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Original Article

Effect of epigallocatechin gallate on ischemia-reperfusion injury: an experimental study in a rat epigastric island flap

Cem Aslan[1], Cenk Melikoglu[2], Irfan Ocal[3], Gulcan Saglam[4], Recep Sutcu[4], Mubin Hosnuter[1]

[1]Department of Plastic and Reconstructive Surgery, University of Katip Celebi, Ataturk Training and Research Hospital, Izmir, Turkey; [2]Department of Plastic and Reconstructive Surgery, University of Sifa, Izmir, Turkey; [3]Department of Pathology, University of Katip Celebi, Ataturk Training and Research Hospital, Izmir, Turkey; [4]Department of Biochemistry, University of Katip Celebi, Ataturk Training and Research Hospital, Izmir, Turkey

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Abstract: Epigallocatechin gallate (EGCG), a polyphenol derived from green tea, is known to have potent antioxidant and anti-inflammatory properties. The aim of this study was to investigate the protective effects of EGCG against ischemia reperfusion injury in the epigastric artery island flap model in rats. The experiment was designed with two groups (control n=40, experiment n=40) of rats with epigastric artery island flaps. Each main group was randomly divided into five sub-groups to apply ischemia at different time intervals (0, 3, 6, 9 and 12 hours). Thirty minutes prior to reperfusion, 100 μmol/kg of EGCG was injected intraperitoneally, and this injection was repeated after 12 hours and continued as a daily injection. Similarly, 2 ml of sterile saline was administered to the rats in the control groups. Superoxide dismutase, glutathione peroxidase, malondialdehyde and tumor necrosis factor alpha levels, together with neutrophil counts, were measured in the tissues taken from the distal portions of the flaps 24 hours after reperfusion. Additionally, flap necrosis was examined on the seventh day after reperfusion. Superoxide dismutase levels were significantly lower in all control groups, and Malondialdehyde and Tumor Necrosis Factor Alpha levels were significantly higher in all control groups. Glutathione peroxidase levels were found to be significantly lower in the control groups after 0, 3, 9 and 12 hours of ischemia. There was no statistically significant difference between the groups undergoing 0, 3, 9 or 12 hours of ischemia with regard to the neutrophil count. Partial flap necrosis occurred in the 9-hour ischemia groups, and significantly lower rates of necrosis were observed in the experimental groups compared to the control groups. The findings of our study showed that EGCG has a protective effect against ischemia-reperfusion injury in skin flaps in the epigastric island flap model.

Keywords: EGCG, green tea, ischemia, reperfusion

Introduction

Ischemia-reperfusion injury can occur after the reperfusion of tissues exposed to prolonged ischemia. Ischemia-reperfusion injury can determine the success or failure of free tissue transfers, composite tissue allotransplantations and replantations. The mechanisms underlying ischemia-reperfusion injury have not been fully elucidated [1]. Possible mechanisms are described as follows:

a) Tissues are exposed to high levels of calcium after the restoration of the blood flow, and thus intracellular calcium levels become much higher. These high levels of calcium activate intracellular enzymes, resulting in detrimental effects on the cellular architecture [2].

b) The restoration of blood flow to the ischemic tissues causes tissue neutrophil accumulation. Neutrophils are responsible for the characteristic tissue damage as a result of their production of reactive oxygen species [1, 2].

c) Adenosine triphosphate breaks down to hypoxanthine as a result of long-lasting ischemia. During reperfusion, xanthine dehydrogenase is converted into xanthine oxidase, which catalyzes the conversion of hypoxanthine (formed from the degradation of ATP during prolonged ischemia) plus oxygen to form xanthine.

Table 1. Average distribution of SOD enzyme levels

Groups	*Control*		*Experimental*		*Total*		*p**
	Avr.±SD	Median	Avr.±SD	Median	Avr.±SD	Median	
0.h	0.05±0	0.05	0.06±0	0.06	0.06±0.01	0.06	0.001
3.h	0.04±0	0.05	0.05±0	0.05	0.05±0.01	0.05	0.001
6.h	0.03±0	0.03	0.04±0	0.04	0.03±0.01	0.03	0.002
9.h	0.02±0	0.02	0.03±0	0.03	0.03±0.01	0.03	0.001
12.h	0.02±0	0.02	0.03±0	0.03	0.02±0.01	0.02	0.001

*Mann Whitney U analysis.

Table 2. Bilateral comparisons of the SOD enzyme levels

	p*	
	Control Groups	Experimental Groups
0.h - 3.h	0.031	0.012
0.h - 6.h	0.011	0.011
0.h - 9.h	0.011	0.011
0.h - 12.h	0.012	0.012
3.h - 6.h	0.011	0.011
3.h - 9.h	0.011	0.011
3.h - 12.h	0.012	0.011
6.h - 9.h	0.034	0.011
6.h - 12.h	0.012	0.011
9.h - 12.h	0.017	0.011

*Wilcoxon signed ranks analysis.

The superoxide anion is a byproduct of this reaction [1-3].

As a result of the above-mentioned mechanisms, the quantity of reactive oxygen species often increases dramatically in ischemic cells during reperfusion. Reactive oxygen species have detrimental effects on cell membranes, especially mitochondrial membranes. Under ischemic conditions (high calcium and high pH) in combination with the presence of reactive oxygen species, a nonspecific mitochondrial permeability transition pore opens in the inner mitochondrial membrane. The opening of this transition pore leads to the depolarization of the membrane potential and to outer mitochondrial membrane rupture. The depolarization of the membrane potential causes adenosine triphosphate depletion, finally resulting in cell necrosis. In addition, proapoptotic agents, such as cytochrome c, are released into the cytosol after mitochondrial membrane rupture, where they activate a program leading to cell apoptosis [2, 3].

Previous studies have shown the protective effects of antioxidant and anti-inflammatory agents in ischemia-reperfusion injury. The therapeutic application of tempol, coenzyme Q10, caffeic acid phenyl ester, extracellular superoxide dismutase, kappa-opioid receptor agonist, fucoidin and vitamin C has been shown to have such activities [4-10]. Most of the studies on ischemia-reperfusion injury have been focused on antioxidant or anti-inflammatory activity. However, Epigallocatechin Gallate (EGCG) shows both high antioxidant [11, 12] and high anti-inflammatory activities [13-15].

EGCG is the most abundant catechin in green tea and also has the highest biological activity of the catechins in green tea [16]. The ROS-scavenging and antioxidant effects of EGCG in reperfusion injury have been reported in previous studies. Büttemeyer et al. reported in a study of lower extremity ischemia-reperfusion in rats that superoxide radical formation was less than 50% in the EGCG-treated group versus the control group [17]. Brückner at al. investigated the therapeutic antioxidant effect of EGCG in a murine model of colitis. They reported that EGCG improved the clinical course and increased overall survival in comparison to untreated groups. Additionally, they reported that EGCG enhanced the expression of SOD and GPX and reduced the production of proinflammatory cytokines [18]. Kakuta at al. researched the protective effects of EGCG in rats with renal ischemia-reperfusion injury. They reported that EGCG protects kidneys from reperfusion injury by augmenting the HO-1 (heme oxygenase-1) gene and blocking macrophages [19].

Townsend et al. investigated the protective effects of EGCG in an experimental study in the myocardial cell culture. They reported that EGCG protects cells from reperfusion injury via the inhibition of STAT-1 activation [20]. Piao et al. investigated the effects of EGCG on ischemia-reperfusion injury in isolated rat hearts. They showed that the coronary flow was better and the end-diastolic pressure was lower in experimental groups. They also showed that EGCG protects cells from apoptosis by increas-

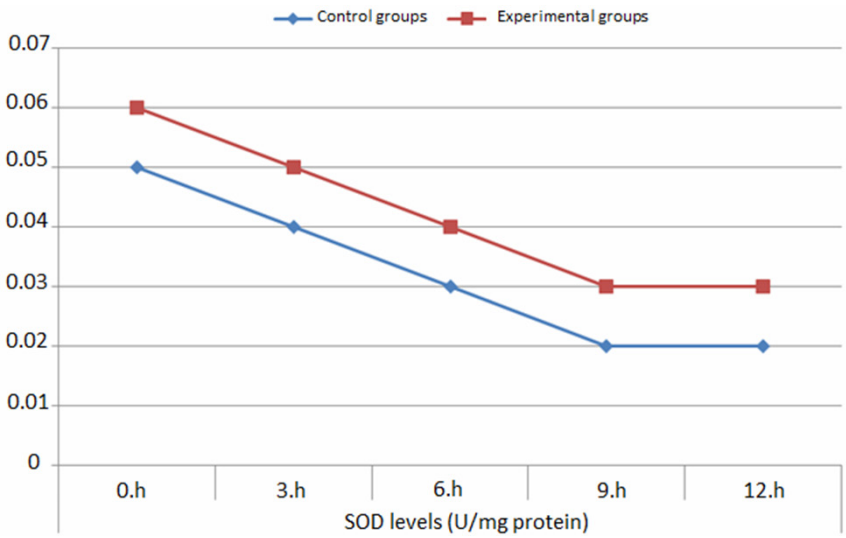

Figure 1. The time dependent changes of the SOD enzyme levels.

Table 3. Average distribution of GPX enzyme levels

Groups	*Control*		*Experimental*		*Total*		*p**
	Avr.±SD	Median	Avr.±SD	Median	Avr.±SD	Median	
0.h	8.85±0.09	8.85	11.31±0.06	11.31	10.08±1.27	10.08	0.001
3.h	8.85±0.09	8.85	9.64±0.07	9.64	9.25±0.41	9.24	0.001
6.h	8.15±0.14	8.15	8.15±0.09	8.15	8.15±0.12	8.15	0,959
9.h	4.88±0.12	4.87	5.37±0.1	5.37	5.12±0.28	5.18	0.001
12.h	2.18±0.07	2.18	4.88±0.03	4.88	3.53±1.4	3.56	0.001

*Mann Whitney U analysis.

Table 4. Bilateral comparisons of the GPX enzyme levels

	p*	
	Control Groups	Experimental Groups
0.h - 3.h	0.414	0.012
0.h - 6.h	0.012	0.011
0.h - 9.h	0.012	0.012
0.h - 12.h	0.012	0.011
3.h - 6.h	0.012	0.012
3.h - 9.h	0.012	0.008
3.h - 12.h	0.012	0.012
6.h - 9.h	0.011	0.012
6.h - 12.h	0.011	0.011
9.h - 12.h	0.011	0.012

*Wilcoxon signed ranks test.

ing the Bcl-2/Bax ratio in experimental groups [21].

In this study, we examined the possible protective effects of EGCG treatment in ischemia-reperfusion injury in the rat epigastric artery island flap model. To determine the efficacy of EGCG, tissue superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities, as well as malondialdehyde (MDA) and tumor necrosis factor-α (TNF-α) levels, neutrophil counts and tissue necrosis areas, were measured after the reperfusion period.

MDA is a stable end product of lipid peroxidation that is generated after free radical damage [22]. SOD catalyzes the transformation of superoxide radicals to hydrogen peroxide and oxygen. Thus, it is an important antioxidant enzyme that protects cells from superoxide radical damage [23]. GPX is the common name of an enzyme family that protects cells from free radicals by catalyzing the transformation of hydrogen peroxide to water [24]. TNF-α is a polypeptide compound and an important member of the cytokine family. TNF-α is synthesized primarily by activated macrophages during inflammation and has many systemic effects, such as the aggregation and activation of neutrophils, nitric oxide synthesis, fibroblast activation and the release of proteolytic enzymes from mesenchymal cells [25].

Materials and methods

Animals

The study protocol was approved by the Ethics Committee on Research Animal Use of Ege University (Turkey). Wistar albino rats of the same age, weighing between 250-300 g, were used for this study. The rats were housed in separate cages under standard conditions, with a 12/12 h light-dark regimen and were fed with standard rat chow and water ad libitum.

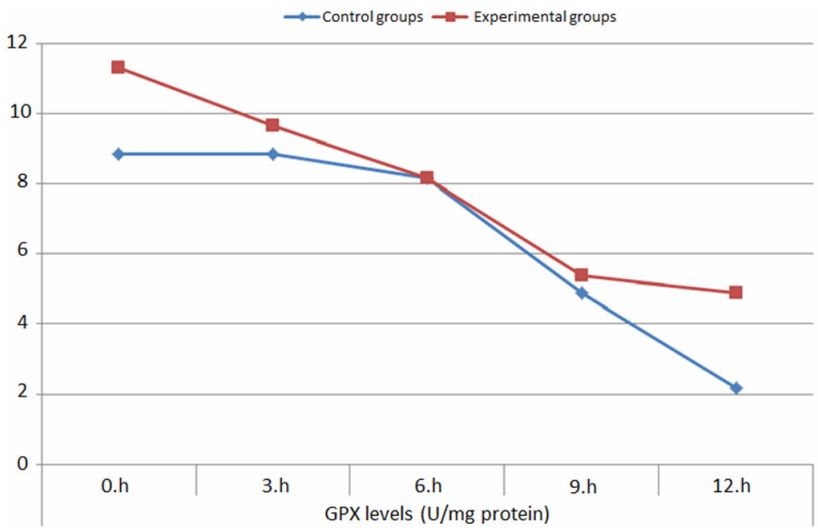

Figure 2. The time dependent changes of the GPX enzyme levels.

Table 5. Average distribution of TNF-α levels

Groups	*Control*		*Experimental*		*Total*		p*
	Avr.±SD	Median	Avr.±SD	Median	Avr.±SD	Median	
0.h	2.81±0.07	2.81	2.29±0.04	2.29	2.55±0.27	2.53	0.001
3.h	3.26±0.1	3.26	2.93±0.06	2.93	3.09±0.19	3.07	0.001
6.h	3.32±0.1	3.32	3.2±0.07	3.2	3.26±0.1	3.2	0.049
9.h	4.15±0.13	4.15	3.26±0.07	3.26	3.7±0.47	3.64	0.001
12.h	4.89±0,08	4.89	4.65±0.03	4.65	4.77±0.14	4.74	0.001

*Mann Whitney U analysis.

Table 6. Bilateral comparisons of the TNF-α levels

	p*	
	Control Groups	Experimental Groups
0.h - 3.h	0.008	0.011
0.h - 6.h	0.012	0.012
0.h - 9.h	0.011	0.011
0.h - 12.h	0.010	0.011
3.h - 6.h	0.049	0.011
3.h - 9.h	0.011	0.008
3.h - 12.h	0.011	0.012
6.h - 9.h	0.011	0.123
6.h - 12.h	0.011	0.011
9.h - 12.h	0.011	0.012

*Wilcoxon signed ranks test.

Anesthesia was achieved by a combination of ketamine and xylazine. The skin of the abdomen was depilated using an animal depilatory agent. The skin of the abdomen was scrubbed with betadine following the removal of the fur.

Tested drug

EGCG (DSM Nutrients Inc./Istanbul/TURKEY) was administered intraperitoneally to rats in doses of 100 μmol/kg. (A maximum of 5 mg of the dry powdered form of EGCG was dissolved in 1 ml sterile saline, based on the information obtained from the manufacturer).

Flap design

The epigastric artery flap in rats includes the area within the boundaries of the xiphoid as an upper limit, the pubis as a lower limit and both axillary lines as a lateral limits. Smaller flaps may be used within these anatomical landmarks. The vascular supply of the flap is provided by the medial branches of the superficial epigastric artery and accompanying veins [26].

In this study, 3*6 cm unilateral flaps were prepared by the same surgeon within the boundaries of the anatomical landmarks. A microvascular clamp was placed on the pedicle after flap elevation. The flaps were resutured to the donor site after clamp application. The animals were reanesthetized, and the microclamps were removed after a period of ischemia. The pedicles were examined to evaluate flap reperfusion after removing the micro clamp. Flaps that had any flow did not detected in the pedicle were not included in this study.

Experimental protocol

Eighty rats were used in this study; 40 were randomly selected for the experimental group, and 40 were randomly selected for the control group. Each group was randomly divided into five subgroups to apply ischemia at different time intervals (0, 3, 6, 9 and 12 hours). In the experimental groups, 100 μmol/kg of EGCG were injected intraperitoneally 30 minutes prior

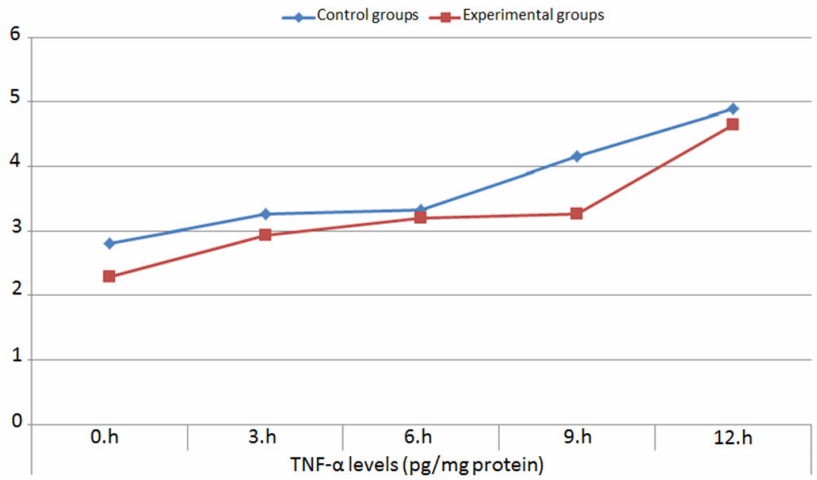

Figure 3. The time dependent changes of the TNF-α levels.

Table 7. Average distribution of the MDA levels

Groups	*Control*		*Experimental*		*Total*		*P**
	Avr.±SD	Median	Avr.±SD	Median	Avr.±SD	Median	
0.h	0.04±0.00	0.04	0.02±0.01	0.02	0.03±0.01	0.03	0.001
3.h	0.06±0.01	0.06	0.03±0.01	0.02	0.04±0.02	0.05	0.001
6.h	0.09±0.01	0.09	0.06±0.01	0.06	0.07±0.02	0.08	0.001
9.h	0.23±0.01	0.24	0.13±0.01	0.13	0.18±0.06	0.18	0.001
12.h	0.28±0.01	0.28	0.21±0.01	0.21	0.24±0.03	0.24	0.001

*Mann Whitney U analysis.

Table 8. Bilateral comparisons of the MDA levels

	p*	
	Control Groups	Experimental Groups
0.h - 3.h	0.010	0.180
0.h - 6.h	0.010	0.010
0.h - 9.h	0.011	0.011
0.h - 12.h	0.011	0.011
3.h - 6.h	0.011	0.011
3.h - 9.h	0.011	0.010
3.h - 12.h	0.011	0.011
6.h - 9.h	0.011	0.011
6.h - 12.h	0.011	0.011
9.h - 12.h	0.011	0.011

*Wilcoxon signed ranks test.

to reperfusion. The same dose was repeated 12 hours after reperfusion and continued four days as a daily injection; additionally, 2 ml of sterile saline were injected to the control groups in the same way. The flap drawings were transferred to acetate on the 7. day without injection.

Biochemical analysis

First, 3 × 1 cm skin biopsies were taken from the most distal end of the flaps 24 hours after reperfusion for biochemical examination. Then, biopsies were immediately stored at -80°C within individual containers. The wounds were sutured immediately after the skin biopsies. MDA and TNF-α levels, as well as SOD and GPX activity, was measured from the skin biopsies.

The MDA levels were detected using the thiobarbituric acid reactivity method of Draper and Hadley [27]. MDA is the stable end product of lipid peroxidation. MDA reacts with thiobarbituric acid and forms a colored complex that gives a maximum absorbance at 532 nm. MDA levels were first detected in nanomoles per ml using the absorption coefficient of the MDA-TBA complex at 532 nm ($1.56 \times 105\ cm^{-1}\ M^{-1}$). The results were obtained in the form of nanomoles/(mg-protein) by dividing the previously obtained values by the homogenate protein.

TNF-α levels were studied by using the enzyme-binding immunosorbent assay (ELISA) method in a semi-automatic device. (Assay Rat TNF-α ELISA kit, lot #: 978614A, Invitrogen, USA).

SOD activity was measured using the method of Woolliams et al [28]. The enzyme concentrations were determined by the use of a standard % inhibitory-concentration chart that had been prepared previously. Enzyme activity was first detected in U/ml. Then, the results were obtained in the form of U/mg-protein by multiplying the values with a dilution coefficient and then dividing by the protein level.

GPX activity was measured using the method of Paglia and Valentine [29]. Glutathione peroxi-

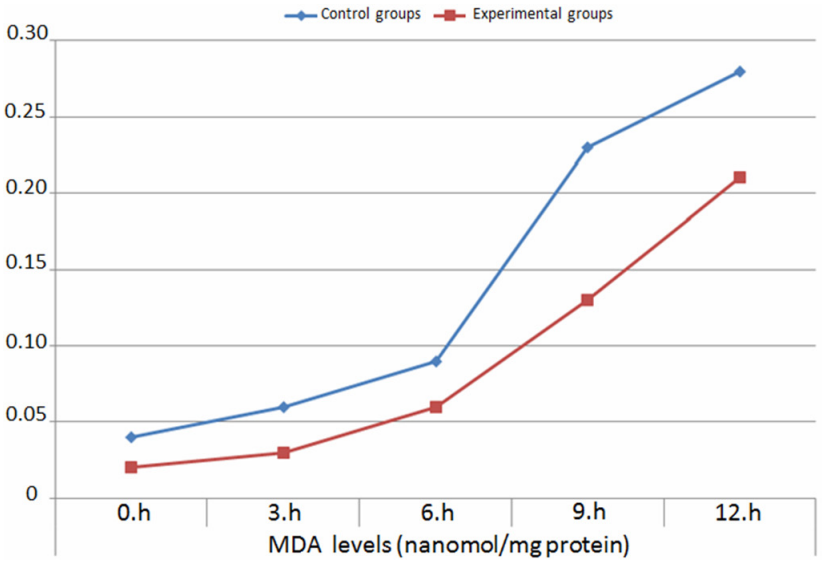

Figure 4. The time dependent changes of the MDA levels.

dase catalyzes the oxidation of glutathione by cumene hydroperoxide. Oxidized glutathione is reduced by glutathione reductase in the presence of NADPH. Meanwhile, NADPH is oxidized to NADP+. The enzyme activity was calculated by measuring the absorbance difference depending on the decrease of NADPH at 340 nm. The amount of homogenate protein was determined by the Lowry method, and the results were obtained in the form of U/mg-protein currency.

Histopathological analysis

A histopathological study of the samples was carried out by the same pathologist through random selection, without knowing which group was included in the tissue sample. Tissue samples were taken from the central portion of the remaining flap tissue after the excision of the distal portion of the flap for biochemical analysis 24 hours after reperfusion. The tissue samples were fixed with formalin, and three-micron-thick sections were taken for histopathological analysis. Neutrophils were counted in the most intense area of 1 mm^2 under a light microscope with hematoxylin and eosin stain.

Assessment of skin flap survival

The surviving portions of the flaps were determined on seventh day. The flap drawings were transferred to acetate for the measurement of surface area. Acetates were placed on standard A4 paper and then scanned and transferred to the computer. Drawings that transferred to the computer were opened as an image in Photoshop CS 5.1 Extended Edition Trial Version. Viable and necrotic portions of the flaps were chosen separately with a magic wand tool. After selection with the magic wand tool, the histogram window was opened, and the pixel numbers were recorded in the expanded mode of the histogram window. The pixel numbers of the 1 cm^2 area that scanned simultaneously with the flap drawings were determined by the method described above. Each of the pixel counts was calculated as cm^2 by dividing the number of pixels in the flaps by that in the 1 cm^2 area. Then, the flap necrosis ratio was given as a proportion of the necrotic areas to the living areas.

Statistical analysis

Statistical analysis was performed using the SPSS 15.0 for Windows package program with a 95% confidence interval. The variables between the two groups were compared using the Mann-Whitney U analysis. Wilcoxon signed-rank analysis was used to evaluate the time-dependent measurements of the MDA, SOD, GPX, TNF-α level and the neutrophil counts on a bilateral basis. Repeated measures ANOVA were used to evaluate the time-dependent changes of the variables at the 0, 3, 6, 9 and 12 hours. $p<0.05$ was considered statistically significant.

Results

Biochemical results

It was observed that SOD enzyme activities were significantly lower in control groups at all ischemic periods ($p<0.05$). All bilateral comparisons of the SOD activity was significantly different between control and experimental groups ($p<0.05$). Detailed statistical evaluations of the SOD activity can be seen in **Tables 1** and **2** and **Figure 1**.

Table 9. Average distribution of flap necrosis area in the 9-hour ischemic subgroups

Groups	*Control*		*Experimental*		*Total*		*P**
	Avr.±SD	Median	Avr.±SD	Median	Avr.±SD	Median	
flap necrosis area	36.29±14.14%	34.68%	22.91±11.54%	20.675%	29.6±14.25%	27.3%	0.049

*Mann Whitney U analysis.

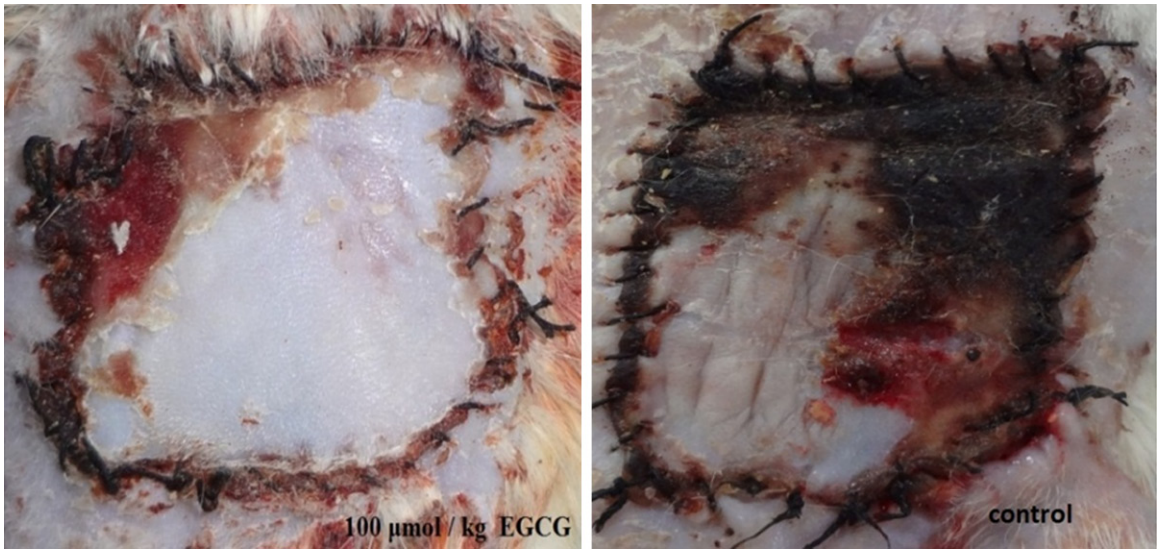

Figure 5. View of the 9-hour ischemic group flaps on day 7.

GPX enzyme activities were significantly lower in control groups at the ischemic periods of 0, 3, 9 and 12 hours ($p<0.05$). Bilateral comparison of the GPX activity was not significantly different between the 0- and 3-hour ischemic periods in the control group ($p>0.05$). All other bilateral comparisons of the GPX activity were significantly different between the control and experimental groups ($p<0.05$). Detailed statistical evaluation of the GPX activity can be seen in **Tables 3** and **4** and **Figure 2**.

Significantly higher TNF-α levels were observed in control groups at the all ischemic periods ($p<0.05$). A bilateral comparison of the TNF-α levels was not significantly different between the 6- and 9-hour ischemic period in the experimental group ($p>0.05$). All other bilateral comparisons of the TNF-α levels were significantly different between the control and experimental groups ($p<0.05$). Detailed statistical evaluation of the TNF-α levels can be seen in **Tables 5** and **6** and **Figure 3**.

MDA levels were significantly lower in the experimental groups at all ischemic periods ($p<0.05$), while the bilateral comparison of the MDA levels was not significantly different between the 0- and 3-hour ischemic periods in the experimental group ($p>0.05$). All other bilateral comparisons of the MDA levels were significantly different between the control and experimental groups ($p<0.05$). A detailed statistical evaluation of the MDA levels can be seen in **Tables 7** and **8** and **Figure 4**.

Skin flap survival

Overall, 3 animals from the ischemic groups were excluded over the 12-hour period because of an inadequate reperfusion of the flaps after clamp removal. These animals were replaced with additional study animals. Flaps that were subjected to 0, 3, or 6 hours of ischemia did not show any necrosis at the end of seventh day. Total necrosis was observed in all flaps subjected to the 12-hour ischemia at the end of the seventh day. Partial necrosis was observed only in the 9-hour ischemia applied flaps, and the necrotic percentages of the flaps in the control groups were significantly higher than the necrosis percentages of the flaps in experimental groups ($p<0.05$) (**Table 9**, **Figure 5**).

Neutrophil counts

There was no significant difference between the control and experimental groups in neutro-

Table 10. Average distribution of the neutrophil counts

Groups	*Control*		*Experimental*		*Total*		*P**
	Avr.±SD	Median	Avr.±SD	Median	Avr.±SD	Median	
0.h	3.25±1.67	3	2.5±1.2	2.5	2.88±1.45	3	0.442
3.h	6.75±3.01	6.5	6.13±2.59	5.5	6.44±2.73	6	0.645
6.h	14.38±2.56	14	11.63±2.07	11.5	13±2.66	13	0.049
9.h	19.63±3.42	18.5	17.88±3.14	17	18.75±3.3	18	0.382
12.h	22.25±2.92	22	20.75±2.82	21	21.5±2.88	22	0.328

*Mann Whitney U Analysis.

Table 11. Bilateral comparisons of the neutrophil counts

	p*	
	Control Groups	Experimental Groups
0.h - 3.h	0.043	0.002
0.h - 6.h	0.000	0.000
0.h - 9.h	0.000	0.000
0.h - 12.h	0.000	0.000
3.h - 6.h	0,001	0.001
3.h - 9.h	0. 000	0.000
3.h - 12.h	0.000	0.000
6.h - 9.h	0.018	0.002
6.h - 12.h	0.001	0.000
9.h - 12.h	0.199	0.046

*Wilcoxon signed ranks test.

phil counts at the 0, 3, 9 and 12 hour ischemic periods ($p>0.05$). The neutrophil counts were significantly higher in the control group than in the experimental group, but only at the 6-hour ischemic period ($p<0.05$). The five time groups were statistically significantly different from each other with regard to the neutrophil count (F: 247.882, p: 0.001, $p<0.05$). Bilateral comparisons of the neutrophil counts were not significantly different between the 9 and 12 hour periods of ischemia in the control group, according to time-dependent measurements ($p>0.05$). All other bilateral comparisons of the neutrophil counts were significantly different between the control and experimental groups ($p<0.05$) (**Tables 10** and **11**, **Figure 6**).

Discussion

In this study, the effects of EGCG on reperfusion injury were investigated in rat epigastric artery flaps in three different ways. These approaches included a biochemical analysis of the distal portion of the flap, neutrophil counts in a specific part of the distal portion of the flap and an assessment of flap necrosis.

Tissue SOD, GPX, MDA and TNF-α levels were evaluated for biochemical analysis. The detailed statistical analysis of the SOD levels showed that SOD activity was significantly lower in the control groups ($p<0.05$). The GPX activity was significantly lower in the control groups compared to the experimental groups ($p<0.05$), except in the 6-hour ischemic period. High levels of SOD and GPX activity may protect the cells from the harmful effects of the free radicals in the EGCG-treated experimental groups. Significantly lower MDA levels in the experimental groups also supported an antioxidant effect.

TNF-α levels and neutrophil counts were evaluated for the possible protective anti-inflammatory effects of EGCG on reperfusion injury. The TNF-α levels were significantly higher in the control groups at all of the ischemic periods ($p<0.05$). Lower TNF-α levels may cause protective effects against reperfusion injury by reducing inflammation. Tissue neutrophil counts were also evaluated in this study; however, there were no significant differences between the control and experimental groups with regard to neutrophil counts at the 0-, 3-, 9- and 12-hour ischemic periods. ($p>0.05$) The possible reason for this lack of a significant difference in the number of neutrophils may be related to the time of the sampling. Tissue samples were taken 24 hours after the reperfusion, which is the peak time for neutrophil infiltration. Had the samples been taken in the early period of reperfusion, for example, 12 hours after reperfusion, the neutrophil counts may have been significantly different between the experimental and control groups. In addition, even though the anti-inflammatory effects of EGCG (13-15) have been reported in previous studies, EGCG may have been ineffective in preventing the migration of neutrophils in reperfusion injury to the skin flaps or neutrophil function may have been affected, instead of the neutrophil migration that occurred. Tissue myeloperoxidase enzyme activity determination would be useful in a similar study to show the quantitative evaluation of the neutrophil infiltration [30].

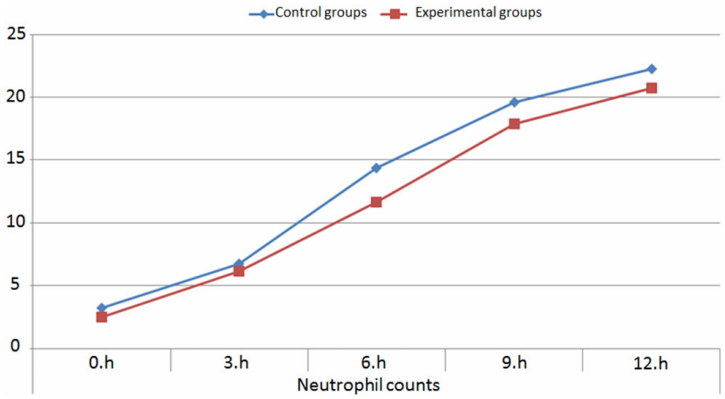

Figure 6. The time-dependent changes of the neutrophil counts.

Flap necrosis was also evaluated to determine the effect of the EGCG on reperfusion injury. Partial necrosis was observed only in flaps that were subjected to the 9-hour ischemia. The necrotic percentages of the flaps in the control groups were significantly higher than those in the experimental groups of the 9-hour ischemic subgroup ($p<0.05$). No flap survival was observed in the flaps of the 12-hour ischemic subgroups. These findings are in agreement with data found in the literature. Klein et al. performed a similar ischemia-reperfusion study with superoxide dismutase transgenic mice. They reported that total necrosis was observed in all of the flaps subjected to a 12-hour ischemia [31]. Similarly, Tosa et al. investigated the protective effects of monoclonal antibodies against the intercellular adhesion molecule-1 in reperfusion injury. They reported that flap survival was only 0.3% (± 1.0) in the experimental group subjected to the 12-hour ischemia [32].

Conclusion

While many studies on the prevention of reperfusion injury are ongoing, a suitable pharmaceutical agent still has not been developed. Due to this situation, the possible protective effects of EGCG against ischemia-reperfusion injury were examined in our study. When tissue MDA, SOD, GPX, and TNF-α levels, together with flap survival, were compared in the 9-hour ischemic control and experimental subgroups, EGCG treatment was found to have a beneficial effect on reperfusion injury.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Cem Aslan or Mubin Hosnuter, Department of Plastic and Reconstructive Surgery, Katip Celebi University School of Medicine, Ataturk Training and Research Hospital, Izmir, Turkey. Tel: +905327126769; E-mail: cemsln@yahoo.com (CA); hosnuter@yahoo.com (MH)

References

[1] Vedder NB. Flap physiology. In: Mathes SJ, Hentz VR, eds. Mathes Plastic Surgery. 2nd edition. Philadelphia: Saunders Elsevier; 2006. pp: 483-506.

[2] Mitchell RN, Cotran RS. Cell injury, cell death and adaptations. In: Kumar V, Cotran RS, Robbins SL, eds. Robbins Basic Pathology. 7th edition. Philadelphia: Saunders Elsevier; 2003. pp: 3-33.

[3] Wang WZ, Baynosa RC and Zamboni WA. Update on Ischemia-Reperfusion Injury for the Plastic Surgeon: 2011. Plast Reconstr Surg 2011; 128: 685-692.

[4] Tran TP, Tu H, Pipinos II, Muelleman RL, Albadawi H and Li YL. Tourniquet-induced acute ischemia-reperfusion injury in mouse skeletal muscles: Involvement of superoxide. Eur J Pharmacol 2011; 650: 328-334.

[5] Bolcal C, Yildirim V, Doganci S, Sargin M, Aydin A, Eken A, Ozal E, Kuralay E, Demirkilic U and Tatar H. Protective effects of antioxidant medications on limb ischemia reperfusion injury. J Surg Res 2007; 139: 274-279.

[6] Ozyurt H, Ozyurt B, Koca K and Ozgocmen S. Caffeic acid phenethyl ester (CAPE) protects rat skeletal muscle against ischemia-reperfusion-induced oxidative stress. Vascul Pharmacol 2007; 47: 108-112.

[7] Park JW, Qi WN, Cai Y, Zelko I, Liu JQ, Chen LE, Urbaniak JR and Folz RJ. Skeletal muscle reperfusion injury is enhanced in extracellular superoxide dismutase knockout mouse. Am J Physiol Heart Circ Physiol 2005; 289: 181-187.

[8] Lin JY, Hung LM, Lai LY and Wei FC. Kappa-opioid receptor agonist protects the microcirculation of skeletal muscle from ischemia reperfusion injury. Ann Plast Surg 2008; 61: 330-6.

[9] Cetin C, Kose AA, Aral E, Colak O, Ercel C, Karabagli Y, Alatas O and Eker A. Protective effect of fucoidin (a neutrophil rolling inhibitor) on ischemia reperfusion injury: experimental study in rat epigastric island flaps. Ann Plast Surg 2001; 47: 540-6.

[10] Ulug BT, Aksungar FB, Mete O, Tekeli F, Mutlu N and Calik B. The effect of vitamin C on ischemia reperfusion injury because of prolonged tourniquet application with reperfusion intervals. Ann Plast Surg 2009; 62: 194-9.
[11] Kim CY, Lee C, Park GH and Jang JH. Neuroprotective effect of epigallocatechin-3-gallate against [beta]-amyloid-induced oxidative and nitrosative cell death via augmentation of antioxidant defense capacity. Arch Pharm Res 2009; 32: 869-881.
[12] Brückner M, Westphal S, Domschke W, Kucharzik T and Lügering A. Green tea polyphenol epigallocatechin-3-gallate shows therapeutic antioxidative effects in a murine model of colitis. J Crohns Colitis 2012; 6: 226-35.
[13] Shin TY, Um JY, Hong SH and Kim HM. Epigallocatechin-3-gallate inhibits secretion of TNF-alpha, IL-6 and IL-8 through the attenuation of ERK and NF-kappaB in HMC-1 cells. Int Arch Allergy Immunol 2007; 142: 335-344.
[14] Katiyar S and Mukhtar H. Green tea polyphenol (2)-epigallocatechin-3-gallate treatment to mouse skin prevents UVB-induced infiltration of leukocytes, depletion of antigen-presenting cells, and oxidative stress. J Leukoc Biol 2001; 69: 719-726.
[15] Joo SY, Song YA, Park YL, Myung E, Chung CY, Park KJ, Cho SB, Lee WS, Kim HS, Rew JS, Kim NS and Joo YE. Epigallocatechin-3-gallate Inhibits LPS Induced NF-κB and MAPK Signaling Pathways in Bone Marrow-Derived Macrophages. Gut Liver 2012; 6: 188-96.
[16] Hu C and Kitts DD. Evaluation of antioxidant activity of epigallocatechin gallate in biphasic model systems in vitro. Mol Cell Biochem 2001; 218: 147-155.
[17] Büttemeyer R, Philipp AW, Schlenzka L, Mall JW, Beissenhirtz M and Lisdat F. Epigallocatechin gallatecan significantly decrease free oxygen radicals in the reperfusion injury in vivo. Transplant Proc 2003; 35: 3116-20.
[18] Brückner M, Westphal S, Domschke W, Kucharzik T and Lügering A. Green tea polyphenol epigallocatechin-3-gallate shows therapeutic antioxidative effects in a murine model of colitis. J Crohns Colitis 2012; 6: 226-35.
[19] Kakuta Y, Okumi M, Isaka Y, Tsutahara K, Abe T, Yazawa K, Ichimaru N, Matsumura K, Hyon SH, Takahara S and Nonomura N. Epigallocatechin-3-gallateprotects kidneys from ischemia reperfusion injury by HO-1 upregulation and inhibition of macrophage. Transpl Int 2011; 24: 514-22.
[20] Townsend PA, Scarabelli TM, Pasini E, Gitti G, Menegazzi M, Suzuki H, Knight RA, Latchman DS and Stephanou A. Epigallocatechin-3-gallate inhibits STAT-1 activation and protects cardiac myocytes from ischemia/reperfusion-induced apoptosis. FASEB J 2004; 18: 1621-1623.
[21] Piao CS, Kim DS, Ha KC, Kim HR, Chae HJ and Chae SW. The Protective Effect of Epigallocatechin-3 Gallate on Ischemia/Reperfusion Injury in Isolated Rat Hearts: An ex vivo Approach. Korean J Physiol Pharmacol 2011; 15: 259-266.
[22] Giakoustidis DE, Giakoustidis AE, Iliadis S, Papageorgiou G, Koliakou K, Kontos N, Taitzoglou I, Botsoglou E, Papanikolaou V, Atmatzidis K, Takoudas D and Antoniadis A. Attenuation of liver ischemia/reperfusion induced apoptosis by epigallocatechin-3-gallate via down-regulation of NF-kappaB and c-Jun expression. J Surg Res 2010; 159: 720-728.
[23] McCord JM and Fridovich I. Superoxide dismutase: the first twenty years. Free Radic Biol Med 1988; 5: 363-9.
[24] Nakagawa Y. Role of mitochondrial phospholipid hydroperoxide glutathione peroxidase (PHGPx) as an antiapoptotic factor. Biol Pharm Bull 2005; 27: 956-960.
[25] Mitchell RN, Cotran RS. Acute and Chronic Inflammation. In: Kumar V, Cotran RS, Robbins SL, eds. Robbins Basic Pathology. 7th edition. Philadelphia: Saunders Elsevier; 2003. pp: 33-61.
[26] Petry JJ and Wortham KA. The anatomy of the epigastric flap in the experimental rat. Plast Reconstr Surg 1984; 74: 410-414.
[27] Drapper HH and Hadley M. Malondialdehiyde determination as index of lipid peroxidation. Methods Enzymol 1990; 186: 421-431.
[28] Woolliams JA, Wiener G, Anderson PH and McMurray CH. Variation in the activities of glutathione peroxidase and superoxide dismutase and in the concentration of copper in the blood various breed crosses of sheep. Res Vet Sci 1983; 34: 69-77.
[29] Paglia DE and Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70: 158-169.
[30] Gurel A, Armutcu F, Hosnuter M, Unalacak M, Kargı E and Altınyazar C. Caffeic Acid Phenethyl Ester Improves Oxidative Organ Damage in Rat Model of Thermal Trauma. Physiol Res 2004; 53: 675-682.
[31] Klein MB, Chan PH and Chang J. Protective effects of superoxide dismutase against ischemia reperfusion injury: development and application of a transgenic animal model. Plast Reconstr Surg 2003; 111: 251-255.
[32] Tosa Y, Lee WP, Kollias N, Randolph MA and May JW Jr. Monoclonal antibody to intercellular adhesion molecule 1 protects skin flaps against ischemia-reperfusion injury: an experimental study in rats. Plast Reconstr Surg 1998; 101: 1586-1594.