.org/10.1002/ehf2.14947 (2025).
- Angelini, A. et al. Sex-specific phenotypes in the aging mouse heart and consequences for chronic fibrosis. Am. J. Physiol.-Heart Circ. Physiol. 323, H285–H300. https://doi.org/10.1152/ajpheart.00078.2022 (2022).
- Yu, D. et al. Resveratrol against Cardiac Fibrosis: Research Progress in Experimental Animal Models. *Molecules (Basel, Switzerland)* 26, 6860 (2021).
- López, B. et al. Diffuse myocardial fibrosis: mechanisms, diagnosis and therapeutic approaches. *Nat. Rev. Cardiol.* 18, 479–498. https://doi.org/10.1038/s41569-020-00504-1 (2021).
- 60. Xiao, F., Qi, J., Ma, S., Sun, L. & Sun, Y. Research progress on the role and mechanism in the change of cardiac structure and function of cardiac fibrosis in the elderly. *Cardiol. Rev.* https://doi.org/10.1097/CRD.000000000000911 (2025).
- Saadat, S. et al. Pivotal role of TGF-β/Smad signaling in cardiac fibrosis: Non-coding RNAs as effectual players. Front. Cardiovasc. Med. 7, 588347. https://doi.org/10.3389/fcvm.2020.588347 (2020).
- Hanna, A. & Frangogiannis, N. G. The role of the TGF-β superfamily in myocardial infarction. *Front. Cardiovasc. Med.* 6, 140. https://doi.org/10.3389/fcvm.2019.00140 (2019).
- Jung, M. H., Seong, P. N., Kim, M. H., Myong, N.-H. & Chang, M.-J. Effect of green tea extract microencapsulation on hypertriglyceridemia and cardiovascular tissues in high fructose-fed rats. *Nutr. Res. Pract.* 7, 366–372. https://doi.org/10.4162/nrp .2013.7.5.366 (2013).
- Islam, M. K. et al. Antinociceptive and antioxidant activity of Zanthoxylum budrunga Wall (Rutaceae) seeds. Sci. World J. 2014, 7. https://doi.org/10.1155/2014/869537 (2014).
- Shen, C. L. et al. Safety evaluation of green tea polyphenols consumption in middle-aged ovariectomized rat model. J. Food Sci. 82, 2192–2205. https://doi.org/10.1111/1750-3841.13745 (2017).
- 66. Liu, W., Rouzmehr, F. & Seidavi, A. Effect of amount and duration of waste green tea powder on the growth performance, carcass characteristics, blood parameters, and lipid metabolites of growing broilers. *Environ. Sci. Pollut. Res.* 25, 375–387. https://doi.org/10.1007/s11356-017-0442-z (2018).
- 67. Wu, H. et al. Establishment and effect evaluation of a stress cardiomyopathy mouse model induced by different doses of isoprenaline. *Exp. Ther. Med.* 25, 166. https://doi.org/10.3892/etm.2023.11865 (2023).
- Sanchís-Ollé, M. et al. Male long-Evans rats: An outbred model of marked hypothalamic-pituitary-adrenal hyperactivity. Neurobiol. Stress 15, 100355. https://doi.org/10.1016/j.ynstr.2021.100355 (2021).
- Snyder, B., Duong, P., Tenkorang, M., Wilson, E. N. & Cunningham, R. L. Rat strain and housing conditions alter oxidative stress and hormone responses to chronic intermittent hypoxia. Front. Physiol. 9, 1554. https://doi.org/10.3389/fphys.2018.01554 (2018).
- Niehaus, W. G. & Samuelsson, B. Formation of malonaldehyde from phospholipid arachidonate during microsomal lipid peroxidation. *Eur. J. Biochem.* 6, 126–130 (1968).
- Tracey, W. R., Tse, J. & Carter, G. Lipopolysaccharide-induced changes in plasma nitrite and nitrate concentrations in rats and mice: pharmacological evaluation of nitric oxide synthase inhibitors. J. Pharmacol. Exp. Ther. 272, 1011–1015 (1995).
- Khan, R. A. Protective effects of Sonchus asper (L) Hill, (Asteraceae) against CCl4-induced oxidative stress in the thyroid tissue of rats. BMC Complement. Altern. Med. 12, 181. https://doi.org/10.1186/1472-6882-12-181 (2012).
- 73. Chance, B. & Maehly, A. Assay of catalase and peroxidases. Methods Enzymol 11, 764-775 (1955).
- Jollow, D., Mitchell, J., Zampaglione, N. & Gillete, J. Bromobenzene induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as a hepatotoxic metabolite. *Pharmacology* 11, 151 (1974).
- 75. Khan, F. et al. Pretreatment of cultured preadipocytes with arachidonic acid during the differentiation phase without a cAMPelevating agent enhances fat storage after the maturation phase. *Prostaglandins Other Lipid Mediat.* **123**, 16–27. https://doi.org/10. 1016/j.prostaglandins.2016.02.003 (2016).

#### Acknowledgements

No funds were received from any public, private, or nonprofit organization for this study. The research was conducted at the Department of Pharmaceutical Sciences at North South University in Bangladesh. The authors of this paper would like to express their gratitude to the Department of Pharmaceutical Sciences at North South University, Bangladesh, for its logistical support. Lutfun Nahar gratefully acknowledges the assistance from the European Regional Development Fund (Project ENOCH #CZ.02.1.01/0.0/0.0/ 16\_019/0000868), as well as the Czech Science Foundation (Projects #23-05474S and #23-05389S).

#### Author contributions

MA: conceptualized, visualized, designed, and directed the project. IJ, MMR, FK, KSA, and MHH: conducted the experiments and drafted the original manuscript. HA, LN, SDS, MAA, and NS: contributed to writing, analysis, and editing of the final draft of the manuscript.

#### Declarations

#### **Competing interests**

The authors declare no competing interests.

#### Additional information

Correspondence and requests for materials should be addressed to H.A. or L.N.

#### Reprints and permissions information is available at www.nature.com/reprints.

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by-nc-nd/4.0/.

© The Author(s) 2025

### Terms and Conditions

Springer Nature journal content, brought to you courtesy of Springer Nature Customer Service Center GmbH ("Springer Nature").

Springer Nature supports a reasonable amount of sharing of research papers by authors, subscribers and authorised users ("Users"), for smallscale personal, non-commercial use provided that all copyright, trade and service marks and other proprietary notices are maintained. By accessing, sharing, receiving or otherwise using the Springer Nature journal content you agree to these terms of use ("Terms"). For these purposes, Springer Nature considers academic use (by researchers and students) to be non-commercial.

These Terms are supplementary and will apply in addition to any applicable website terms and conditions, a relevant site licence or a personal subscription. These Terms will prevail over any conflict or ambiguity with regards to the relevant terms, a site licence or a personal subscription (to the extent of the conflict or ambiguity only). For Creative Commons-licensed articles, the terms of the Creative Commons license used will apply.

We collect and use personal data to provide access to the Springer Nature journal content. We may also use these personal data internally within ResearchGate and Springer Nature and as agreed share it, in an anonymised way, for purposes of tracking, analysis and reporting. We will not otherwise disclose your personal data outside the ResearchGate or the Springer Nature group of companies unless we have your permission as detailed in the Privacy Policy.

While Users may use the Springer Nature journal content for small scale, personal non-commercial use, it is important to note that Users may not:

- 1. use such content for the purpose of providing other users with access on a regular or large scale basis or as a means to circumvent access control;
- 2. use such content where to do so would be considered a criminal or statutory offence in any jurisdiction, or gives rise to civil liability, or is otherwise unlawful;
- 3. falsely or misleadingly imply or suggest endorsement, approval, sponsorship, or association unless explicitly agreed to by Springer Nature in writing;
- 4. use bots or other automated methods to access the content or redirect messages
- 5. override any security feature or exclusionary protocol; or
- 6. share the content in order to create substitute for Springer Nature products or services or a systematic database of Springer Nature journal content.

In line with the restriction against commercial use, Springer Nature does not permit the creation of a product or service that creates revenue, royalties, rent or income from our content or its inclusion as part of a paid for service or for other commercial gain. Springer Nature journal content cannot be used for inter-library loans and librarians may not upload Springer Nature journal content on a large scale into their, or any other, institutional repository.

These terms of use are reviewed regularly and may be amended at any time. Springer Nature is not obligated to publish any information or content on this website and may remove it or features or functionality at our sole discretion, at any time with or without notice. Springer Nature may revoke this licence to you at any time and remove access to any copies of the Springer Nature journal content which have been saved.

To the fullest extent permitted by law, Springer Nature makes no warranties, representations or guarantees to Users, either express or implied with respect to the Springer nature journal content and all parties disclaim and waive any implied warranties or warranties imposed by law, including merchantability or fitness for any particular purpose.

Please note that these rights do not automatically extend to content, data or other material published by Springer Nature that may be licensed from third parties.

If you would like to use or distribute our Springer Nature journal content to a wider audience or on a regular basis or in any other manner not expressly permitted by these Terms, please contact Springer Nature at

onlineservice@springernature.com