



## Effect of electrical stimulation on oxidative stress in tissues in a rat model with incision wound

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Electrical stimulation (ES) are known to have beneficial effects in wound healing. However, the effect of electrical stimulation in wound therapy on the oxidant and antioxidant levels of various tissues in the body remains unclear. Here, we investigated the effects of electrical stimulation on the oxidative stress capacity of tissues in a rat model with a surgical incision wound on the lateral line of the femoral region. Rats divided in two groups: control and ES group. A longitudinal skin incision was made only from the right lateral line of all rats. ES was applied 200  $\mu$ s, 20 Hz, 2mA for 20 min during 15 days. Some oxidative stress parameters (malondialdehyde (MDA), reduced glutathione (rGSH), glucose-6-phosphate dehydrogenase (G6PD), glutathione peroxidase (GPx) and catalase (CAT)) in tissues (brain, heart, kidney, liver, testis, gastrocnemius and quadriceps femoris muscle) were obtained spectrophotometrically. The ES reduced oxidative stress by decreasing MDA and simultaneously increasing different antioxidants in different tissues. The results suggest that incision wound could induce oxidative stress in tissues and electrical stimulation post-incision wound may have ameliorative effect.

**Keywords:** Electrotherapy, Glucose-6-phosphate dehydrogenase, Malondialdehyde, M. gastrocnemius, M. quadriceps femoris

Wounds are still a problem, often possessing severe complications. Healing is a complicated process that consists a series of biochemical and cellular reactions initiated in response to a damage that restores the function and integrity of injured tissues<sup>1</sup>. Healing of a wound consists inflammation, cell proliferation and contraction of collagen lattice formation stages by releasing eicosanoids, leukotrienes, prostaglandins and reactive oxygen species. In addition, reactive oxygen species has an essential role in healing and serve as cellular messengers that drive numerous aspects of molecular and cell biology<sup>2</sup>. In previous studies<sup>3-5</sup> investigating the effects of electrical stimulation (ES) therapy on wound healing, it has been presented that ES therapy possesses beneficial effects on wound healing and also accelerates wound healing. Rouabhia *et al.*<sup>5</sup> presented that ES promotes the proliferation of human keratinocytes, increases the production of keratin 5 and 14, and increases the phosphorylation of extracellular signal-regulated protein kinase and mitogen-activated protein kinases, so that ES can be

useful in supporting skin wound healing by activating keratinocytes. In addition, it has been found that the use of ES for wound healing is effective at the stages of non-inflammation, proliferation and maturation, and early scar formation<sup>6</sup>. Wang *et al.*<sup>7</sup> reported that the flexible ES-chitosan dressing may promoted healing of diabetic wounds by accelerating angiogenesis, enhancing epithelialization, and inhibiting scar formation. Moreover, Mehmandoust *et al.*<sup>8</sup> in a study in which they investigated the effects of ES application on wound healing, showed that there was a significant difference in the percentage reduction in the wound surface and an increase in wound closure rate between the treatment and control groups. Previous studies presented that the use of electroacupuncture can attenuate the oxidative stress in different organs and tissues such as liver and kidneys and random skin flaps in anesthetized rats<sup>10,11</sup>. To date, no studies on the effects of ES on oxidant and antioxidant system of tissues in a rat model with incision wound have been published. Hence, in the present study, we investigated the effect of ES on the oxidative stress induced by the surgical incision wound in various (brain, heart, kidney, liver, testis, gastrocnemius muscle and quadriceps femoris muscle) tissues.

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## Material and Methods

Fourteen, two-months-old male Wistar Albino rats weighing 250–300 g (Experimental Research Application and Research Center, Hatay Mustafa Kemal University) were used in this study, distributed equally into two groups (n=7 each); the control group and the ES group. A longitudinal skin incision was performed in all rats. However, ES was applied only to rats in the ES group. The study protocol was approved by the Local Ethical Committee of Experimental Animal Ethics of Hatay Mustafa Kemal University and was performed entirely according to ethical rules (Approval no: 2021/03-01, 27/04/2021). Animals were maintained in a temperature and humidity controlled environment with a 12 h light/dark cycle. Food and water were available *ad libitum*. All animal surgical and experimental procedures were carried out in accordance with the care guidelines of the laboratory of animal resources of Hatay Mustafa Kemal University.

Before the operative procedure, the lateral part of the femur was shaved and the area was cleaned with an antiseptic solution. Anesthesia was provided with a combination of xylazine hydrochloride and ketamine hydrochloride. In both the control and ES groups, a longitudinal skin incision was made, starting from the lateral line of the right thigh and the trochanter major level only. The skin and subcutaneous tissue were opened and closed in layers with simple separate sutures using 2-0 absorbable suture material (Vicryl, Ethicon, Canada) 15 min later.

The ES parameters were calibrated to those used in previous studies<sup>12,13</sup>. ES was commenced 3 hours after the operation. It was applied with two electrodes (biomedical carbon film electrodes, Stimrodes), one of the electrodes was placed on quadriceps femoris muscle especially 5 mm proximal to the injury site and the other was placed on gastrocnemius muscle of right extremity. ES was applied to rats in ES group at 200  $\mu$ s current time, 20 Hz frequency, 2 mA amplitude for 20 min parameters with an ES device (Chattanooga Intellect, Primera, England). ES was administered for 15 days from the first day post-incision wound. Rats were not anesthetized for this process. Shortly, stimulations were performed for 20 min while the rats were conscious.

At the 16<sup>th</sup> day of the study, anaesthesia was produced by means of a cocktail prepared by using xylazine and ketamine hydrochloride. Before

performing necropsy, brain, heart, kidney, liver, testis, gastrocnemius muscle and quadriceps femoris muscle tissues were removed. The tissues were stored at  $-80^{\circ}\text{C}$  until analyses.

## Analyses

The tissues were individually homogenized at 1:10 (w/v) in sterile phosphate buffer (pH: 7.4) with ultrasonic homogenizer (Bandelin Electronic UW 2070, Germany) in cooled tubes with ice. The homogenates were immediately centrifuged 5000 rpm for 30 minutes at  $+4^{\circ}\text{C}$ . The supernatant was aliquoted and stored at  $-80^{\circ}\text{C}$  until analyses<sup>14,15</sup>.

The samples were used for spectrophotometrical (UV 2100 UV-VIS Recording Spectrophotometer Shimadzu, Japan) analysis of total protein, malondialdehyde (MDA), reduced glutathione (rGSH), glucose-6-phosphate dehydrogenase (G6PD), glutathione peroxidase (GPx) and catalase (CAT). The total protein contents of tissues were determined by the method of Lowry *et al*<sup>16</sup>. MDA was assayed by the method of Ohkawa *et al*<sup>17</sup>. An end product of lipid peroxidation was measured by a reaction with thiobarbituric acid yielding a coloured substance. This coloured adduct was read at 532 nm. Results were presented as  $\mu\text{mol/g}$  protein. rGSH were analyzed according to the method described by Ellman<sup>18</sup> method. It is a kinetic method based on the principle of the reduction of 5,5'-dithiobis (2-nitrobenzoic) acid to trinitrobenzoate by glutathione. The optical density of the reduced disulphide compound absorbance can be measured by spectrophotometry at 412 nm. Results are presented as  $\mu\text{mol/g}$  protein. G6PD in tissue extracts was measured and according to the method described by Beutler<sup>19</sup>. The activity measurement was monitored by the increase in absorption at 340 nm due to the reduction of nicotinamide adenine dinucleotide phosphate ( $\text{NADP}^+$ ). GPx was determined according to the method developed by Beutler<sup>20</sup>. According to this method, GPx catalyzes the conversion of rGSH to oxidized glutathione in the presence of hydrogen peroxide. Oxidized glutathione, formed by GPx in an environment where hydrogen peroxide is present, is converted back to GSH with the help of glutathione reductase and NADPH. The activity was calculated by the spectrophotometric measurement of the absorbance difference in optical density caused by the conversion of NADPH to  $\text{NADP}^+$  at 340 nm. Results were presented as U/g protein for G6PD and GPx. CAT was measured by the method of Aebi<sup>21</sup>. The rate of decomposition of hydrogen peroxide was

measured spectrophotometrically at 240 nm. Results were presented as k/g protein.

#### Statistical analysis

All statistical analysis was performed using statistical package for the social sciences (IBM SPSS, USA) for Windows 22 package program. Statistical comparison was performed using either unpaired Student's t-test or Mann Whitney U-test. When the data was not normally distributed the Mann Whitney U-test was used. All the biochemical values were expressed as mean  $\pm$  standard error (Mean  $\pm$  SE). Significant differences were considered when  $P < 0.05$ .

#### Results

MDA levels of brain ( $P < 0.001$ ), kidney ( $P < 0.05$ ), gastrocnemius muscle ( $P < 0.001$ ) and quadriceps femoris muscle ( $P \leq 0.001$ ) decreased but also liver MDA ( $P \leq 0.01$ ) increased significantly in ES group compared with control group. There were no significant changes in MDA levels of heart and testis between the groups. rGSH levels of kidney ( $P < 0.001$ ), liver ( $P \leq 0.001$ ), gastrocnemius muscle ( $P < 0.001$ ) and testis ( $P < 0.01$ ) increased but also quadriceps femoris muscle rGSH level ( $P \leq 0.001$ )

decreased significantly in ES group compared with control group. There were no significant changes in rGSH levels of brain and heart between the groups. G6PD activities of brain ( $P < 0.001$ ), heart ( $P \leq 0.001$ ), kidney ( $P < 0.001$ ), liver ( $P < 0.01$ ) and gastrocnemius muscle ( $P < 0.001$ ) increased significantly in ES group compared with control group. There were no significant changes in G6PD activity of quadriceps femoris muscle and testis between the groups. GPx activities of brain ( $P \leq 0.001$ ), heart ( $P < 0.01$ ), kidney ( $P \leq 0.001$ ), liver ( $P < 0.001$ ), gastrocnemius muscle ( $P < 0.001$ ) and testis ( $P < 0.05$ ) increased significantly in ES group compared with control group. Quadriceps femoris muscle GPx activity was not different in both groups. CAT activities of brain ( $P < 0.01$ ), kidney ( $P < 0.001$ ), liver ( $P < 0.001$ ), gastrocnemius muscle ( $P < 0.001$ ) and testis ( $P \leq 0.01$ ) increased but also quadriceps femoris muscle CAT ( $P \leq 0.001$ ) decreased significantly in ES group compared with control group. Heart CAT was not different in both groups.

The results of antioxidant and lipid peroxidation levels in the brain of control and ES groups were presented in Table 1. ES significantly decreased MDA (26%,  $P < 0.001$ ) but also increased G6PD (77%,  $P < 0.001$ ), GPx (17%,  $P < 0.001$ ) and CAT

Table 1 — Effect of ES on the levels of lipid peroxide (in terms of MDA) and antioxidants in brain, kidney, liver, and testis

Parameters	Control Group (n=7)		ES Group (n=7)		P - Significance
	Mean $\pm$ SE	Min-Max	Mean $\pm$ SE	Min-Max	
<b>Brain</b>					
MDA ( $\mu\text{mol/g}$ protein)	1.15 $\pm$ 0.03***	1.03-1.28	0.85 $\pm$ 0.04	0.72-1.05	$P=0.000$ 't' test
rGSH ( $\mu\text{mol/g}$ protein)	16.05 $\pm$ 1.33	11.77-21.98	18.57 $\pm$ 1.22	13.74-22.31	$P=0.189$ 't' test
G6PD (U/g protein)	18.71 $\pm$ 0.42	17.12-20.63	33.16 $\pm$ 1.19***	27.89-37.91	$P=0.000$ 't' test
GPx (U/g protein)	318.92 $\pm$ 8.48	289.46-348.62	372.84 $\pm$ 9.31***	342.55-405.72	$P=0.001$ 't' test
CAT (k/g protein)	0.012 $\pm$ 0.0004	0.010-0.014	0.017 $\pm$ 0.0012**	0.013-0.021	$P=0.007$ Mann Whitney U
<b>Kidney</b>					
MDA ( $\mu\text{mol/g}$ protein)	13.83 $\pm$ 0.99*	11.04-17.81	10.64 $\pm$ 0.45	8.5-12.13	$P=0.017$ Mann Whitney U
rGSH ( $\mu\text{mol/g}$ protein)	4.50 $\pm$ 0.16	3.88-5.11	9.57 $\pm$ 0.45***	7.35-10.98	$P=0.000$ T test
G6PD (U/g protein)	5.94 $\pm$ 0.19	5.23-6.57	8.57 $\pm$ 0.31***	7.58-10.13	$P=0.000$ T test
GPx (U/g protein)	262.30 $\pm$ 4.32	241.24-280.47	460.99 $\pm$ 12.86***	424.14-500.43	$P=0.001$ Mann Whitney U
CAT (k/g protein)	0.569 $\pm$ 0.0207	0.471-0.641	0.721 $\pm$ 0.0205***	0.659-0.799	$P=0.000$ 't' test
<b>Liver</b>					
MDA ( $\mu\text{mol/g}$ protein)	23.40 $\pm$ 2.78	14.79-35.96	32.89 $\pm$ 1.60*	27.01-38.18	$P=0.012$ 't' test
rGSH ( $\mu\text{mol/g}$ protein)	17.35 $\pm$ 1.01	13.70-20.77	24.99 $\pm$ 0.83***	21.96-28.42	$P=0.000$ 't' test
G6PD (U/g protein)	4.93 $\pm$ 0.25	4.13-6.31	7.19 $\pm$ 0.50**	5.68-9.64	$P=0.002$ 't' test
GPx (U/g protein)	281.78 $\pm$ 4.14	267.59-294.89	330.29 $\pm$ 8.29***	300.42-366.80	$P=0.000$ 't' test
CAT (k/g protein)	0.456 $\pm$ 0.0236	0.335-0.543	0.706 $\pm$ 0.0130***	0.663-0.764	$P=0.000$ 't' test
<b>Testis</b>					
MDA ( $\mu\text{mol/g}$ protein)	23.61 $\pm$ 2.58	18.59-38.23	24.33 $\pm$ 1.92	17.33-30.46	$P=0.620$ Mann Whitney U
rGSH ( $\mu\text{mol/g}$ protein)	29.98 $\pm$ 0.90	26.22-33.36	39.37 $\pm$ 2.54**	32.30-48.94	$P=0.004$ Mann Whitney U
G6PD (U/g protein)	17.84 $\pm$ 0.43	16.02-19.18	19.03 $\pm$ 0.79	16.60-21.65	$P=0.211$ 't' test
GPx (U/g protein)	338.86 $\pm$ 10.05	302.46-390.20	367.07 $\pm$ 7.84*	334.26-393.27	$P=0.047$ 't' test
CAT (k/g protein)	0.024 $\pm$ 0.0021	0.017-0.031	0.033 $\pm$ 0.0021**	0.027-0.040	$P=0.010$ 't' test

[Data were given as Mean  $\pm$  SE. Mean values with different superscripts within a row differ significantly (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ). ES, Electrical stimulation; MDA, Malondialdehyde; rGSH, Reduced glutathione; G6PD, Glutathione 6 phosphate dehydrogenase; GPx, Glutathione peroxidase; and CAT, Catalase]

(42%,  $P < 0.01$ ) as compared to control rats. Table 2 demonstrated the results of antioxidant and lipid peroxidation levels in the heart of control and ES groups. A significant increase by 225% ( $P < 0.001$ ) in G6PD and 22% ( $P < 0.01$ ) in GPx was observed in ES group. The results of antioxidant and lipid peroxidation levels in the kidney of control and ES groups were presented in Table 1. A significant increase by 112% ( $P < 0.001$ ) in rGSH, 44% ( $P < 0.001$ ) in G6PD, 76% ( $P < 0.001$ ) in GPx and 27% ( $P < 0.001$ ) in CAT was observed in ES group, whereas MDA resulted in significant decrease by 23% ( $P < 0.05$ ). Table 1 showed the results of antioxidant and lipid peroxidation levels in the liver of control and ES groups. A significant increase by 41% ( $P < 0.05$ ) in MDA, 44% ( $P < 0.001$ ) in rGSH, 46% ( $P < 0.01$ ) in G6PD, 17% ( $P < 0.001$ ) in GPx and 55% ( $P < 0.001$ ) in CAT was determined in ES group. The results of antioxidant and lipid peroxidation levels in the M. gastrocnemius of control and ES groups were presented in Table 2. MDA resulted in significant decrease by 66 % ( $P < 0.001$ , whereas a significant increase by 29% ( $P < 0.001$ ) in rGSH, 61% ( $P < 0.001$ ) in G6PD, 27% ( $P < 0.001$ ) in GPx and 27% ( $P < 0.001$ ) in CAT was found in ES group. Table 2 demonstrated the results of antioxidant and lipid peroxidation levels in the M. quadriceps femoris of control and ES groups. A significant decrease by 64% ( $P < 0.001$ ) in MDA, 68% ( $P < 0.001$ ) in rGSH, and

13% ( $P < 0.001$ ) in CAT was determined in ES group. The results of antioxidant and lipid peroxidation levels in the testis of control and ES groups were presented in Table 1. A significant increase by 31% ( $P < 0.01$ ) in rGSH, 8% ( $P < 0.05$ ) in GPx, and 38% ( $P < 0.01$ ) in CAT was determined in ES group. Since an altered oxidant-antioxidant capacity in different tissues are associated with the application of ES, a positive correlation may be drawn showing a possible contribution of altered antioxidant systems in wound healing.

### Discussion

Operative interventions are performed in many cases in the femoral region, such as total hip prostheses, tumoral formations, femoral fractures, vascular pathologies, tendon-related pathologies and surgical treatment of nerve ruptures<sup>22-27</sup>. Thus, an incision wound occurs on the lateral line of the femoral region. As the problems in wound healing proceed to cause significant morbidity and mortality, wound healing is a continuing challenge in rehabilitation medicine<sup>6</sup>. In previous studies<sup>3-5</sup> investigating the effects of ES therapy on wound healing, ES has been shown to benefit tissue repair in a variety of wound types<sup>28</sup>. However, our literature search did not reveal any study regarding the effects of ES on oxidant and antioxidant system of tissues in a rat model with incision wound. To our knowledge,

Table 2 — Effect of ES on the levels of lipid peroxide (in terms of MDA) and antioxidants in heart, M. gastrocnemius, and M. quadriceps femoris

Parameters	Control Group (n=7)		ES Group (n=7)		P - Significance
	Mean±SE	Min-Max	Mean±SE	Min-Max	
<b>Heart</b>					
MDA (μmol/g protein)	4.89±0.40	3.61-6.57	3.93±0.29	2.56-4.90	$P = 0.078$ 't' test
rGSH (μmol/g protein)	0.78±0.02	0.72-0.84	0.83±0.06	0.67-1.07	$P = 0.902$ Mann Whitney U
G6PD (U/g protein)	0.57±0.05	0.41-0.80	1.85±0.27***	1.02-2.75	$P = 0.001$ Mann Whitney U
GPx (U/g protein)	272.40±10.92	225.36-310.18	332.13±11.21**	284.65-375.45	$P = 0.002$ 't' test
CAT (k/g protein)	0.048±0.0028	0.038-0.059	0.050±0.0016	0.045-0.056	$P = 0.575$ 't' test
<b>M. gastrocnemius</b>					
MDA (μmol/g protein)	15.38±0.99***	11.56-18.78	5.26±0.43	3.91-6.74	$P = 0.000$ 't' test
rGSH (μmol/g protein)	1.41±0.05	1.23-1.63	1.82±0.06***	1.53-2.05	$P = 0.000$ 't' test
G6PD (U/g protein)	2.26±0.14	1.80-2.74	3.63±0.13***	3.21-4.15	$P = 0.000$ 't' test
GPx (U/g protein)	315.39±7.38	282.25-336.71	400.59±4.01***	382.98-411.30	$P = 0.000$ 't' test
CAT (k/g protein)	0.011±0.0004	0.009-0.012	0.014±0.0002***	0.013-0.015	$P = 0.000$ 't' test
<b>M. quadriceps femoris</b>					
MDA (μmol/g protein)	36.52±1.97***	29.18-43.98	13.15±0.49	10.78-14.68	$P = 0.001$ Mann Whitney U
rGSH (μmol/g protein)	6.02±0.40***	4.69-7.68	1.90±0.04	1.70-2.03	$P = 0.001$ Mann Whitney U
G6PD (U/g protein)	2.39±0.09	2.06-2.66	2.33±0.09	2.15-2.88	$P = 0.646$ 't' test
GPx (U/g protein)	429.57±20.56	351.51-502.64	428.23±14.45	393.19-507.52	$P = 0.958$ 't' test
CAT (k/g protein)	0.008±0.0004***	0.007-0.010	0.007±0.0001	0.006-0.007	$P = 0.001$ Mann Whitney U

[Data were given as Mean ± SE. Mean values with different superscripts within a row differ significantly (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ). ES, Electrical stimulation; MDA, Malondialdehyde; rGSH, Reduced glutathione; G6PD, Glutathione 6 phosphate dehydrogenase; GPx, Glutathione peroxidase; and CAT, Catalase]

this is the first study to investigate the effects of ES on the MDA and antioxidative parameters (rGSH, G6PD, GPx, CAT) in tissues (brain, heart, kidney, liver, testis, gastrocnemius muscle and quadriceps femoris muscle) in a rat model with incision wound where was created on the lateral line of the femoral region.

Oxidants production is required for wound healing. However, overproduction of oxidants results in oxidative stress can damage cells and tissues and also prevent wound healing<sup>2</sup>. Antioxidants have cytoprotective effects by reducing, deactivating and removing oxidants, as well as regulating the process of wound healing. Thus, balance in oxidants and antioxidants is essential for wound healing<sup>29</sup>. MDA is a detrimental substance that influences the ion change in the cell membranes and disrupts the permeability, reacts with amino acids and nucleosides, blocks cellular metabolism, leading to cytotoxicity and its level is known as a marker of oxidative stress<sup>30</sup>. Previous studies<sup>31,32</sup> reported a significant increase in lipid peroxide levels, as measured by MDA in the skin of rats, ten days after dorsal incision wound. Similarly, in the present study, longitudinal incision wound caused an increase in brain, kidney, gastrocnemius muscle and quadriceps femoris muscle MDA. Electroacupuncture is an electrically driven acupuncture that a therapeutic technique using percutaneous thin two needles, in which the stimulation frequency and intensity can be regulated<sup>9</sup>. Liu *et al.*<sup>33</sup> reported that electroacupuncture attenuated the hippocampal postoperative cognitive dysfunction induced increase in hippocampal MDA at day one following operation. Furthermore, electroacupuncture decreased MDA in rat brains with ischemic-reperfusion<sup>34</sup>. In the present study, the brain MDA were found to be significantly lower in the ES group than control group. Our findings agree with the results reported by Liu *et al.*<sup>33</sup> and Siu *et al.*<sup>34</sup> Lima *et al.*<sup>11</sup> presented that electroacupuncture 3 Hz induces changes in plasma and skin MDA, but the use of electroacupuncture 100 Hz promotes a significant increase in plasma and skin MDA. Moreover, one study reported that the use of electroacupuncture (10 Hz and 100 Hz) induced a significant increase in kidney MDA<sup>10</sup>. However, some studies have demonstrated the effects of electroacupuncture in decreasing the MDA of plasma<sup>35</sup>, brain<sup>33,34</sup> and ovary<sup>35</sup> in different experimental diseases. Similar to these literatures, this study showed that ES reduces the MDA in different

organs and tissues such as brain, kidney, gastrocnemius muscle and quadriceps femoris muscle. However, liver MDA increased significantly in ES group compared with control group. A similar finding was obtained for liver MDA by Silva *et al.*<sup>10</sup> in an experimental study using electroacupuncture. Wang *et al.*<sup>36</sup> indicated that electroacupuncture reduces the MDA of pig heart muscle in experimental model of ischemia-reperfusion. In the current study, there was no significant change in heart MDA between the groups. Acioli *et al.*<sup>37</sup> studied the protective effects of abdominal electroacupuncture on oxidative stress and inflammation due to testis torsion/detorsion in rats and concluded that the application of electroacupuncture (2 and 10 Hz) increase MDA in testis and plasma. In this study, the application of ES induced no significant changes in testis MDA.

It has been reported that the wound is a skin damage and leads to the disequilibrium in oxidant and antioxidant balance and induces excessive oxidant production. Thus, the skin needs to use a defense mechanism to protect the oxidative balance, such as rGSH, G6PD, GPx and CAT<sup>38,39</sup>. GSH which is known as a non-enzymatic antioxidant, regulates the early phases of wound healing; therefore, lack of GSH may play a role in delaying the wound healing<sup>40</sup>. In the present study decreasing GSH in kidney, liver, gastrocnemius muscle and testis observed in the control group. Silva *et al.*<sup>10</sup> demonstrated that the electroacupuncture (10 and 100 Hz) induces a significant increase in liver and kidney GSH. Acioli *et al.*<sup>37</sup> presented that electroacupuncture stimulation (2 Hz and 10 Hz) promoted significant increase in testis GSH. In keeping with the previous researches,<sup>10,37</sup> in the present study indicated that the use of ES (20 Hz) caused a significant increase in kidney, liver, gastrocnemius muscle and testis GSH in ES group. Nevertheless, quadriceps femoris muscle GSH decreased significantly in rats submitted to ES group compared with control group. In addition, there were no significant changes in brain and heart GSH between the groups. G6PD provides to maintain and regenerate the intracellular GSH pool and plays a very important role in the cell response to the oxidative stress<sup>42</sup>. In the present study, the use of ES (20 Hz) indicates a significant increase in brain, heart, kidney, liver and gastrocnemius muscle activities of G6PD. In contrast with current study, Silva *et al.*<sup>10</sup> reported that the electroacupuncture (10 and 100 Hz) induces a significant decrease in kidney and liver G6PD. GSH serves as a coenzyme necessary for GPx,

an essential antioxidant enzyme for the detoxification of hydrogen peroxide resulting from lipid peroxidation<sup>42</sup>. Studies<sup>40,43</sup> demonstrated that skin GPx activity was markedly decreased in rats after wounding. In accordance with the previous data, present study showed that incision wound cause of the decrease in tissues GPx. Shen *et al.*<sup>44</sup> reported that treatment with electroacupuncture significantly increases serum GPx in rats with cerebral ischemia and reperfusion injury. In this study indicated that treatment with ES increases GPx of brain, heart, kidney, liver, gastrocnemius muscle and testis in rats with incision wound. CAT is one of the peroxide-removing enzymes like GPx and a common antioxidant enzyme present almost in all living tissues that utilize oxygen<sup>42</sup>. It has been reported that a significant decrease in CAT was observed at two days<sup>43</sup> and seven days<sup>40</sup> postwounding in the skin of rats. Similarly, in this study demonstrated that CAT of brain, kidney, liver, gastrocnemius muscle and testis decreased at fifteen days post-incision wound in rats. However, ES administration to rats with incision wound significantly increased the mentioned tissues CAT. These findings provides that the effect of ES in which high levels of antioxidants accompanied by reduced levels of markers of free radical damage plays an essential role in accelerating wound healing in rats with incision wound.

As for strengths and weaknesses of the present study in relation to others, there is no published research that can be directly compared with the current study. The data collected support the hypothesis that 15 days 30 min 200  $\mu$ s, 20 Hz, 2mA ES session reduces oxidative stress by decreasing MDA (in brain, kidney, gastrocnemius muscle and quadriceps femoris muscle) and simultaneously increasing rGSH (in kidney, liver, testis and gastrocnemius muscle), G6PD (in brain, heart, kidney, liver and gastrocnemius muscle), GPx (brain, heart, kidney, liver, testis and gastrocnemius muscle) and CAT (brain, kidney, liver, testis and gastrocnemius muscle) in a rat model with incision wound. Thus, ES applications may promote increased different antioxidant levels in different tissues of rats, ensuring antioxidative protection to rats with incision wound.

## Conclusion

Consequently, the present study suggests that performance of incision wound could induce oxidative stress and electrical stimulation may inhibit the oxidative stress to produce protective and also ameliorative effect at post-incision wound. Moreover, this protection of electrical stimulation procedure can be sufficient and

potentially regulates the redox environment. Therefore, this area needs to be explored further, including the development of suitable experimental animal models.

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## Conflict of Interest

Authors declare no competing interests.

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