ISSN 2475-5451

Research Article

International Journal of Diabetes & Metabolic Disorders

Enhancing Effect of Epigallocatechin-3-Gallate (EGCG) on Liver Antioxidant Activity in Mice Exposed to Cardiovascular Disease Risk

Emmanuel Banzubaze[1*], Julius Mulindwa[2], Silver Ochwo[3], Eddie Wampande[3]

[1]*Department of Medical Biochemistry, Kampala International University, Kampala, Uganda*

[2]*Department of Biochemisrty and Sports Science, School of Biosciences, College of Natural Sciences, Makerere University, Kampala, Uganda.*

[3]*Department of Bio-molecular Resources and Bio-lab Sciences, School of Bio-security, Biotechnical and Laboratory Sciences, College of Veterinary Medecine, Animal Resources & Bio-security, Makerere University, Kampala, Uganda.*

[*]Corresponding author:
Emmanuel Banzubaze,Department of Medical Biochemistry, Kampala International University, Kampala, Uganda.

Submitted: 14 Jul 2022; **Accepted**: 20 Jul 2022; **Published**: 26 Jul 2022

Citation: *Emmanuel Banzubaze, Julius Mulindwa, Silver Ochwo, Eddie Wampande. (2022). Enhancing Effect of Epigallocatechin-3-Gallate (EGCG) on Liver Antioxidant Activity in Mice Exposed to Cardiovascular Disease Risk. Int J Diabetes MetabDisord, 7(2), 129-142.*

Abstract

Objective: *Epigallocatechin-3-gallate (EGCG) is a fundamental polyphenol compound present in green tea. Green tea has been credited for centuries with providing significant health benefits. The aim of this study was to test whether the antioxidant component of EGCG can enhance hepatic antioxidant enzyme activity and consequently increase endogenous antioxidant defense in mice exposed to cardiovascular disease.*

Groups of male mice were subjected to different diet (standard fat diet, high fat diet and low-fat diet), exercise (voluntary, chronic and sedentary lifestyle) and EGCG supplementation. EGCG powder was dissolved in 4 ml of drinking water to deliver a daily dose of 30 mg/kg.Mice were anesthetized with isoflurane (Abbott, Cham, Switzerland) 2% (v/v) in a 20% O2 and 80% air mixture by inhalation in a closed container, then euthanized by manual cervical dislocation and were put on the animal's bed to collect liver tissue for analysis of antioxidant enzyme activities.We compared the variation in antioxidant enzyme activity between the control and treated groups using one-way analysis of variance (ANOVA) for□-value < 0.05% was considered statistically significant.

Results: *In this study we showed that mice on a high-fat diet and sedentary lifestyle had significantly decreased superoxide dismutase 1 (p-value = 0, 001 after diets treatment, p-value = 0, 009 after exercise treatment), glutathion peroxidase 1 (p- value = 0, 016 after diets treatment, p-value = 0, 0022 after exercise treatment), catalase (p- value = 0, 019 after diets treatment,p-value = 0,005 after exercise treatment) and thioredoxin reductase 1(p- value = 0,000 after diets treatment, p-value = 0,027 after exercise treatment) activities. EGCG enhanced the activity of antioxidant enzymes in mice subjected to high fat diet or subjected to sedentary lifestyle.*

Keywords: High Fat Diet, Sedentary lifestyle, cardiovascular disease, Antioxidant, Epigallocatechin-3-gallate.

Introduction

Tea (Camellia sinensis) has become one of the most consumed beverages in the world, not only because of its special flavor, but also for its potential benefits to human health[1, 2]. Epigallocatechin-3-gallate (EGCG) is a fundamental polyphenol compound present in green tea (GT) [3]. GThas been credited for centuries with providing significant health benefits, including use as a stimulant, a diuretic, or an astringent to promote wound healing [4]. A subacute toxicity study report of green tea extract stated that drinking green tea every day is safe and has no adverse effects in mice [5]. Green tea has been proven to have many useful biological effects that are attributed to its rich polyphenolic active substances, especially catechins and catechin derivatives, including (−) -epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC) and epicatechin-3-gallate (ECG) [6]. A number of epidemiological and experimental studies showed that green tea catechins have

been provided with a variety of physiological and medicinal properties and can be used not only a health-preserving beverage, but also a raw material for health foods and nutritional supplements in traditional diets. Many scientific documents have confirmed many medicinal effects of green tea catechins, such as antioxidant, antibacterial, anti-inflammatory and anti-tumor activities [7–8]. The benefits of GThave been associated with cancer prevention weight management and lowering cholesterol level and cardiovascular risk [9-13]. EGCG has strong antioxidant activity and works as a free radical scavenger as well as an inducer of phase II enzyme activities [14]. The strong antioxidant capacity of GTmay help to slow the initial development of atherosclerosis [15]. Regulation of free radicals generated by mitochondria seems to be dependent on a variety of antioxidant enzymes including, Superoxide dismutase 2 (SOD2), Glutathione Peroxidase 1(GPX1), Catalase (CAT) and Thioredoxin (TRX) [16]. Antioxidant enzymes neutralize free radicals to help restore cell function and prevent further damage to cell membranes [17]. Catalase enzyme inactivates reactive oxygen species, GPX enzyme by regulating the availability of nitric oxide and selenium by increasing glutathione peroxidase activity and protects against CVD [18]. TRX is a multifunctional stress-inducible protein, which protects cells from various types of stresses and plays an important role in maintaining a reduced environment in cells through thiol-disulfide exchange reactions [19, 20].

In this study, we have investigated the protective or ameliorating effects of EGCG in enzymatic activity of antioxidant enzymes in mice against the fat rich diet (HFD) and sedentary lifestyle (SL). The aim of our study was to test whether the antioxidant component of EGCG can enhance hepatic antioxidant enzyme activity in mice exposed to CVD risk.Our findings demonstrated that epigallocatechin-3-gallate (EGCG) can enhance the activity of antioxidant enzymes in mice on a high fat diet and in mice under a sedentary lifestyle. Our findings demonstrated that EGCG can be used as a functional food additive that can improve liver damage caused by high fat diet and a sedentary lifestyle by renewing the activities of antioxidant enzymes.

Main text

Materials and Methods

Ethics Statement

The procedures and protocols of our study were approved by the Animal Ethics Committee of Kampala International University and Uganda national council of science and technology, Approval number is NS 645. Animals were cared for in accordance with the Guiding Principles in the Care and use of experimental animals of the European Council of the animal and the US National Institutes of Health Guide to the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996) [21].

Animals and Experimental Groups

A total of sixty, three-month-old Albino swiss male mice used for experimentation were acquired from animal facility of College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB) of Makerere University. The mice were housed in standard cages, maintained at a normal temperature (27 ± 2°C) and humidity (55 ± 5%), and exposed to a 12-h light/dark cycle. Mice were divided into twelve groups of 5 mice each. The first six groups were exercised with normal diet and the other six received diet treatment as follows: (1) voluntary exercise (VE), (2) chronic exercise (CE), (3) sedentary lifestyle (SL), (4) VE+EGCG, (5) CE+EGCG, (2) SL+EGCG, (7) normal diet (ND) (3% fat), (8) high fat diet (HFD) (16% fat); (9), low fat diet (LFD) (0.2% fat), (10) ND+ EGCG, (11) HFD+EGCG and (12) LFD+EGCG (Figure 1 and Table S1)[22]. EGCG powder was dissolved in 4 ml of drinking water to deliver a daily dose of 30 mg/kg. For sedentary lifestyle, one mouse was reared in its own cage. For chronic and voluntary exercise, mice from each group were housed together in their respective cages.

Animals, Preparation, Anesthesia, Euthanasia, Liver Tissue SamplesandMeasurement of Activities of Hepatic Antioxidant Enzymes

The experiments were carried out in compliance with European animal protection laws. A three-month-old male Swiss Albino mouse for each group was studied after 0, 2, 4 and 6 weeks of the experiment. These mice were anesthetized with isoflurane (Abbott, Cham, Switzerland) 2% (v/v) in a 20% O2 and 80% air mixture by inhalation in a closed container, then euthanized by manual-cervical dislocation and were put on the animal's bed to take liver sample[23-25]. A total of 48 mice were sacrificed (12 mice after 0, 2, 4 and 6 weeks).Hepatic tissues were collected in tubes and were used to determine the activities of the antioxidant enzymes: SOD2, Gpx1, Cat and TrxR1. SOD2 activity was measured using the SOD2 Typed Colorimetric Assay Kit (Elabscience, Catalog Number: E-BC-K022) according to the manufacturers' instructions as cited by Hu and coleagues;Fanand colleagues [26, 27]. The activity of GPx1 was determined by the Mouse Glutathione Peroxidase 1(GPX1) ELISA Kit; Catalog Number: E-EL-M0950 (96T)] from Elabscience as recommended by the manufacturer's protocoland as cited by Fan and colleagues; Bilgiç and colleagues [27, 28].Catalase Activity was determined by Catalase (CAT) Assay Kit; Catalog Number: E-BC-K031 from ElabScience. Steps were performed as recommended by the manufacturer's protocolinstructions and as cited by Marcusand coleaguesand as cited by Mbiantchaand coleagues [29, 30]. Theactivity of TrxR1 was determined by the Mouse Thioredoxin Reductase 1 (TrxR1) ELISA Kit, Catalog Number: SEA 703Mu (96T)] and steps were performed as recommended by the manufacturer's protocol] and as cited by Marcusand coleagues [29].

Statistical Analysis

The values are expressed as mean ± standard deviation (SD). Differences between groups were assessed by a one-way analysis of variance (ANOVA). Comparison of variances for the expression of activities of antioxidant enzymes was used to analyze the significance of differences between control and treated groups using ANOVA (GraphPad Prisms Software version 6, San Diego, CA, USA).

Results

Effect of Egcg And Diet on Enzyme Activity of Antioxidant Enzymes in Mice

We observed marked differences in the trendof the enzymatic activities of Sod2, Gpx1, Cat and TrxR1in the liver of mice treated with EGCG under different diets (Figure 2, Fig S1).In comparison to the normal diet (ND) and low-fat diet (LFD), the high fat diet (HFD) showed lowest levels of Sod2 (Fig 2A) and highest levels of Gpx1 (Fig 2B) by the 6th week. However, mice treated with EGCG showed reverse effect of increased levels of Sod2 and decreased Gpx1.

After analyzing the results by One Way ANOVA analysis with Tukey multiple comparisons of all pairs in the columns, our results showed that a high fat diet decreases the enzymatic activities of antioxidant enzymes. This decrease in enzymatic activity is observed for all the antioxidant enzymes. Our results on the enzymatic activities showed that the enzymatic activities of Sod2 and TrxR1 (Figure 2 A and D) are more expressed compared to the enzymatic activities of Gpx1 and Cat when we compare the values of their p-value (Figure 2 B and C).

When EGCG is supplemented,results have shown that the enzymatic activities of all antioxidant enzymes are increased especially for HFD compared to HFD without EGCG. For HFDwithout EGCG, Figure 2 gives the linesgraphsin descending while for HFDwith EGCG we have line graphs that start to decrease from 0 to 2 weeks but start to increase at from 2 weeks of the experiment. For the enzymatic activities of Sod2 and Gpx1 of the HFDwith EGCG, the linear increase started from two weeks while for the enzymatic activities of Cat and TrxR1, the increase started at 0 weeks.Results also showed that there is a statistically significant relationship between diets without EGCG supplementation and the enzymatic activity of all antioxidant enzymes except catalase where its p-value is greater 0.05 (Table 1: A and Figure S4).

Effect of EGCG and Exercise on Enzyme Activity of Antioxidant Enzymes in Mice

Figure 3 shows the evolution of the effect of EGCG and exercise for the enzymatic activities of Sod2, Gpx1, Cat and TrxR1 and Figure S3 shows the averages of the effect of EGCG on the enzymatic activities of these antioxidant enzymes.Results of the activities of antioxidant enzymes in the liver have shown that there are significant relationships between enzymatic activities and various treatments in mice subjected to exercise before and after EGCG supplementation at a level of 5% significance since the p-value is less than 0.05 (Table 1: B and Figures3 and S3). Results showed that a sedentary lifestyledecreases the enzymatic activities of antioxidant enzymes. This decrease in enzymatic activity is observed for all the antioxidant enzymes, (Figure 3, S2 and S5).When EGCG is supplemented, Results have shown that the enzymatic activities of all antioxidant enzymes are increased especially for SL compared to SL without EGCG. For a sedentary lifestylewithout EGCG, the enzymatic activities of Sod2 and TrxR1 showed a dramatic decrease from 4 weeks. For a sedentary lifestyle with EGCG supplementation, results showed that the enzymatic activities of all antioxidant enzymes began to increase linearly (Figure 3).Our results on the enzymatic activities showed that all the enzymatic activities of all antioxidant enzymes are more expressed but the enzymatic activity of TrxR1 is less expressed compared to the enzymatic activities of Sod2, Gpx1 and Cat (Figure 3).

Correlation Between Diet and Exercise, Diet and Exercise Supplemented by Egcg On Enzyme Activity of Antioxidant Enzymes in Mice

The study of the correlation between diet and exercise, diet and exercise supplemented with EGCG on the enzyme activity of SOD2, GPx1, Cat and TrxR1 in mice, revealed that it exists a significant association between diet and exercise in mice when treated with or without EGCG supplementation for antioxidant enzyme activities in the liver at the 5% level of significance since the p-value is less than 0.05 except for Cat (Figure 4C) for the association between diet and exercise and GPx1 for the association between diet and EGCG-supplemented exercise (Figure 5B).

Discussion

In this study, we hypothesized that human metabolic diseases such as obesity, nonalcoholic steatohepatitis (NASH), and metabolic syndrome can be mimicked in rodents using genetic, dietary, and physical inactivity interventions and would lead to a decrease in the endogenous defense capacity of antioxidant enzymes. This hypothesis led us to study the effect of EGCG in mice subjected to a high-fat diet and a sedentary lifestyle to assess the activity of antioxidant enzymes SOD2, GPX1, CAT and TRXR1 and therefore assess the ability to endogenous antioxidant defense. The three strategies mentioned above lead to body weight gain, hyperglycemia, hyperinsulinemia, hepatic steatosis and heart failure [31]. Dietary patterns are considered to be more similar to metabolic diseases in humans and in rodents such as mice, but there is currently no standard composition and duration for these diets. In our last publication, we showed that a high-fat diet and a sedentary lifestyle lead to an increase in total cholesterol, LDL cholesterol, triglycerides and glucose [32]. James and coworkers claimed that nutrient overload generated by HFD in laboratory animals resembles conditions of overeating and physical inactivity that are major risk factors for the development of metabolic syndrome in humans [33]. These conditions, when combined, are able to overwhelm the ability of adipose tissue to handle excess energy, resulting in increased efflux of non-esterified fatty acids (NEFA) and release of pro-inflammatory cytokines and adipokines that can lead to ectopic fat deposition in the liver, muscle, and heart [34]. In the liver, the accumulation of fat can be toxic [35]. Non-alcoholic fatty liver disease (NAFLD) includes several liver abnormalities related to the accumulation of fat in hepatocytes, including simple fatty liver disease, a benign condition that can progress to severe liver cirrhosis [35]. Non-alcoholic fatty liver disease (NAFLD) is characterized by excessive hepatic fat accumulation via lipid and/ or carbohydrate intake [36]. Metabolic disorders including dyslipidaemia, obesity, and type 2 diabetes often comorbid with NAFLD [37]. In fact, increased occurrence of NAFLD has paralleled recent

increases in the prevalence of obesity and diabetes [38]. Clinically, NAFLD shares similar risk factors with cardiovascular disease and is associated with the development of hepatocellular carcinomas [39-41]. NAFLD livers exhibit an infiltration of inflammatory macrophages and other myeloid cells in the hepatic parenchymal area, implicating these mechanisms in the pathogenesis of NAFLD [42]. Hepatic lipid elevation and inflammation has also been associated with NAFLD, however, the precise mechanism by which hepatic lipid accumulation and inflammation contribute to NAFLD remains poorly understood[43].

Currently, the accepted pathophysiological model for NAFLD is the "two-shot" model. First, there is accumulation of triglycerides (TAG) and free fatty acids (FFA) in the liver as a result of changes in fatty acid influx, synthesis, oxidation and transport. The second hit, triggered by the first hit, includes oxidative imbalance, decreased hepatic ATP production, insulin resistance, and induction of pro-inflammatory cytokines as a result of mitochondrial dysfunction, lipid peroxidation and activation of inflammatory pathways [44].

In this study, we have investigated the protective or ameliorating effects of EGCG in enzymatic activity of antioxidant enzymes in mice against HFD and SL. The antioxidant enzymes Sod2, Gpx1, CAT and TrxR1 are involved in scavenge of free radicals, disposal of superoxide anions and hydrogen peroxide [45]. These activities constitute the first line of cellular defense against oxidative injury [46]. Dobrzynska and colleagues confirmed that green tea extract had a protective effect on ethanol-induced oxidative stress in different tissues (including brain, liver, and blood), which could be attributed its ability to strengthen the antioxidant defense system in laboratory animals[47]. In addition, a study conducted by Tsai and colleagues proved that green tea extract had a protective effect by decreasing oxidative stress, restoring the activity of antioxidant enzymes and decreasing the incidence of hepatic fibrosis[48]. However, there are few animal models available for green tea to study high-fat diet and sedentary lifestyle damage in the liver.

The results showed thataHFD and a SL significantly decreased the enzyme activities of antioxidant enzymes. However, when EGCG is supplemented, anHFD and a SL significantly increased the enzyme activities. These results suggest that EGCG attenuates disorders of lipid metabolism induced by HFD, a SL and excessive hepatic fat deposition. However, the detailed mechanism of lipid metabolism disorder induced by HFD, SL and excessive hepatic fat deposition needs to be investigated further in further studies. The antioxidant defense system would maintain the dynamic balance of free radical production and scavenging, so changes in free radicals necessarily affect antioxidant function and therefore increase the enzymatic activity.Similar results have been reported in other studies in rats, mice, or fish species during antioxidant supplementation[49]. We also observed that the mean enzymatic activities of SOD2, GPX1, CAT and TRXR1 in liver tissue were significantly improved for mice fed aHFD and in mice under sedentary lifestyle during administration of EGCG. Administration of EGCG perhaps acted by regulating the activities of these antioxidant enzymes. Earlier studies reported that EGCG is a good scavenger of superoxide radicals, hydroxylradicals and peroxynitrite radicals [50]. Our study showed an increase in the activities of the antioxidant enzymes when there was supplementation of EGCG. Our results have been supported by other authors. Ramesh et al[51]. reported increased CAT, SOD, GPx, and GST activities in cardiac tissue and hemolysate of rats fed an atherogenic diet. Conversely, Khan et al[52]. discovered inconsistencies in enzyme activity dependent on the location of tissue extraction.Further research may be needed to analyze the effects of antioxidant enzymes on muscle, heart and liver function.EGCG have been reported to increase antioxidant enzyme activities, which is consistent with our results. In this study, antioxidant activity was significantly higher in mice fed the HFD and in SL supplemented with EGCG[53]. Similar results have been reported in animal and human studies, suggesting that GT may be an effective treatment for the prevention of oxidative processes involved in atherosclerosis[54].

In conclusion, although additional studies are needed, it could be suggested thatGTP could partly protect hepatocytes through antioxidative, anti-inflammatory and antiapoptotic mechanisms against liver injury induced by HFD and a SL.

Conclusion

In this study, we investigated whether physiological levels of EGCG trigger changes in liver antioxidant enzyme activity related to cardiovascular health in a diet and exercise intervention study in mice. The study demonstrated that the antioxidant EGCG increased the activity of antioxidant enzymes. The study also revealed that EGCG can be used as a functional food additive that can improve liver damage caused by HFD and sedentary lifestyle by renewing the activities of antioxidant enzymes.

Limitations

The limitations of this studywere mainly experimental. It was suggested that the enzymatic activity depends on the site of tissue extraction. Therefore it would have been interesting to compare the enzymatic activities of different tissues such as muscle tissue as well as cardiac tissue but this couldn't be done with the limitted resources. Further research may be needed to further analyze the effects of antioxidant enzymes on muscle, heart, and liver function.

Abbreviations

ATP: Adenosine triphosphate
CE: Chronic exercise
CoVAB: College of Veterinary Medicine, Animal Resources and Biosecurity (Makerere University)
CVD: Cardiovascular disease
EGCG: Epigallocathin-3-gallate
FFA: Free fatty acids
GPX1: Glutathione Peroxidase 1
GT: Green tea
GTP: Green tean polyphenol
HDL: High density lipoprotein cholesterol

HFD: High-fat diet
LFD: Low fat diet
LDL: Low-density lipoproteincholesterol
NAFLD: Non-alcoholic fatty liver disease
NEFA: Non-esterified fatty acids
ND: Normal diet
SL: Sedentary lifestyle
SOD2: Superoxide dismutase 2
TRX: Thioredoxin
TrxR1: Thioredoxin Reductase 1
TC: Total cholesterol
TAG: Triglycerides
VE: Voluntary exercise
VLDL: Very low-densitylipoprotein cholesterol.

Author Contributions

Banzubaze Emmanuel is the research designer. In addition to the design of the research, this Banzubaze Emmanuel did the experimentation, the analysis of the results, the discussion and the drafting of the manuscript. The other authors were the directors of the research. To do this, they read and made corrections to errors of substance and form in the manuscript.

Declaration

In research, there is no competing interest as the research has been experimental on laboratory animal models (mice).

Ethics Approval

The research has been approved by the Animal Ethics Committee of Kampala International University and the Uganda national council of science and technology; Approval number is NS 645.

Consent to Publish

Before submitting the manuscript, there was consent of the authors for its submission

Founding Source

This work was supported by National Institute of Public Health of Bujumbura, Burundi

Availability of Data and Materials

I declare on my honor that the data and materials used in the writing of this manuscript are available in the file entitled "manuscript data submit". I also declare that this data may be made public by the scientific community for research purposes or during review of the manuscript by editors or reviewers.

Acknowledgements

We thank all the people who contributed to this paper. We thank all the people who contributed to this paper. We thank Pandasaphan MUZOORA for allowing me to use the Medical Biochemistry Research Laboratory of the Department of Biomolecular Resources & Biolab Sciences of Makerere University.We thank Geoffrey and AboubacarMusoba in the sample collection, Daniel Sempebwa and Daisy Wannyana in the analysis of the samples.

Figures

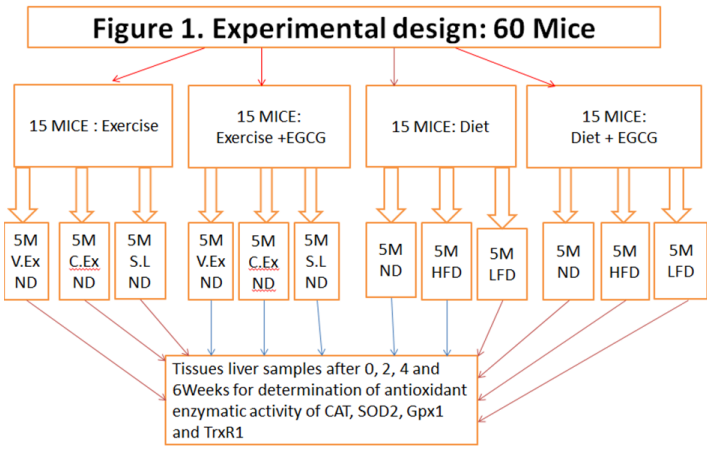
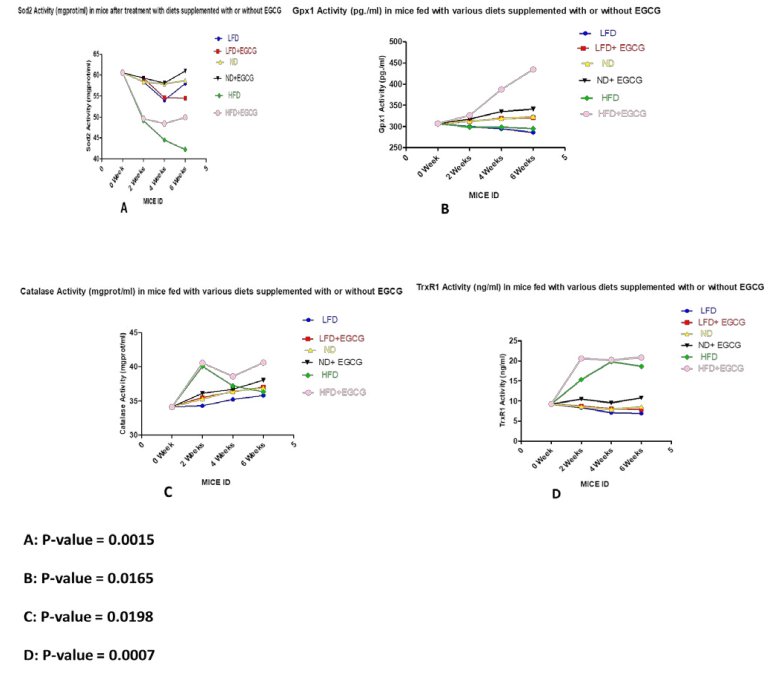


Figure 1: Experimental design for the study.

CAT is catalase, CE is chronic exercise, EGCG is epigallocatechin-3-gallate,GPX1 isglutathione peroxidase 1, HFD is high fat diet, LFD is low fat diet, M is mice, ND is normal diet, SL is sedentary lifestyle, SOD2 is Superoxide dismutase 2, TrxR1 is Thioredoxin Reductase 1and VE is voluntary exercise



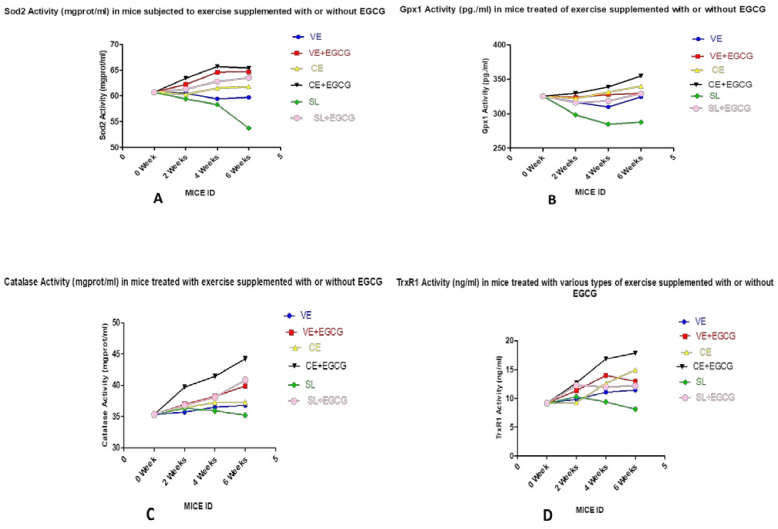
A: P-value = 0.0015

B: P-value = 0.0165

C: P-value = 0.0198

D: P-value = 0.0007

Figure 2: Effect of EGCG and diet on the enzymatic activities of antioxidant enzymes in Mice. Sod2 Activity *(mgprot/ml)* in mice after treatment with various diets supplemented with or without EGCG **(A)**,Gpx 1 Activity results(pg. /ml)in mice after treatment with various diets supplemented with or without EGCG **(B)**, Catalase Activity *(mgprot/ml)* in mice after treatment with various diets supplemented with or without EGCG **(C)** andTrxR1 Activity results(ng/ml) in mice after treatment with various diets supplemented with or without EGCG **(D)**.



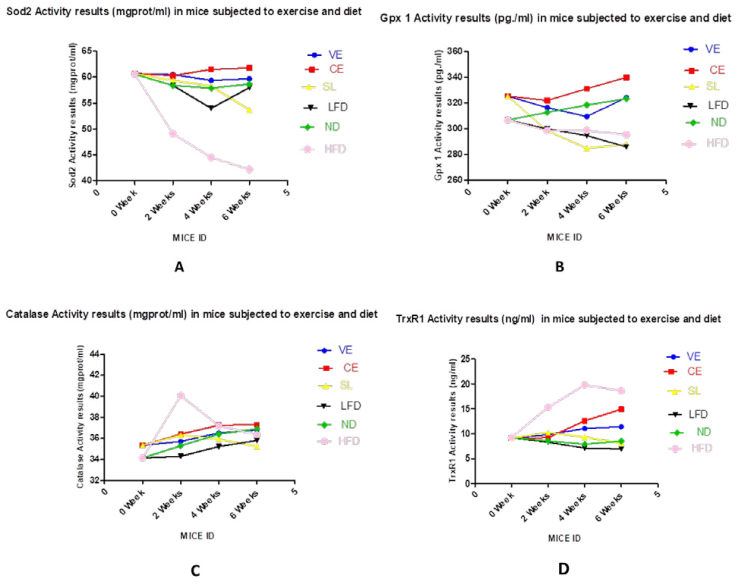
A: P-value = 0.0091

B: P-value = 0.0022

C: P-value = 0.0052

D: P-value = 0.0207

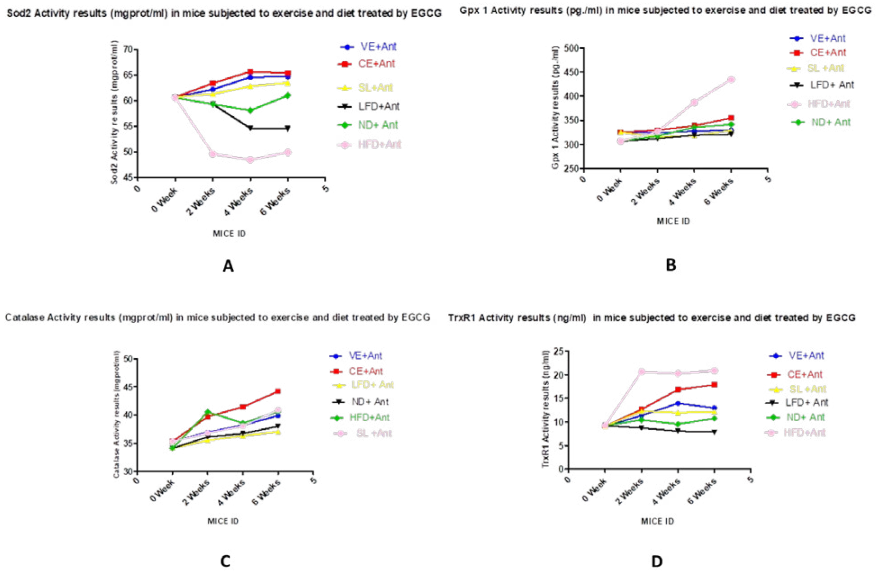
Figure 3: Effect of EGCG and Exercise on the enzymatic activities of antioxidant enzymes in Mice. Sod2 Activity *(mgprot/ml)* in mice after treatment with various exercise supplemented with or without EGCG **(A)**,Gpx 1 Activity results(pg./ml)in mice after treatment with various exercise supplemented with or without EGCG **(B)**, Catalase Activity *(mgprot/ml)* in mice after treatment with various exercise supplemented with or without EGCG **(C)** andTrxR1 Activity results(ng/ml) in mice after treatment with various exercise supplemented with or without EGCG **(D).**



A: P-value = 0, 0018
B: P-value = 0, 0013
C : P-value = 0,1541
D : P-value = 0,0031

Figure 4: Correlation between diet and exerciseon enzyme activity of antioxidant enzymes in mice. Sod2 Activity *(mgprot/ml)* in mice after treatment with diet and exercise **(A)**,Gpx 1 Activity results(pg./ml)in mice after treatment with diet and exercise **(B)**, Catalase Activity *(mgprot/ml)* in mice after treatment with diet and exercise **(C)** andTrxR1 Activity results(ng/ml) in mice after treatment with diet and exercise **(D).**

Gpx1+ EGCG	0,1020	Ns
Gpx1 with or without EGCG	0,0165	s
Cat	0,2131	ns
Cat+ EGCG	0,0346	s
Cat with or without EGCG	0,0198	s
TrxR1	0,0241	s
TrxR1+ EGCG	0,0160	s
TrxR1 with or without EGCG	0,0007	s
A. P-value of effect of EGCG on antioxidant enzymes activities after exercise treatment		
Name of enzyme	P-value	Level of significant (s or ns)
Sod2	0,1505	ns
Sod2 + EGCG	0,0228	s
Sod2 with or without EGCG	0,0091	s
Gpx1	0,0320	s
Gpx1+ EGCG	0,0471	s
Gpx1 with or without EGCG	0,0022	s
Cat	0,1836	ns
Cat+ EGCG	0,0206	s
Cat with or without EGCG	0,0052	s
TrxR1	0,2746	ns
TrxR1+ EGCG	0,0968	ns
TrxR1 with or without EGCG	0,0207	s



A : P-value = 0,0010
B : P-value = 0,0702
C : P-value = 0,0010
D : P-value = 0,0012

Figure 5: Correlation between diet and exercise supplemented by EGCG on enzyme activity of antioxidant enzymes in mice. Sod2 Activity *(mgprot/ml*) in mice after treatment with diet and exercise supplemented with EGCG **(A)**,Gpx 1 Activity results(pg./ml)in mice after treatment with diet and exercise supplemented with EGCG **(B)**, Catalase Activity *(mgprot/ml)* in mice after treatment with diet and exercise supplemented with EGCG **(C)** andTrxR1 Activity results(ng/ml) in mice after treatment with diet and exercisesupplemented with EGCG **(D).**

Table 1: P-value of effect of EGCG on antioxidant enzymes activities

A. P-value of effect of EGCG on antioxidant enzymes activities after diets treatment		
Name of enzyme	P-value	Level of significant (s or ns)
Sod2	0,0263	s
Sod2 + EGCG	0,0276	s
Sod2 with or without EGCG	0,0015	s
Gpx1	0,0413	s
Gpx1+ EGCG	0,1020	ns
Gpx1 with or without EGCG	0,0165	s
Cat	0,2131	ns
Cat+ EGCG	0,0346	s
Cat with or without EGCG	0,0198	s
TrxR1	0,0241	s
TrxR1+ EGCG	0,0160	s
TrxR1 with or without EGCG	0,0007	s
B. P-value of effect of EGCG on antioxidant enzymes activities after exercise treatment		
Name of enzyme	P-value	Level of significant (s or ns)
Sod2	0,1505	ns
Sod2 + EGCG	0,0228	s
Sod2 with or without EGCG	0,0091	s

Gpx1	0,0320	s
Gpx1+ EGCG	0,0471	s
Gpx1 with or without EGCG	0,0022	s
Cat	0,1836	ns
Cat+ EGCG	0,0206	s
Cat with or without EGCG	0,0052	s
TrxR1	0,2746	ns
TrxR1+ EGCG	0,0968	ns
TrxR1 with or without EGCG	0,0207	s

Supplementary data

Figures

Figures of enzymatic activities of antioxidant enzymes

Effect of EGCG and Diet on Enzyme Activity of Antioxidant Enzymes in Mice

Figure S1 shows the averages of the effect of EGCG and diet on the enzymatic activities of Superoxide dismutase 2 (Sod2), Glutathione Peroxidase 1 (GPx1), Catalase (Cat) and Thioredoxin Reductase (TrxR1) during the experimental period of 0 to 6 weeks. The results of the activities of antioxidant enzymes in the liver have shown that there are significant relationships between enzymatic activities and various treatments in mice subjected to diet before and after EGCG supplementation at a level of 5% significance since the p-value is less than 0.05.

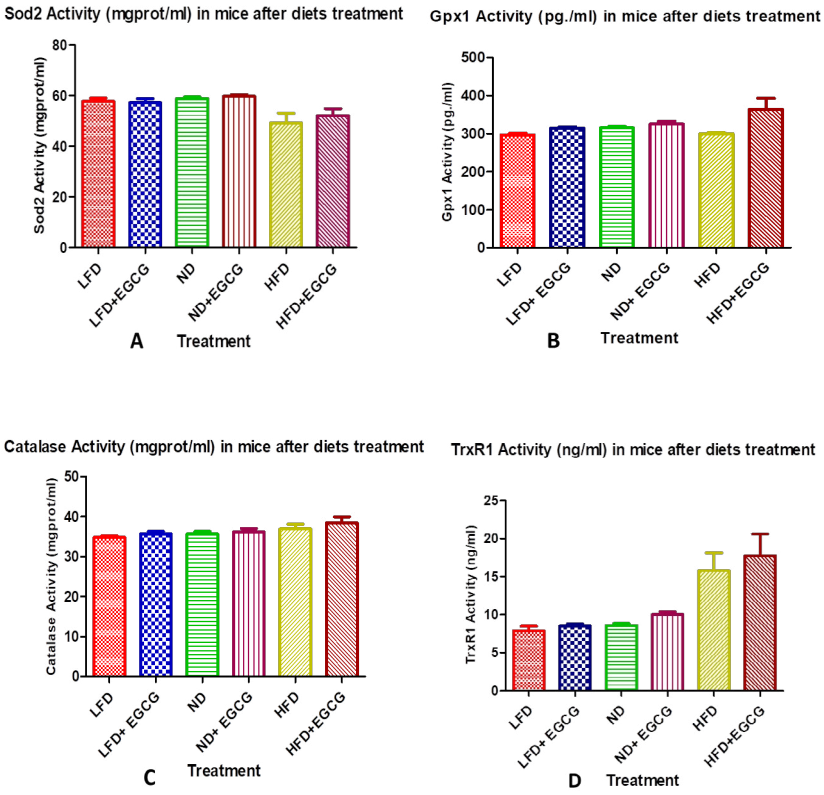


Figure S1: Averages of the Effect of EGCG and Diet on Enzyme Activities of Antioxidant Enzymes in Mice.
Means of Sod2 Activity *(mgprot/ml)* in mice after treatment with various diets supplemented with or without EGCG **(A)**,means of Gpx 1 Activity results(pg./ml)in mice after treatment with various diets supplemented with or without EGCG **(B)**, means of Catalase Activity *(mgprot/ml)* in mice after treatment with various diets supplemented with or without EGCG **(C)** andmeans of TrxR1 Activity results(ng/ml) in mice after treatment with various diets supplemented with or without EGCG **(D)**

Effect of EGCG and exercise on Enzyme Activity of Antioxidant Enzymes in Mice

Figure S2 shows the averages of the effect of EGCG and exercise on the enzymatic activities of Sod2, Gpx1, Cat and TrxR1 during the experimental period of 0 to 6 weeks. The results of the activities of antioxidant enzymes in the liver: Superoxide dismutase 2 (Sod2), Glutathione Peroxidase 1 (GPx1), Catalase (Cat) and Thioredoxin Reductase (TrxR1) have shown that there are significant relationships between enzymatic activities and various treatments in mice subjected to exercise before and after EGCG supplementation at a level of 5% significance since the p-value is less than 0.05.

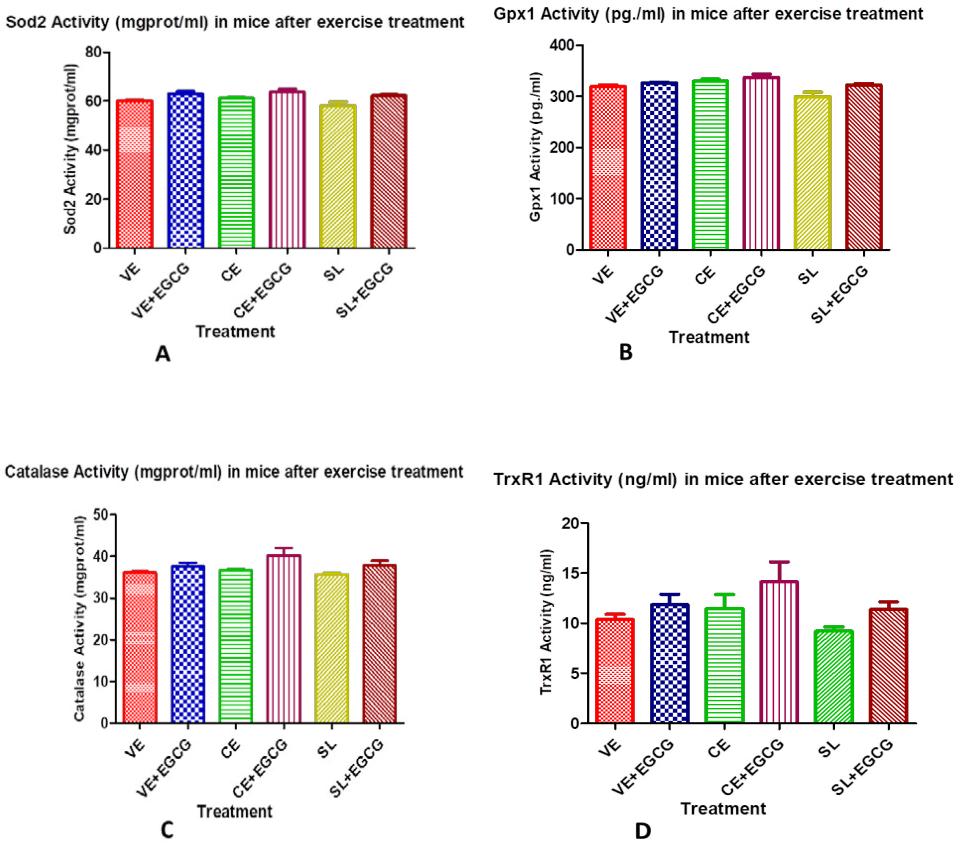


Figure S2: Averages of the Effect of EGCG and exercise on Enzyme Activities of Antioxidant Enzymes in Mice.
Means of Sod2 Activity *(mgprot/ml)* in mice after treatment with various exercise supplemented with or without EGCG **(A)**,means of Gpx 1 Activity results(pg./ml)in mice after treatment with various exercise supplemented with or without EGCG **(B)**, means of Catalase Activity *(mgprot/ml)* in mice after treatment with various exercise supplemented with or without EGCG **(C)** andmeans of TrxR1 Activity results(ng/ml) in mice after treatment with various exercise supplemented with or without EGCG **(D)**

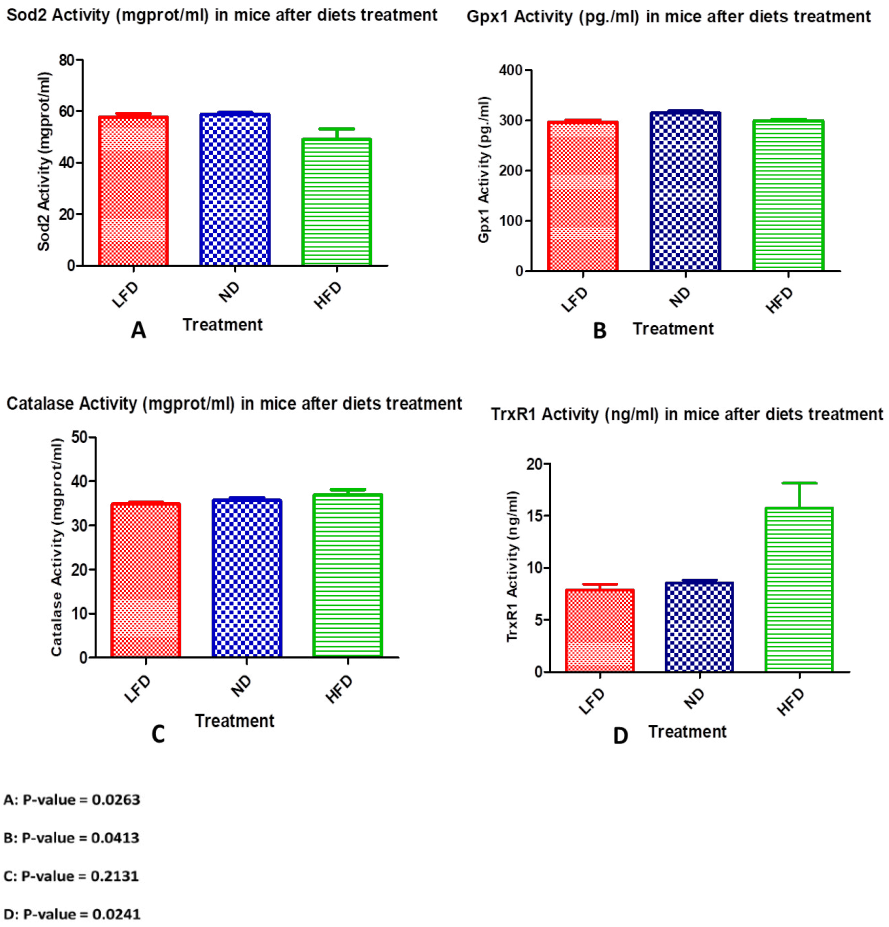


Figure S3:Averages of Enzyme Activities of Antioxidant Enzymes in Mice after diets treatment.
Means of Sod2 Activity *(mgprot/ml)* in mice afterdiets treatment **(A)**,means of Gpx 1 Activity results(pg./ml)in mice diets after treatment **(B)**, means of Catalase Activity *(mgprot/ml)* in mice after diets treatment **(C)** andmeans of TrxR1 Activity results(ng/ml) in mice afterdiets treatment **(D)**.

Effect of exercise on Enzyme Activity of Antioxidant Enzymes in Mice

Figure S4 shows the averages of exercise on the enzymatic activities of Sod2, Gpx1, Cat and TrxR1 during the experimental period of 0 to 6 weeks. When considering the effect of physical exercise on the enzymatic activities of antioxidant enzymes, our results have shown that there are no statistically significant relationships between physical processing and enzyme activity for all antioxidant enzymes exceptGpx1 because their p- value is greater than 0.05.

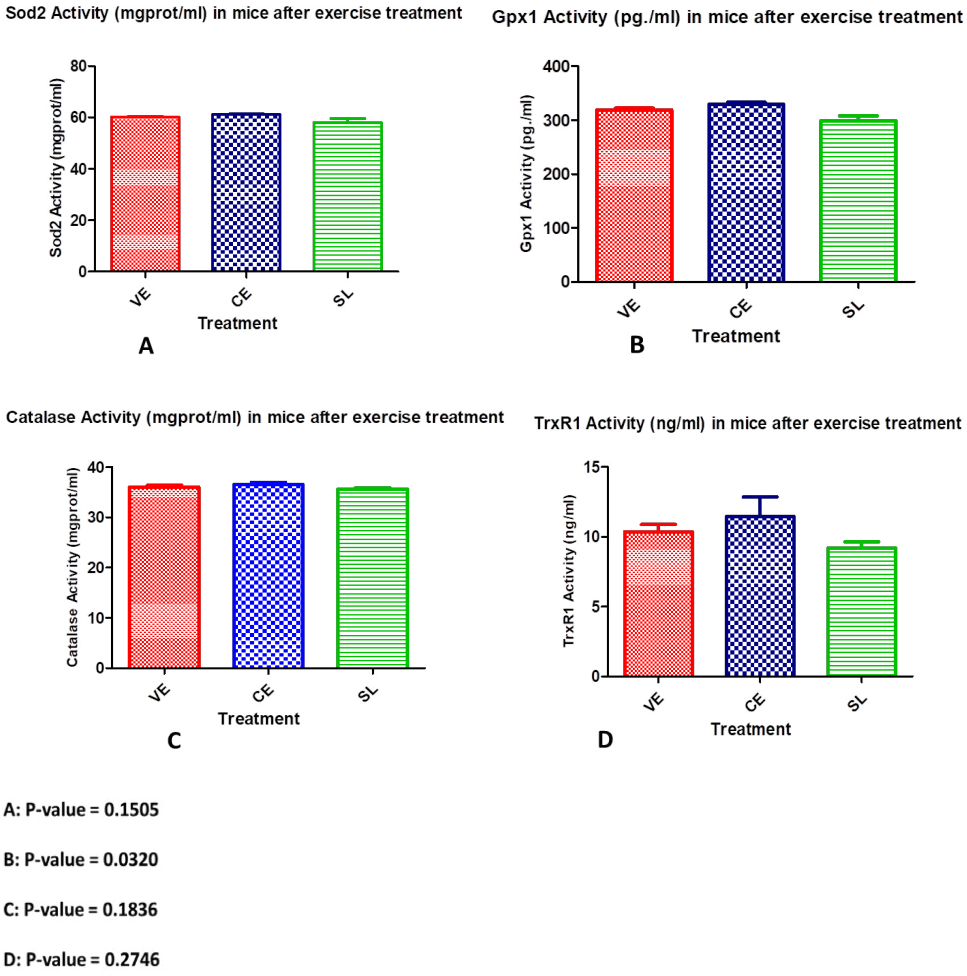


Figure S4: Averages of Enzyme Activities of Antioxidant Enzymes in Mice after exercise treatment.
Means of Sod2 Activity *(mgprot/ml)* in mice afterexercise treatment **(A)**,means of Gpx 1 Activity results(pg./ml)in mice afterexercise-treatment **(B),** means of Catalase Activity (mgprot/ml) in mice after exercise treatment **(C)** andmeans of TrxR1 Activity results(ng/ml) in mice after exercisetreatment **(D)**.

Tables

Feed formulation and pallet preparation

The formulation of the feeds and the preparation of the pallets were carried out at the nutrition laboratory of COVAB, Makerere University. Three types of feeds were prepared: a high fat diet (16% fat), a normal diet (3% fat) and a low-fat diet (0.2% fat) as shown in table S1. To obtain the different food proportions, the following nutrients were mixed: maize bran which is rich in carbohydrate, soy bean-full which is rich in fat and protein, sunflower which is rich in fiber and protein, cotton seed cake which is rich in fat and protein, rice bran that is rich in carbohydrate and fiber, shell that is rich in minerals, fish that is rich in fat and animal protein and cassava that has a very low nutritional value. To obtain the rations/percentages of these three types of food, we weighed an amount for each nutrient, carefully mixed them and then proceeded to extract the fat contained in the mixture. After formulation of the feeds, pellets were made with pallet making machine.

Table S1: Percentage composition of the amount of nutrients used in feed formulation fed to mice

Nutrient name	Quantity of nutrient for a diet containing 16% in fat	Quantity of nutrient for a diet containing 3% in fat	Quantity of nutrient for a diet containing 0.2% in fat
1. Maize Bran	39	80	80
2. Soya Bean-Full	45	4	0
3. Sunflower	5	4	0
4. Cotton Seed Cake	5	2	0
5. Rice Bran	3	2	0
6. Shell	1	4	6
7. Fish	2	4	6
8. Cassava	0	0	8
Total composition	100	100	100

References

1. Dou, Q. P. (2019). Tea in health and disease. Nutrients, 11(4), 929.
2. Yang, C. S., Wang, X., Lu, G., &Picinich, S. C. (2009). Cancer prevention by tea: animal studies, molecular mechanisms and human relevance. Nature Reviews Cancer, 9(6), 429-439.]
3. Fujiki, H., Sueoka, E., Watanabe, T., &Suganuma, M. (2015). Primary cancer prevention by green tea, and tertiary cancer prevention by the combination of green tea catechins and anticancer compounds. Journal of cancer prevention, 20(1), 1.
4. Lee, M. S., Kim, C. T., & Kim, Y. (2009). Green tea (–)-epigallocatechin-3-gallate reduces body weight with regulation of multiple genes expression in adipose tissue of diet-induced obese mice. Annals of Nutrition and Metabolism, 54(2), 151-157.
5. Hsu, Y. W., Tsai, C. F., Chen, W. K., Huang, C. F., & Yen, C. C. (2011). A subacutetoxicityevaluation of green tea (Camellia sinensis) extract in mice. Food and Chemical Toxicology, 49(10), 2624-2630.
6. Dufresne, C., & Farnworth, E. (2000). Tea, Kombucha, and health: a review. Food research international, 33(6), 409-421.
7. Higdon, J. V., & Frei, B. (2003). Tea catechins and polyphenols: health effects, metabolism, and antioxidant functions.
8. Friedman, M. (2007). Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas. Molecular nutrition & food research, 51(1), 116-134.
9. Neužil, J., Thomas, S. R., & Stocker, R. (1997). Requirement for, promotion, or inhibition by α-tocopherol of radical-induced initiation of plasma lipoprotein lipid peroxidation. Free Radical Biology and Medicine, 22(1-2), 57-71.
10. Qin, Y., Wang, H. W., Thirupathi, K., &Wook, K. (2010). Chitosan green teapolyphenolcomplex as a released control compound for woundhealing. Chinese Journal of Traumatology, 13(02), 91-95.
11. Yang, C. S., & Wang, X. (2010). Green tea and cancer prevention. Nutrition and cancer, 62(7), 931-937.
12. Wang, P., Aronson, W. J., Huang, M., Zhang, Y., Lee, R. P., Heber, D., & Henning, S. M. (2010). Green teapolyphenols and metabolites in prostatectomytissue: implications for cancer prevention. Cancer Prevention Research, 3(8), 985-993.
13. Westerterp-Plantenga, M. S. (2010). Green tea catechins, caffeine and body-weight regulation. Physiology & Behavior, 100(1), 42-46.
14. Basu, A., Sanchez, K., Leyva, M. J., Wu, M., Betts, N. M., Aston, C. E., & Lyons, T. J. (2010). Green teasupplementation affects body weight, lipids, and lipidperoxidation in obese subjectswithmetabolic syndrome. Journal of the American College of Nutrition, 29(1), 31-40.
15. Powers, J. S., L Jackson Roberts, I. I., Tarvin, E., Hongu, N., Choi, L., &Buchowski, M. (2008). Oxidative stress and multi-organfailure in hospitalizedelderly people. Journal of the American Geriatrics Society, 56(6), 1150.
16. Łuczaj, W., &Skrzydlewska, E. (2004). Antioxidant properties of black tea in alcohol intoxication. Food and chemical toxicology, 42(12), 2045-2051.
17. Rodrigo, R., González, J., &Paoletto, F. (2011). The role of oxidative stress in the pathophysiology of hypertension. Hypertension Research, 34(4), 431-440.
18. Powers, J. S., L Jackson Roberts, I. I., Tarvin, E., Hongu, N., Choi, L., &Buchowski, M. (2008). Oxidative stress and multi-organfailure in hospitalizedelderly people. Journal of the American Geriatrics Society, 56(6), 1150.
19. Alberts J.A. B, Lewis J, et al. (2002). Molecular Biology of the Cell "Peroxisomes", Garland Science; New York.
20. Shioji, K., Nakamura, H., Masutani, H., &Yodoi, J. (2003). Redox regulation by thioredoxin in cardiovascular diseases. Antioxidants and Redox Signaling, 5(6), 795-802.
21. Clark J. Derrell, Gerald F. Gebhart, Janet C. Gonder, Michale E. Keeling, Dennis F. Kohn. (1997). The 1996 Guide for the Care and Use of Laboratory Animals, 7th ed, 1996. National Academy Press, 125 p, ISBN 0-309-05377-3.
22. Samuelsson, A. M., Matthews, P. A., Argenton, M., Christie, M. R., McConnell, J. M., Jansen, E. H., ... & Taylor, P. D. (2008). Diet-induced obesity in female mice leads to offspring hyperphagia, adiposity, hypertension, and insulin resistance: a novel murine model of developmental programming. Hypertension, 51(2), 383-392.
23. Gail C. Golabet al. (2013). AVMA Guidelines for the Euthanasia of Animals: 2013 Edition.
24. ILAR J. (2012). Effects of Isoflurane Anesthesia on the Cardiovascular Function of the C57BL/6 Mouse
25. Grandjean, J., Schroeter, A., Batata, I., & Rudin, M. (2014). Optimization of anesthesia protocol for resting-state fMRI in mice based on differential effects of anesthetics on functional connectivity patterns. Neuroimage, 102, 838-847.

26. Hu, M., Zhao, Y., Cao, Y., Tang, Q., Feng, Z., Ni, J., & Zhou, X. (2020). DRP1 promotes lactate utilization in KRAS-mutant non-small-celllung cancer cells. Cancer science, 111(10), 3588-3599.
27. Fan, X., Han, J., Zhu, L., Chen, Z., Li, J., Gu, Y., ... & Shang, J. (2020). Protective activities of Dendrobium huoshanense CZ Tang et SJ Cheng polysaccharide against high-cholesterol diet-induced atherosclerosis in zebrafish. Oxidative medicine and cellular longevity, 2020.
28. Bilgiç, A., ÇolakSivri, R., &Kılınç, İ. (2018). 8-F2-isoprostane, thioredoxin and thioredoxin reductase levels in children with obsessive–compulsive disorder. Nordic Journal of Psychiatry, 72(7), 484-488.
29. Marcus, A. J., Iezhitsa, I., Agarwal, R., Vassiliev, P., Spasov, A., Zhukovskaya, O., ... & Ismail, N. M. (2019). Intraocular pressure-lowering effects of imidazo [1, 2-a]-and pyrimido [1, 2-a] benzimidazole compounds in rats with dexamethasone-induced ocular hypertension. European journal of pharmacology, 850, 75-87.
30. Mbiantcha, M., Khalid, R., Dawe, A., Mehreen, A., Atsamo, D. A., Ateufack, G., ... & Izhar, A. (2019). Antihypernociceptive and neuroprotective effects of Combretin A and Combretin B on streptozotocin-induced diabetic neuropathy in mice. Naunyn-Schmiedeberg's Archives of Pharmacology, 392(6), 697-713.
31. Panchal, S. K., & Brown, L. (2010). Rodent models for metabolic syndrome research. Journal of Biomedicine and Biotechnology, 2011.
32. Banzubaze, E., Mulindwa, J., Ochwo, S., &Wampande, E. (2022). Epigallocatechin-3-gallate (EGCG) as a Potential Therapeutic Against Cardiovascular Disease Risk in Mice. J Anesth Pain Med, 2022; Volume 7 | Issue 3 |; 79-90.
33. James, A. M., Collins, Y., Logan, A., & Murphy, M. P. (2012). Mitochondrial oxidative stress and the metabolic syndrome. Trends in endocrinology & metabolism, 23(9), 429-434.
34. White, P. J., &Marette, A. (2014). Potential role of omega-3-derived resolution mediators in metabolic inflammation. Immunology and cell biology, 92(4), 324-330.
35. Scorletti, E., & Byrne, C. D. (2013). Omega-3 fatty acids, hepatic lipid metabolism, and nonalcoholic fatty liver disease. Annual review of nutrition, 33, 231-248.
36. Tsuru, H., Osaka, M., Hiraoka, Y., & Yoshida, M. (2020). HFD-induced hepatic lipid accumulation and inflammation are decreased in Factor D deficient mouse. Scientific reports, 10(1), 1-10.
37. Parry, S. A., & Hodson, L. (2017). Influence of dietary macronutrients on liver fat accumulation and metabolism. Journal of Investigative Medicine, 65(8), 1102-1115.
38. Marchesini, G. et al. (2003). Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology (Baltimore, Md.)37, 917–923.
39. Younossi, Z. M (2019). Non-alcoholic fatty liver disease—A global public health perspective. J. Hepatol. 70, 531–544.
40. Byrne, C. D. &Targher, G. NAFLD (2015).A multisystem disease. J. Hepatol. 62, S47-64.
41. Buzzetti, E., Pinzani, M., &Tsochatzis, E. A. (2016). The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism, 65(8), 1038-1048.
42. van der Heide, D., Weiskirchen, R., & Bansal, R. (2019). Therapeutic targeting of hepatic macrophages for the treatment of liver diseases. Frontiers in immunology, 10, 2852.
43. Than, N. N., & Newsome, P. N. (2015). A concise review of non-alcoholic fatty liver disease. Atherosclerosis, 239(1), 192-202.
44. Day, C. P., & James, O. F. (1998). Steatohepatitis: a tale of two "hits"?. Gastroenterology, 114(4), 842-845.
45. Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., &Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. The international journal of biochemistry & cell biology, 39(1), 44-84.
46. Sudhahar, V., Kumar, S. A., & Varalakshmi, P. (2006). Role of lupeol and lupeol linoleate on lipemic–oxidative stress in experimental hypercholesterolemia. Life sciences, 78(12), 1329-1335.
47. Dobrzyńska, I., Szachowicz-Petelska, B., Ostrowska, J., Skrzydlewska, E., &Figaszewski, Z. (2005). Protective effect of green tea on erythrocyte membrane of different age rats intoxicated with ethanol. Chemico-biological interactions, 156(1), 41-53.
48. Tsai, C. F., Hsu, Y. W., Ting, H. C., Huang, C. F., & Yen, C. C. (2013). The in vivo antioxidant and antifibrotic properties of green tea (Camellia sinensis, Theaceae). Food chemistry, 136(3-4), 1337-1344.
49. Wang, W. F., Luo, J., Yao, S. D., Lian, Z. R., Zhang, J. S., Lin, N. Y., ... & Hu, T. X. (1993). Interaction of phenolic antioxidants and hydroxyl radicals. Radiation Physics and Chemistry, 42(4-6), 985-987.
50. Niu, H., Jia, Y., Hu, P., Meng, Z., & Lei, J. (2014). Effect of dietaryvitamin E on the growth performance and nonspecificimmunity in sub-adult turbot (Scophthalmusmaximus). Fish &shellfishimmunology, 41(2), 501-506.
51. Ramesh, E., Elanchezhian, R., Sakthivel, M., Jayakumar, T., Senthil Kumar, R. S., Geraldine, P., & Thomas, P. A. (2008). Epigallocatechin gallate improves serum lipid profile and erythrocyte and cardiac tissue antioxidant parameters in Wistar rats fed an atherogenic diet. Fundamental & clinical pharmacology, 22(3), 275-284.
52. Khan, S. A., Priyamvada, S., Arivarasu, N. A., Khan, S., &Yusufi, A. N. K. (2007). Influence of green tea on enzymes of carbohydrate metabolism, antioxidant defense, and plasma membrane in rat tissues. Nutrition, 23(9), 687-695.
53. Hirano, R., Sasamoto, W., Matsumoto, A., Itakura, H., Igarashi, O., & Kondo, K. (2001). Antioxidant ability of various flavonoids against DPPH radicals and LDL oxidation. Journal of nutritional science and vitaminology, 47(5), 357-362.
54. Henning, S. M., Niu, Y., Lee, N. H., Thames, G. D., Minutti, R. R., Wang, H., ... & Heber, D. (2004). Bioavailability and antioxidant activity of tea flavanols after consumption of green tea, black tea, or a green tea extract supplement. The American journal of clinical nutrition, 80(6), 1558-1564.