

Available online at [GSC Online Press Directory](https://www.gsconlinepress.com/)

GSC Biological and Pharmaceutical Sciences

e-ISSN: 2581-3250, CODEN (USA): GBPSC2

Journal homepage: <https://www.gsconlinepress.com/journals/gscbps>

(RESEARCH ARTICLE)



## The investigation of the preventive effects of Coenzyme Q10 and Berberine for tourniquet induced ischemia-reperfusion injury on skeletal muscle in rat hindlimb

Apaydin Yildirim Betul \* and Batil Annour Adoum

*Ataturk University Veterinary Faculty, Department of Biochemistry, Erzurum, Turkey.*

Publication history: Received on 12 December 2019; revised on 20 December 2019; accepted on 23 December 2019

Article DOI: <https://doi.org/10.30574/gscbps.2019.9.3.0238>

### Abstract

In this study, the role of CoQ10 and berberine in the treatment of ischemia-reperfusion injury is researched by analyzing the biochemical effects of CoQ10 and berberine administered after experimental ischemia-reperfusion injury in rats. 32 Sprague-Dawley 200-250 g male rats were randomly divided into four equal groups as weighting, including: control, ischemia-reperfusion (I/R) and experimental groups treated with CoQ10 (10 mg/kg dose, 3 times with 8 h intervals) and berberine (200 mg/kg dose, 3 times with 8 h intervals) that were administered by intragastric way to the I/R+ CoQ10 and I/R+ berberine group before two hours ischemia two hours reperfusion with tourniquet. In the sham group, only gastrocnemius muscle were removed and given no CoQ10 and Berberine. By the completion of reperfusion; all rats were anesthetized with ketamine and xylazine, sacrificed after taking samples to measure levels of blood lactate dehydrogenase (LDH), creatine kinase (CK); MDA, MPO, GSH, SOD, GPx and CAT in blood and muscle tissues. Compared to control group, ischemic-reperfusion injury significantly increased plasma LDH, CK; MDA and MPO levels, decreased GSH, SOD, GPx and CAT activities of blood and muscle tissues. Treatments groups showed significantly decreased MDA and MPO levels, increased GSH levels, SOD, GPx and CAT activities compared to the IR group. Our results suggest that CoQ10 and Berberine may protect or treat against oxidative damage on ischemia-reperfusion injury and that can be beneficial treating association with lower extremity ischemia-reperfusion injury.

**Keywords:** Berberine; Coenzyme Q10; Ischemia; Rat; Reperfusion

### Abbreviations

Reactive oxygen species (ROS), Creatine kinase (CK), Lactate dehydrogenase (LDH), Total protein (TP), Malondialdehyde (MDA), Reduced glutathione (GSH), Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Catalase (CAT).

### 1. Introduction

Ischemia is one of the most widespread damage that are mostly caused by decreased blood flow to an organ various reasons such as transplantation, thrombosis, surgical procedures, hypovolemia, energy production stopping and lack of oxygen and nutrients that occurs in vascular substrates of the tissues [1]. While reconstituting blood flow to tissues in an ischemia may contribute to their survival, it may also cause simultaneous damage during reperfusion. This is known as ischemia-reperfusion (I/R) injury.

The rat hind limb ischemia-reperfusion (I/R) injury and extremity vascular injury model resembles human diseases including extremity vascular injury [2], peripheral artery disease [3] and tourniquet application [4], which might sometimes lead to multiple organ failure and death.

\* Corresponding author

E-mail address: [betul\\_apaydin@hotmail.com](mailto:betul_apaydin@hotmail.com)

Coenzyme Q10 (CoQ10) is a vitamin like nutrient which known as ubidecarenone or ubiquinone and lipid-soluble compound. It is found in the mitochondria, cell membranes, lipoproteins in blood and all tissues'cell in the body. CoQ10's primary role in cellular energy production, the electron transport chain, along the inner mitochondrial membrane. It is a component in oxidative phosphorylation converting products of proteins, fats and carbohydrates metabolism into energy as ATP [5]. It has been considered that CoQ10 may be effective in treatment particular pathological events as an antioxidant supplementation.

Berberine is an isoquinoline derived alkaloid extracted from *Rhizoma Coptidis*, which has been widely used in clinical owing to its multiple biochemical and pharmacological effects [6, 7]. Berberine, is an isoquinoline alkaloid, major component of *Cortex phellodendri* and *Rhizoma coptidis* which medicinal use in both Chinese and Ayurvedic medicine. Recently, berberine has been shown to powerful neuroprotective effects against ischemic damage [8, 9]. Berberine exerts antitumor, antiviral, antioxidant, neuroprotective, antiapoptotic and anti-inflammatory properties in various animal models of central nervous system related Alzheimer's disease, the anterior part of brain ischemia, Parkinson's disease (PD), depression, and anxiety disorders [10, 11].

Although the mechanisms of hind limb I/R injury are complicated, accumulating evidence has suggested that reactive oxygen species (ROS) and inflammation play a crucial role in the pathogenesis of hind limb I/R injury. Cellular damage is a critical and widespread clinical event after reperfusion of beforehand viable ischemic tissues in lower extremity. The mechanism of ischemia-reperfusion is multifactorial and involves divergent biological mechanisms, such as ion accumulation, immune activation and the formation of toxic reactive oxygen species (ROS) defined as free radicals [12, 13]. ROS are the most important key molecules in the reperfusion injury and toxic substances produced in various clinical conditions [14, 15]. Oxidative stress is known as a disturbance between the antioxidant and prooxidant balance resulting in cell injury by oxidation of lipids, proteins and DNA [16]. In tourniquet-related surgery or major vascular surgery procedures involving ischemia-reperfusion injury. In this reason, it is not possible to distinguish between oxidative stress caused by surgical procedures and oxidative stress caused by ischemia-reperfusion.

Ischemia/reperfusion injury in skeletal muscles can be fixed by antioxidant agents which binds free oxygen radicals. To date, a number of drugs, physical methods and chemicals have been investigated to protect skeletal muscle against ischemia- reperfusion injury. It is also of clinical importance to know the preventive effects of muscle damage during tourniquet-induced ischemia and reperfusion. The purpose of this study was to investigate the effect of both CoQ10 and berberine on lower extremity muscle I/R injury, which may happen often after the tourniquet method.

---

## 2. Material and methods

### 2.1. Chemicals

Coenzyme Q10 was bought from SOLGAR. All of other chemicals and Berberine used were of analytical level and were bought from the Sigma Chemical Co. (St. Louis, MO, USA). Coenzyme Q10 and Berberine protected from sunlight.

### 2.2. Animals

The experimental protocol were conducted according to the ethical norms approved by the Ethic Committee of Experimental Animal Teaching and Researcher Center (No: 06.10.2017, 36643897-000-E.1700276378-132). Rats were obtained from the Medical and Experimental Application and Research Center (ATADEM), Erzurum, Turkey. Thirty-two Sprague–Dawley male rats weighing between 200–250 g were used in this experimental study. Throughout the animal experiments were processed following the internationally accepted ethical guidelines for the care of laboratory animals. Prior to the experiments, rats were accommodated in the standard laboratory conditions with a temperature-controlled room 21°C (+/- 2) with natural light and dark cycle (12 / 12 hours light:dark) and humidity (55+/-5%) were fed and water ad libitum, in order to adapt to the laboratory conditions. Before from the study, animals were fasted overnight, but were allowed free access to water. All rats were anesthetized with ketamine (60 mg / kg) and xylazine (8 mg / kg). Except for the control group, common femoral arteries and collateral flow were occluded tightly with rubber tourniquets, the proximal of the left extremity and ischemia was confirmed by cyanosis and temperature drop in the left lower limbs of the other 3 groups were applied to 2 hours of ischemia and 2 hours of later tourniquate was released and reperfusion was initiated. Reperfusion was verified by edema, the extremity return to normal temperature, receiving pulse and the extremity colour changed to pink. Seven animals were used for each group of study. Rats were divided into the following groups.

### 2.2.1. Control Group

0.5 mL 0.25% CMC was administered by gastric gavage 3 times at 8 hour intervals. Blood samples were obtained from the aorta abdominalis under anesthesia for biochemical analyzes. Tissue specimens were taken under anesthesia from the left extremity.

### 2.2.2. I/R Group

0.5 mL 0.25% CMC was administered by gastric gavage 3 times at 8 hour intervals. Under the anesthesia, 2 hours tourniquet was applied to the lower extremity (low temperature and cyanotic claw marks the occurrence of ischemia). Subsequently, the tourniquets were opened and reperfusion was applied for 2 hours (the pinking of the claws and the increase in temperature indicate reperfusion) and afterwards, the abdomen was opened with midline incision, and blood samples for biochemical analysis were taken from *Aorta abdominalis*. Tissue samples were taken from the left extremity.

### 2.2.3. I/R+CoQ10 Group

Prior to ischemia formation, rats were dosed with gastric gavage at a dose of 10 mg/kg (3 times at 8 hour intervals) from 0.5 mL CoQ10 (It was dissolved in 0.25% CMC). After 30 minutes, under anesthesia, 2 hours tourniquet was applied to the lower extremity. Subsequently, the tourniquets were opened and reperfusion was applied for 2 hours, and afterwards, blood and tissue samples were taken as they were in the sham group.

### 2.2.4. I/R+Berberine Group

Prior to ischemia, rats were given 200 mg/kg (3 times at 8 hour intervals) from 0.5 mL Berberine (It was dissolved in 0.25% CMC) by gastric gavage. After 30 minutes, under anesthesia, 2 hours tourniquet was applied to the lower extremity. Subsequently, the tourniquets were opened and reperfusion was applied for 2 hours, and afterwards, blood and tissue samples were taken as they were in the sham group.

## 2.3. Biochemical analysis in plasma

Whole blood was collected into lithium heparinised tubes from *Aorta abdominalis*. Plasma was obtained from these whole blood samples by centrifugation (3000 rpm for 10 min) and used for the determination of the biochemical parameters. Lactate dehydrogenase, creatine kinase activities were analyzed in the plasma using commercially available diagnostic kits with a Beckman Coulter AU5800 (USA) auto analyser. Plasma MDA [17], MPO [18], GSH levels [19], GPx [20], SOD [21] and CAT activities [22] were measured with Biotek ELISA Reader (Bio Tek  $\mu$ Quant MQX200 Elisa reader/USA). The protein concentration was also measured in the plasma according to the method of Lowry et al [23].

## 2.4. Biochemical analyzes in the gastrocnemius muscle

The muscle tissues were homogenized in Qiagen TissueLyser II using a buffer of 1.15% KCl to obtain 1:10 (w/v) 0.1 M phosphate buffer (pH 7.4) to obtain a 1:10 (w/v) homogenate. Homogenates MDA [24], MPO [18], GSH [25, 26] levels, GPx [20], SOD [21] and CAT [22] activities were measured with Biotek ELISA Reader (Bio Tek  $\mu$ Quant MQX200 Elisa reader/USA). The protein concentration was also measured in the supernatant according to the method of Lowry et al [23].

## 2.5. Statistical analysis

Data were analyzed using SPSS software package, version 22.00. Statistical analysis was done by one-way analysis of variance (ANOVA). Post-hoc Tukey's test was used to compare the biochemical parameters between the groups. P values <0.05 were considered as significant. The results are expressed as mean $\pm$ standard error (SEM) for each group.

---

## 3. Results

Results of plasma biochemical parameters were shown in Table 1. In this study, plasma CK, LDH, MDA levels and MPO activities of the I/R group increased significantly compared with the control group. CK, LDH, MDA levels and MPO activities in I/R+CoQ10 and I/R+Berberine groups was found to be significantly decreased compared with I/R group. Besides, plasma GSH levels, GPx, SOD and CAT activities significantly reduced in the IR group compared with control group. Also, it was found that plasma GSH levels, GPx, SOD and CAT activities were increased by treatment of CoQ10 and Berberine compared with the IR group.

**Table 1** CK, LDH, MDA, MPO, GSH, GPx, SOD and CAT values of all groups in plasma.

GROUPS	CK(U/mL)	LDH (U/mL)	MDA (mmol/L)	MPO (U/mL)	GSH (mmol/L)	GPx (U/mL protein)	SOD (U/mL protein)	CAT (kU/L)
C	1.56±0.04 <sub>d</sub>	2.66±0.05 <sub>d</sub>	19.67±0.10 <sub>c</sub>	10.45±0.16 <sub>d</sub>	3.37±0.05 <sup>a</sup>	0.27±0.01 <sub>a</sub>	18.66±0.12 <sub>a</sub>	251.23±2.42 <sub>a</sub>
IR	4.79±0.09 <sub>a</sub>	6.69±0.06 <sub>a</sub>	28.56±0.09 <sub>a</sub>	20.34±0.08 <sub>a</sub>	2.37±0.11 <sup>c</sup>	0.20±0.00 <sub>c</sub>	10.97±0.14 <sub>d</sub>	158.54±0.29 <sub>d</sub>
IR+COQ10	2.29±0.06 <sub>b</sub>	3.95±0.18 <sub>b</sub>	20.50±0.08 <sub>b</sub>	15.55±0.16 <sub>b</sub>	2.91±0.04 <sup>b</sup>	0.24±0.00 <sub>b</sub>	14.22±0.22 <sub>c</sub>	210.39±3.25 <sub>c</sub>
IR+Berberin <sub>e</sub>	1.94±0.03 <sup>c</sup>	3.24±0.07 <sub>c</sub>	19.60±0.27 <sub>c</sub>	14.18±0.08 <sub>c</sub>	3.11±0.05 <sup>a</sup> <sub>b</sub>	0.25±0.00 <sub>b</sub>	15.88±0.27 <sub>b</sub>	236.14±3.25 <sub>b</sub>
P	0.000 <sub>***</sub>	0.000 <sub>***</sub>	0.000 <sub>***</sub>	0.000 <sub>***</sub>	0.000 <sub>***</sub>	0.000 <sub>***</sub>	0.000 <sub>***</sub>	0.000 <sub>***</sub>

a,b,c,d Values followed by the different letters in the same columns are significantly different (\*\*\*) P<0.001).

Results of muscle tissues biochemical parameters were shown in Table 2. In this study, muscle tissue MDA levels and MPO activities of the I/R group increased significantly compared with the control group. MDA levels and MPO activities in I/R+CoQ10 and I/R+Berberine groups was found to be significantly decreased compared with I/R group. Besides, muscle tissue GSH levels, GPx, SOD and CAT activities significantly reduced in the IR group compared with control group. Also, it was found that muscle tissue GSH levels, GPx, SOD and CAT activities were increased by treatment of CoQ10 and Berberine compared with the IR group.

There was a statistically significant difference between the groups when they were compared among themselves by means of biochemical parameters in plasma and skeletal muscle tissue (P<0.001) (Table 1-2).

**Table 2** MDA, MPO, GSH, GPx, SOD and CAT values of all groups in muscle tissues.

GROUPS	MDA (nmol/g tissue)	MPO (U/mg tissue)	GSH (mmol/g tissue)	GPx (U/g protein)	SOD (EU/mg protein)	CAT (kU/g tissue)
C	18.00±0.19 <sup>c</sup>	6.21±0.11 <sup>b</sup>	2.15±0.00 <sup>a</sup>	0.16±0.00 <sup>a</sup>	26.51±0.28 <sup>a</sup>	277.87±0.41 <sup>a</sup>
IR	55.84±1.15 <sup>a</sup>	12.06±0.22 <sup>a</sup>	1.92±0.02 <sup>c</sup>	0.11±0.00 <sup>c</sup>	20.04±0.18 <sup>d</sup>	175.46±1.10 <sup>c</sup>
IR+COQ10	25.07±0.15 <sup>b</sup>	5.33±0.11 <sup>c</sup>	2.11±0.01 <sup>b</sup>	0.14±0.00 <sup>b</sup>	22.81±0.19 <sup>c</sup>	250.40±1.55 <sup>b</sup>
IR+Berberine	20.26±0.20 <sup>c</sup>	6.21±0.66 <sup>b</sup>	2.14±0.00 <sup>ab</sup>	0.15±0.00 <sup>b</sup>	25.14±0.28 <sup>b</sup>	253.03±1.54 <sup>b</sup>
P	0.000 <sub>***</sub>	0.000 <sub>***</sub>	0.000 <sub>***</sub>	0.000 <sub>***</sub>	0.000 <sub>***</sub>	0.000 <sub>***</sub>

a,b,c,d Values followed by the different letters in the same columns are significantly different (\*\*\*) P<0.001).

#### 4. Discussion

Tourniquet-induced ischemia leads to progressive vascular, nerve and muscle injury. It is clear that skeletal muscles are more exposed to ischemia than other tissues. Damage to muscles is the most critical aspect of reperfusion in the limbs. The degree of skeletal muscle damage is directly related to the severity and duration of ischemia. Accurate determination of the time of muscle death is difficult because the microscopic and macroscopic changes are very small. Researcher showed that 3 h ischemia in the muscle rat led to significant muscle damage. The injury was very severe after 4, 5 and 6 h ischemia that these led to 3 % loss of functional activity control [27]. In general, as reperfusion progresses, systemic inflammatory response syndrome and multiple respiratory, excretory and circulatory system organ failure follow local muscle necrosis and edema [28, 29].

Cellular damage after reperfusion of previously viable ischemic tissues in lower extremity is a common and critical clinical incident. When blood flow is re-established after reperfusion, secondary metabolites such as oxygen free radicals are produced as a result of oxidation of metabolites and are spread throughout the body via systemic circulation. Skeletal muscle I/R injury could be elicited by multiple pathological etiologies, which can cause significant injury with serious remote organ dysfunction [30, 31].

The role of oxidative stress in the setting of hind limb I/R injury is well demonstrated in previous studies [31-33]. I/R process promoted oxidative stress with over production of ROS that might directly damage cellular membranes by lipid peroxidation [34]. In the present study, the effects of CoQ10 and Berberine on tourniquet- induced ischemia-reperfusion injury were investigated. We found that CoQ10 and Berberine treatment can significantly attenuate the hind limb I/R injury.

We investigated the effects of CoQ10 and Berberine on the level of plasma CK, LDH, plasma and muscle tissue MDA levels, MPO activities, GSH levels, GPx, SOD and CAT activities. Our data showed that the level of plasma CK, LDH, plasma and muscle tissue MDA and MPO activities were increased in I/R group compared with control group, while GSH levels, GPx, SOD and CAT activities were reduced. Administration of CoQ10 and Berberine could decrease the level of plasma CK, LDH, plasma and muscle tissue MDA and MPO activities and increase GSH levels, GPx, SOD and CAT activities, our results thus suggesting that CoQ10 and Berberine could protect against I/R in rat.

---

## 5. Conclusion

On the basis of these results, we can suggest that CoQ10 and Berberine in reducing I/R injury in gastrocnemius muscle tissues, this antioxidants can be effective to reduce I/R injury of skeletal muscles. The main goal of the present study was to examine CoQ10 and Berberine effects on I/R injury of gastrocnemius muscle. Further research is needed to elucidate the action mechanism in hindlimb I/R and must be more detailed in order to fully establish this kind of conclusions.

---

## Compliance with ethical standards

### *Acknowledgments*

The authors would like to thanks Ataturk University in Research and Application Unit of School of Veterinary Faculty for the application support.

### *Disclosure of conflict of interest*

Betul Apaydin Yildirim and Annour Adoum Batil declare that they have no conflicts to declare. This article does not contain any studies with human or animal subjects performed by any of the authors.

### *Statement of ethical approval*

The experimental protocol were conducted according to the ethical norms approved by the Ethic Committee of Experimental Animal Teaching and Researcher Center (No: 06.10.2017, 36643897-000-E.1700276378-132). Rats were obtained from the Medical and Experimental Application and Research Center (ATADEM), Erzurum, Turkey.

---

## References

- [1] Kumar V, Abbas AK and Aster JC. (2018). Robbins basic pathology. Elsevier Health Sciences. Philadelphia, PA.
- [2] Percival TJ and Rasmussen TE. (2012). Reperfusion strategies in the management of extremity vascular injury with ischaemia. *British Journal of Surgery*, 99(1), 66–74.
- [3] Hiatt WR, Armstrong EJ, Larson CJ and Brass EP. (2015). Pathogenesis of the limb manifestations and exercise limitations in peripheral artery disease. *Circulation Research*, 116(9), 1527–1539.
- [4] Estebe JP, Davies JM and Richebe P. (2011). The pneumatic tourniquet: Mechanical, ischaemia-reperfusion and systemic effects. *European Journal of Anaesthesiology*, 28(6), 404–411.
- [5] Molyneux SL, Young JM, Florkowski CM, Lever M and George PM. (2008). Coenzyme Q10: is there a clinical role and a case for measurement? *Clin Biochem Rev*, 29(2), 71–82.

- [6] Dong H, Wang N, Zhao L and Lu F. (2012). Berberine in the treatment of type 2 diabetes mellitus: a systemic review and meta-analysis. *Evid Based Complement Alternat Med*, 591-654.
- [7] Tillhon M, Guaman Ortiz LM, Lombardi P and Scovassi AI. (2012). Berberine: new perspectives for old remedies. *Biochem Pharmacol*, 84, 1260-1267.
- [8] Zhang X, Zhang X, Wang C, Li Y, Dong L, Cui L, Wang L, Liu Z, Qiao H, Zhu C, Xing Y, Cao X, Ji Y and Zhao K. (2012). Neuroprotection of early and short-time applying berberine in the acute phase of cerebral ischemia: up-regulated pAkt, pGSK and pCREB, down-regulated NF-kappaB expression, ameliorated BBB permeability. *Brain Research*. 1459, 61–70.
- [9] Hu J, Chai Y, Wang Y, Kheir MM, Li H, Yuan Z, et al. (2011). PI3K p55 $\gamma$  promoter activity enhancement is involved in the anti-apoptotic effect of berberine against cerebral ischemia-reperfusion. *European Journal of Pharmacology*, 674(2), 132–142
- [10] Lee B, Sur B, Yeom M, Shim I, Lee H and Hahm DH. (2012). Effect of berberine on depression and anxiety like behaviors and activation of the noradrenergic system induced by development of morphine dependence in rats. *Korean J Physiol Pharmacol*, 16, 379-386.
- [11] Qin-Wei Z and Yong-Guang LI. (2016). Berberine attenuates myocardial ischemia reperfusion injury by suppressing the activation of PI3K/AKT signaling. *Exp Ther Med*, 11, 978-984.
- [12] Turer AT and Hill JA. (2010). Pathogenesis of myocardial ischemia-reperfusion injury and rationale for therapy. *Am J Cardiol*, 106, 360-8.
- [13] Kılıç Y, Özer A, Tatar T, Zor MH, Kirisci M, Kartal H, Dursun AD, Billur D, Arslan M and Kucuk A. (2017). Effect of picoside II on hind limb ischemia reperfusion injury in rats. *Drug Des Devel Ther*, 11, 1917–1925.
- [14] Hensley K, Robinson KA, Gabbita SP, Salsman S and Floyd RA. (2000). Reactive oxygen species, cell signaling, and cell injury. *Free Radic Biol Med*, 28(10), 1456–1462.
- [15] Braunersreuther V and Jaquet V. (2012). Reactive oxygen species in myocardial reperfusion injury: from physiopathology to therapeutic approaches. *Curr Pharm Biotechnol*, 13, 97-114.
- [16] Garcia-de-la-Asuncion J, Perez-Solaz A, Carrau M et al. (2012). Different oxidative stress marker levels in blood from the operated knee or the antecubital vein in patients undergoing knee surgery: a tourniquet-induced ischemia-reperfusion model. *Redox Rep*, 17, 194-9.
- [17] Yoshioka T, Kawada K, Shimada T and Mori M. (1979). Lipid peroxidation in maternal and cord blood and protective mechanism against activated oxygen toxicity in the blood. *Am J Obstet Gynecol*, 135, 372-376.
- [18] Krawisz JE, Sharon P and Stenson WF. (1984). Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity: assessment of inflammation in rat and hamster models. *Gastroenterology*, 87(6), 1344-1350.
- [19] Tietze F. (1969). Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione. *Anal Biochem*. 27, 502-522.
- [20] Matkovics B, Szabo L and Varga IS. (1988). Determination of enzyme activities in lipid peroxidation and glutathione pathways. *Laboratoriumi Diagnosztika*. 15, 248–249.
- [21] Sun Y, Oberley LW and Li Y. (1988). A simple method for clinical assay of superoxide dismutase. *Clin Chem*, 34, 497-500.
- [22] Goth L. (1991). A simple method for determination of serum catalase activity and revision of serum catalase activity and revision of reference range. *Clin Chim Acta*, 196, 143–152.
- [23] Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193, 265-275.
- [24] Placer ZA, Cushman LL and Johnson BC. (1966). Estimation of product of lipid peroxidation (malonyldialdehyde) in biochemical systems. *Anal Biochem*, 16, 359-364.
- [25] Ball CR. (1996). Estimation and identification of thiols in rat spleen after cysteine or glutathione treatment relevance to protection against nitrogen mustards. *Biochemical Pharmacology*, 15, 809-816.
- [26] Fernandez V and Videla LA. (1981). Effect of acute and chronic ethanol ingestion on the content of reduced glutathione of various tissues of the rat. *Experientia*, 37, 392–394.

- [27] Subramanian P, Mirunalini S, Pandi-Perumal SR, Trakht I and Cardinali D. (2007). Melatonin treatment improves the antioxidant status and decreases lipid content in brain and liver of rats. *Eur J Pharmacol*, 571(2), 116–119
- [28] Sirmali R, Armağan A, Öktem F, et al. (2015). Protective effects of erdosteine, vitamin E, and vitamin C on renal injury induced by the ischemia- reperfusion of the hind limbs in rats. *Turk J Med Sci*, 45(1), 33–37.
- [29] Erer D, Özer A, Demirtaş H, et al. (2016). Effects of alprostadil and iloprost on renal, lung and skeletal muscle injury following hindlimb ischemia-reperfusion injury in rats. *Drug Des Devel Ther*, 10, 2651–2658.
- [30] Takhtfooladi MA, Asghari A, Takhtfooladi HA and Shabani S. (2015). The protective role of curcumin on testicular tissue after hindlimb ischemia reperfusion in rats. *International urology and nephrology*, 47(10), 1605-1610.
- [31] Ertekin A and Apaydin Yildirim B. (2018). Protective effects of Hesperidin and Coenzyme Q10 on experimental gastrocnemius muscle ischemia/reperfusion model in rats. *International Journal of Veterinary Health Science&Research*, 6(1), 219-224.
- [32] Takhtfooladi MA, Jahanshahi A, Sotoudeh A, Daneshi MH, Khansari M and Takhtfooladi HA. (2013).The antioxidant role of n-acetylcysteine in testicular remote injury after skeletal muscle ischemia and reperfusion in rats. *Polish Journal of Pathology*, 64(3), 204–209.
- [33] Wang XT, Tian Y, Xu WX et al. (2015). Protective effects of modeled superoxide dismutase coordination compound (MSODa) against ischemia/reperfusion injury in rat skeletal muscle. *Cellular Physiology and Biochemistry*, 37(2), 465–476.
- [34] Wang G, Yao J, Li Z, Zu G, Feng D, Shan W and Tian X. (2016). miR-34a-5p inhibition alleviates intestinal ischemia/reperfusion induced reactive oxygen species accumulation and apoptosis via activation of SIRT1 signaling. *Antioxidants & redox signaling*, 24(17), 961-973.

---

#### How to cite this article

Apaydin YB and Batil AA. (2019) The investigation of the preventive effects of Coenzyme Q10 and Berberine for tourniquet induced ischemia-reperfusion injury on skeletal muscle in rat hindlimb. *GSC Biological and Pharmaceutical Sciences*, 9(3), 127-133.

---