Effects of Picroside II on Myocardial Ischemia-Reperfusion Injury in Streptozotocin-Induced Rats

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Abstract

Aim: Diabates mellitus, is a chronic metabolic disorder accompanied by an increase in oxidative stress. Ischemia-reperfusion injury is a cascade of events initiated by tissue ischemia. The cellular damage produced by reperfusion leads to an active inflammatory response. This study was performed to investigate the effect of picroside II on myocardial ischemia-reperfusion injury in rats with streptozotocin-induced diabetes.

Materials and Methods: Animals were equally (n:6) divided for five groups as follows; Control (C), diabetes [D], diabetes+picroside II [DP], diabetes+I/R [DIR], and diabetes+I/R+ picroside II [DIRP]. In DIR group, a left anterior descending artery branch was occluded for 60 minutes, the reperfused for 120 minutes. In DIRP group, picroside II was administrated via 10 mg/kg intraperitoneal route 30 minutes before ligating the left anterior descending artery. At the end of the study, myocardial tissues were taken for total oxidant status and total antioxidant status level determinations.

Results: Total oxidant status levels were significantly higher in DIR group, when compared with C, DP, and DIRP groups (*p*:0.001, *p:0*.019, and *p*:0.031, respectively). Total antioxidant status levels were significantly higher in DIR group, when compared with C, DP, and DIRP groups (*p*:0.006, *p*:0.024, and *p:0*.007, respectively).

Conclusion: These results indicate that administration of picroside II may have protective effects against I/R injury.

Key words: Ischemia-reperfusion, total oxidant status, total antioxidant status, picroside II, myocardial tissue, rat

Streptozosin ile Diyabet Oluşturulan Ratlarda Pikrozid II'nin Miyokard İskemi Reperfüzyon Hasarı Üzerine Etkisi

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ÖZET

Giriş: Diyabetes mellitus, oksidatif stres artışının eşlik ettiği kronik metabolik bir hastalıktır. İskemi-reperfüzyon (İ/R) hasarı doku iskemisi tarafından başlatılan olayların bir kaskadıdır. Reperfüzyon sonucunda oluşan hücre hasarı inflamatuar yanıtı aktive eder. Bu çalışma, Streptozosin kaynaklı diyabeti olan ratlarda miyokard İ/R hasarı üzerine Pikrozid II'nin etkisini araştırmak amacıyla yapıldı.

Materyal ve Metod: Hayvanlar, beş gruba (n=6) olacak şekilde ayrıldı; Kontrol grubu (K), Diyabet grubu [D], Diyabet + Pikrozid II grup [DP], Diyabet + İ/R grubu [DİR] ve Diyabet + İ/R + Pikrozid II grubu [DİRP]. DİR grubunda, sol ön inen arter dalı iskemi amaçlı 60 dakika süre ile kapatılmış ardından reperfüzyon için akım sağlanarak 120 dakika beklenmiştir. DİRP grubuna sol ön inen arter dalı kapatılmadan 30 dakika önce Pikrozid II, 10 mg/kg olmak üzere intraperitoneal olarak verildi. Çalışmanın sonunda, miyokard doku örnekleri total oksidan durum ve total antioksidan durum seviyesinin ölçülmesi için alındı.

Bulgular: Toplam oksidan durum seviyeleri DİR grubunda, diğer gruplarla karşılaştırıldığında (K, DP, DİRP) anlamlı yüksek bulunmuştur (p:0.001, p:0.019, ve p:0.031,

sırası ile). Total antioksidan durum seviyeleri DİR grubunda, diğer gruplarla karşılaştırıldığında (K, DP, DİRP) anlamlı olarak yüksek bulunmuştur (p:0.006, p:0.024, ve p:0.007, sırası ile).

Sonuç: Bu bulgular Pikrozid II'nin İ/R hasarına karşı koruyucu etkiye sahip olabileceğini göstermektedir.

Anahtar Kelimeler:, İskemi-reperfüzyon, Total oksidan seviye, Total antioksidan seviye, Pikrozid II, Miyokard dokusu, Rat

Introduction

Ischemic heart disease is a leading cause of morbidity and mortality worldwide (1). Oxygen-derived free radicals are important agents of tissue injury during ischemia and reperfusion (2). In diabetic patients and diabetic rats studies have shown that, oxygen free radicals and lipid peroxidation are significantly increased, and oxidative stress is an important agent of the etiology and progression of diabetes (3).

Picrorhiza scrophulariiflora belongs to the plant family, Scrophulariaceae. The roots of this plant are beneficial to health and often used in traditional Chinese medicine to treat a number of conditions, including dyspepsia, chronic diarrhea, and upper respiratory ailments (4). Numerous published studies have shown that picroside II has a wide range of pharmacological effects, including, antioxidant (5-7)**,** anticarcinogenic (8), and immune modulating activities (9).

The aim of the present study was to examine the potential protective effects of picroside II on myocardial ischemia-reperfusion (I/R) in a diabetic rat model, using biochemical aspects.

Materials and Methods

Experimental Groups

A total of 30 adult Wistar-albino rats, weighing between 210 to 300 g were used in this study. The present study was approved by the Gazi University Institutional Local Animal Care and Use Committee. All animals received humane care, in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and the Use of Laboratory Animals" prepared by the National Academy of Science and published by the National Institutes of Health (NIH publication no. 85–23, revised in 1985). Rats were housed in cages at an average temperature of 22°C in a light-dark cycle-controlled environment with free access to food and tap water.

Study Design

Animals were equally (n:6) divided for five groups as follows; Control (C), diabetes [D], diabetes+ picroside II [DP], diabetes+I/R [DIR], and diabetes+I/R+ picroside II [DIRP]). The rats were kept alive for four weeks after the streptozotocin injection to allow the development of chronic diabetes before they were exposed to I/R. Picroside II (i.p) (Sigma Aldrich Co. Ltd. [CAS No: 39012-20-9, purity greater than 98%, molecular Formula: $C_{23}H_{28}O_{13}$]) was administered via 10 mg/kg^{-} 30 minutes before ligating the left anterior descending artery to the DIRP group. A small plastic snare was threaded through the ligature and placed in contact with the heart. The artery could then be occluded by applying tension to the ligature for 60 minutes, then reperfusion was achieved by releasing the tension for 120 minutes. However, after the above procedure, the coronary artery was not occluded or reperfused in the C, DC, or DP rats. At the end of the reperfusion period, all rats were sacrified under anesthesia, and myocardium was taken for biochemical analyses.

Diabetes was performed with streptozotocin (Sigma Chemical, St. Louis, MO, USA) by giving a single doses of 55 mg/kg intraperitoneally (i.p). 72 hours after the injection the blood glucose levels were measured. If the blood glucose levels exceed 250 mg/dL, then we said the

rats become diabetic. 100 mg/kg (i.p) of ketamine were administered to the rats for anesthesia. The trachea was cannulated for artificial respiration. The chest was shaved, and each animal was fixed in a supine position on the operating table. The chest was opened by a left thoracotomy, followed by sectioning the fourth and fifth ribs about 2 mm to the left of the sternum. Positive-pressure artificial respiration was started immediately with room air, using a volume of 1.5 mL/100 g body weight, at a rate of 60 strokes/min. Sodium heparin (500 IU/kg) was administered through the peripheral vein from the tail. The heart was exteriorized with gentle pressure on the right side of the rib cage after the pericardium was incised. An 8/0 silk suture attached to a 10 mm micropoint reverse-cutting needle was quickly placed under the left main coronary artery. The heart was then carefully replaced in the chest and the animal was allowed to recover for 20 minutes.

Biochemical Examination

The heart tissue was collected into a sterile Eppendorf tube and kept at -80°C until being analyzed for total antioxidant/oxidant status and oxidative stress index. The sample was removed from the Eppendorf tube and dissolution without allowing tissue left quickly weighed 80 to100 mg using a No. 22 surgical scalpel. These tissue pieces were crushed in liquid nitrogen in a porcelain bowl. The powdered tissue was transferred to the homogenization tube, and for every gram of tissue, the dilution of 1/10 140 mM KCl solution was added. Maintaining homogenization in the homogenization tube, a glass beaker full of snow was used to avoid raising the temperature, and the homogenization rocess was complete in two minutes at 50 rpm in a speed homogenizer. After homogenization, the Eppendorf tubes were covered with Parafilm and then centrifuged for 10 minutes at 3,000 rpm. After centrifugation, the supernatant was put into another Eppendorf tube for measurement of total oxidant status (TOS) and total antioxidant status (TAS).

Measurement of myocardial tissue TOS

Myocardial tissue TOS levels were determined using a commercially available kit, developed by Erel (10) (REL Assay Diagnostics, Mega Tip, Gaziantep, Turkey). In this method, the oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ions. Glycerol molecules, which are abundantly present in the reaction medium, enhance the oxidation reaction. The ferric ions produces a colored complex with Xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide, and the results are expressed as μmol H_2O_2 equivalent/L. Hydrogen peroxide and other derivatives of peroxides, produced physiologically in organisms and occurring in higher concentrations under some pathologic conditions, diffuse into plasma. The level of total peroxide was measured and expressed as TOS in this study.

Measurement of myocardial tissue TAS

Myocardial tissue TAS levels were determined using a commercially available kit developed by Erel (REL assay diagnostics, Mega Tip, Gaziantep, Turkey) (11). In this method, hydroxyl radical, which is the most potent radical, is produced via a Fenton reaction. In the classical Fenton reaction, the hydroxyl radical is produced by mixing a ferrous ion solution and a hydrogen peroxide solution. In the most recently developed assay by Erel, the same reaction is used. In the assay, a ferrous ion solution, which is present in Reagent 1, is mixed with hydrogen peroxide, which is present in Reagent 2. The sequentially produced radicals, such as brown-colored dianisidinyl radical cation produced by the hydroxyl radical, are also potent radicals. In this assay, we measured the antioxidative effect of the sample against the potent free radical reactions initiated by the hydroxyl radical. The assay has excellent precision values, lower than 3%. The results are expressed as mmol Trolox equivalents.

Statistical Analyses

The Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) 20.0 program was used for statistical analyses. The Kolmogorov-Smirnov test was used for the comparisons to determine the distribution of all variable groups. We assessed the variations in TOS and TAS levels by using the Kruskal-Wallis test. The Bonferroni-adjusted Mann-Whitney U test was used after the Kruskal-Wallis test to determine which group differs from the others. Results were expressed as mean ± standard deviation (mean ± SD). P values less than 0.05 were considered as statistically significant.

Results

There was a statistically significant difference between the groups when they were compared among themselves by means of TOS levels in myocardial tissue (*p*: 0.026). TOS levels were significantly higher in DIR group when compared with C, DP, and DIRP groups (*p*: 0.001, *p*: 0.019, and *p*: 0.031, respectively). In addition, the DC groups TOS enzyme activity was significantly higher than the C groups (*p*: 0.023) (Figure 1).

A statistically significant difference was found among the groups when they were compared among themselves for TAS levels in myocardial tissue (*p*: 0.012). TAS levels were significantly higher in DIR group when compared with C, DP, and DIRP groups (*p*: 0.006, *p*: 0.024, and *p*: 0.007, respectively). In addition, TAS enzyme activity of DC groups was significantly higher than the C groups activity (*p*: 0.032) (Figure 2). TAS and TOS levels were shown in Table 1.

Discussion

Jennings and colleagues (12), were decribe the I/R injury in 1960. At that time the study of reperfusion injury has become significant to various studies done on the cerebrovascular, hepatic, renal, and cardiovascular systems.

Reactive oxygen species (ROS) generation, intracellular calcium overload, adenosine triphosphate depletion, myocardial apoptosis, and endothelial dysfunction are all considered the end results of an I/R cascade (13, 14). The disclosure of these mechanisms of several drugs has yielded encouraging results in animals and a few have been tested in humans; however, none of these modalities has been widely accepted (15, 16).

In this study, we examined the effect of picroside II on I/R injury in myocardial streptozotocin-induced diabetic rats and in the control group, with the relationship between oxidant and antioxidant effects of picroside II. The study reflects total antioxidant protection against the attacks of free radicals in the organism (TAS) and the total value of oxidative stress (TOS) markers used.

Restoration of the blood supply to the ischemic tissue results in ROS generation. Excessive ROS production causes lipid peroxidation in cell membranes and oxidative damage to DNA and proteins (17). A number of agents, such as levosimendan and dexmedetomidine, have been proposed as useful against I/R-induced myocardial injury (18, 19).

Picroside II, an iridoid glycoside, has been demonstrated to have multiple pharmacologic actions, including decreasing oxidative stress, inhibiting apoptosis, and downregulating the expression of related inflammatory factors (20, 21).

Studies have also observed the kidney and myocardial protective effect of picrocide II by decreasing oxidative stress and downregulating the expression of related inflammatory factors (22, 23).

In two publications (24, 25), a reversible and dose-related inhibition of oxygen production (by neutrophils) was demonstrated in animal models of ischemic myocardial damage in the presence of iodine. In addition, iodine has proven to significantly decrease malondialdehyde (MDA) in animal models of abdominal aortic I/R (26), induce liver peroxidation (27), and reduce hydrogen peroxide-induced pathological glaucomatous changes in cultured cells (28). Proinflammatory cytokines, such as tumor necrosis factor-alpha and interleukin-1 beta, and adhesion molecule, such as the intercellular adhesion molecule-1 (ICAM-1 mRNA), levels were reduced with picroside II (22).

In another study of the effect of picroside II on I/R damaged models, MDA in serum decreased and superoxide dismutase with glutathione peroxidase increased (23, 29).

In this study, we used a novel measurement method to evaluate the extent of oxidative stress in rat myocardium after I/R. This provides a useful method for the rapid evaluation of TAS and TOS, valuable parameters in conditions involving oxidative stress. TOS indicates the total oxidative products in tissue. Oxidative products such as ROS, reactive nitrogen species, hydrochloric acid, MDA, and lipid peroxides constitute TOS (6). In our study, TOS levels significantly increased after myocardial I/R. We also found that I/R+picroside II significantly reduced TOS levels. TAS levels significantly increased in Group DIR. Our findings are consistent with previous papers reporting the antioxidant effects of picroside II on animal models of organ injury induced by myocardial I/R (22, 23). The mechanism of protective effect of picroside II against myocardial injury cannot be explained only by its antioxidative effect, since I/R injury is a complex process. We have hypothesized that to some extent it may also have an antioxidative effect on myocardial injury. Our findings need to be supported by further studies evaluating different oxidative parameters.

Conclusion

Biochemical findings of this study demonstrate that, administration of picroside II may have protective effects, against myocardial injury induced by left anterior descending artery I/R injury and encourage us to investigate this agent in different dosage strategies with alternate administration protocols. Further studies evaluating histological and other biochemical parameters are required to confirm our findings and to elucidate the exact mechanisms of action before clinical use.

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Table 1. Total oxidant status (TOS) and total antioxidant status (TAS) levels of the study groups. [Mean ± SD)]

	Group C (*n* = 6)	**Group DC (*n* = 6)**	**Group DP (*n* = 6)**	**Group DIR (*n* = 6)**	**Group DIRP (*n* = 6)**	***P*****
TOS (µmol H_2O_2 /L**)**	15.08 ± 8.95*	23.4 5 ± 2.76&	19.48 ± 3.37*	28.98 ± 4.83	20.72 ± 6.86*	0.026
TAS (mmol TroloxEquiv)	0.48 ± 0.14*	0.67 ± 0.15&	0.52 ± 0.22*	0.80 ± 0.18	0.48 ± 0.19*	0.012

*P***: $p < .05$ is considered to be significant using Kruskal-Wallis test.

* $p < .05$ compared to the Group DIR and &$p < .05$ compared to Group C

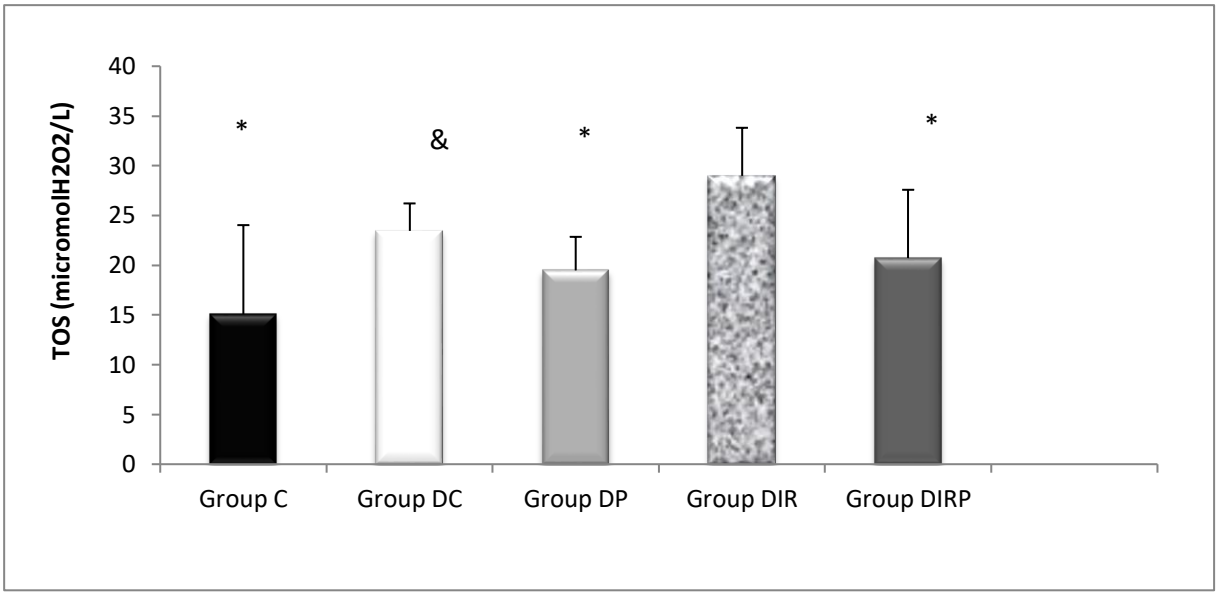

Figure 1. Myocardial tissue total oxidant status (TOS) level [mean ± SD)]

* $p < .05$ compared to Group DIR and &$p < .05$ compared to Group C

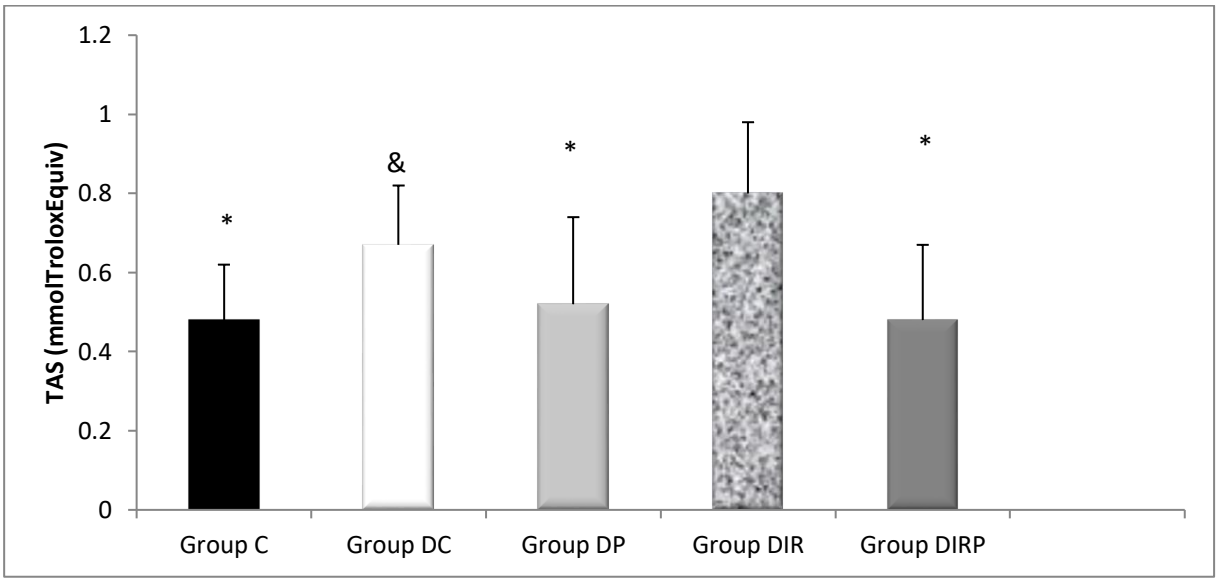

Figure 2. Myocardial tissue total antioxidant status (TAS) level [mean ± SD)]

* $p < .05$ compared to Group DIR and &$p < .05$ compared to Group C