EFFECTS OF ERDOSTEINE, VITAMIN C AND E ON ISCHEMIA/REPERFUSION INDUCED PANCREATIC INJURY IN RATS

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ABSTRACT

Objective: *Ischemia/reperfusion injury may lead to acute pancreatitis through oxidative injury. Administration of different types of free radical scavengers could prevent the pancreatic injury. The aim of this study was to evaluate the protective effects of erdosteine and vitamin C and E on oxidative stress and pancreatic damage in experimental ischemia/reperfusion-induced pancreatitis.*

Material and Method: *Forty rats were divided into four groups: control, ischemia reperfusion, erdosteine administration before ischemia/reperfusion, and vitamins C and E administration before ischemia/reperfusion. Ischemia/reperfusion was performed by occlusion of both hind limbs of the animals. Erdosteine, vitamin C and E were administered for 3 days before ischemia/reperfusion. End of the reperfusion period the entire pancreas was rapidly excised for histological analysis and oxidative stress [malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and reduced glutathione (GSH)] assays.*

Results: *Ischemia/reperfusion produced a significant increase in MDA levels (p=0.002) and CAT activities (p=0.001) in pancreatic tissue when compared with control group. Administration of erdosteine, vitamin C and E before ischemia/reperfusion injury prevented the increase in MDA levels (p=0.002 and p=0.007, respectively) and CAT activities (p=0.008 and p=0.002, respectively). Ischemia/reperfusion produced decreased GSH-Px activity in pancreatic tissue when compared with control group. Administration of erdosteine and vitamins C and E before ischemia/reperfusion injury prevented a significant decrease in GSH-Px activities (p=0.014 and p=0.022, respectively). Nevertheless, GSH levels and SOD activity were not significantly different among groups. The histological analysis showed edema, vacuolization, polymorphonuclear neutrophil (PMN) infiltration and necrosis in ischemia/reperfusion group.*

Conclusion: *The administration of erdosteine and vitamins C and E had a modest protective effect on the oxidative stress and pancreatic injury induced ischemia/reperfusion.*

Key Words: *Erdosteine, ischemia-reperfusion, pancreas, antioxidant, rat.* ***Nobel Med 2012; 8(2): 49-54***

SIÇANLARDA İSKEMİ/REPERFÜZYONA BAĞLI PANKREATİK HASARDA ERDOSTEİN, C VE E VİTAMİNİNİN ETKİSİ

ÖZET

Amaç: İskemi/reperfüzyon hasarı, oksidatif hasar yoluyla akut pankreatite yol açabilir. Bu çalışmanın amacı, deneysel olarak oluşturulmuş iskemi/reperfüzyona bağlı pankreatitte, erdostein C ve E vitamininin oksidatif stres ve pankreas hasarı üzerine koruyucu etkilerini değerlendirmektir.

Materyal ve Metod: Kırk sıçan 4 gruba ayrıldı: kontrol, iskemi/reperfüzyon, iskemi/reperfüzyon öncesi erdostein verilenler, iskemi/reperfüzyon öncesi C ve E vitamini verilenler. Hayvanlar her iki arka ayağı bağlanarak iskemi/reperfüzyon oluşturuldu. İskemi/reperfüzyon öncesi 3 gün erdostein, C ve E vitamini verildi. Reperfüzyon periyodunun sonunda histolojik analiz, oksidatif stres [malandialehit (MDA), süperoksit dismutaz (SOD), katalaz (CAT), glutatyon peroksidaz (GSH-Px) ve azalmış glutatyon (GSH)] ölçümleri için tüm pankreas çıkarıldı.

Bulgular: Kontrol grubu ile karşılaştırıldığında iskemi/reperfüzyon, pankreas dokusundaki MDA düzeyleri (p=0,002) ve CAT aktivitesinde (p=0,001) belirgin artışa sebep oldu. İskemi/reperfüzyon hasarı öncesi erdostein, C ve E vitamini verilmesi MDA düzeylerindeki ve CAT aktivitesindeki artışı önledi. (MDA için p=0,002 ve p=0,007, CAT için p=0,008 ve p=0,002). Kontrol grubu ile karşılaştırıldığında, iskemi/reperfüzyon pankreas dokusunda GSH-Px aktivitesini azalttı. Hasar öncesi erdostein, C ve E vitamini verilmesi ile GSH-Px aktivitesindeki belirgin düşüş önlendi. (p=0,0014 ve p=0,0022). Gruplar arasında GSH düzeyleri ve SOD aktivitesi açısından fark yoktu. Histolojik incelemede iskemi/reperfüzyon grubunda ödem, vakuolizasyon, polimorfonüklear nötrofil (PMN) infiltrasyon ve nekroz izlendi.

Sonuç: Erdostein, C ve E vitamininin iskemi/reperfüzyona bağlı pankreas hasarı ve oksidatif stres üzerinde orta derecede koruyucu etkisi vardır.

Anahtar Kelimeler: Erdostein, iskemi-reperfüzyon, pankreas, antioksidan, sıçan **Nobel Med 2012; 8(2): 49-54**

INTRODUCTION

Ischemic injury may play an important role in the pathogenesis of acute pancreatitis.[1,2] Development of acute pancreatitis secondary to ischemia has been observed after cardiac surgery and systemic shock.[3,4] Furthermore, the reperfusion following ischemia of pancreatic tissue could cause further injury. Experimental models have shown that ischemia/reperfusion causes hemorrhagic and necrotizing pancreatitis.[5,6] Clinically, this situation is observed after transplantation of the pancreas, and ischemia/reperfusion injury may lead to graft pancreatitis.[7] The production of oxygen free radicals and activation of neutrophils have been identified among the possible mechanisms of pancreatic damage during ischemia/reperfusion injury.[5,6,8] Overproduction of oxygen free radicals in the pancreas overwhelms the scavenging capacity of endogenous antioxidants, leading to cell injury through peroxidation of the lipid component of mitochondrial and cellular membranes.[9]

Studies in different experimental pancreatitis models have shown that the administration of different types of free radical scavengers could decrease the severity of pancreatic injury. The administration of antioxidants such as caffeic acid phenethyl ester, melatonin, N-acetyl cysteine, ascorbic acid, and grapefruit seed extract improved pancreatic injury and oxidative damage in several models including ischemia/reperfusion-induced acute pancreatitis, cerulein-induced acute pancreatitis and dibutyltin dichloride-induced chronic pancreatitis.[10-14]

Erdosteine (N-carboxymethylthioacetyl-homocysteine thiolactone), which is a mucolytic agent, contains two sulphydryl groups that are available for free radical scavenging.[15] In experimental studies, erdosteine prevented oxidative stress induced by both toxic agents in various types of tissues and ischemia/reperfusion (I/R) in kidney, lung and spinal cord.[16-22]

The most frequently reported antioxidant vitamins are vitamin C and E.[23,24] Vitamin C is a hydrophilic molecule that can scavenge several radicals, among them the hydroxyl radical. Vitamin E is a lipophilic antioxidant interfering with the chain reaction of lipid peroxidations.[23] It is likely that vitamin C and E act in a synergistic manner, by vitamin E primarily being oxidized to the tocopheroxyl radical and then reduced back to tocopherol by vitamin C.[23] Therefore, we decided to use the lipophilic antioxidant vitamin E and the hydrophilic antioxidant vitamin C together as an antioxidant agent in our rat model for I/R-induced injury.

However, there is no report about the relation between erdosteine, vitamin C, vitamin E and oxidative stress in→

pancreas tissue. The aim of this study was to evaluate the preventive effect of erdosteine, vitamin C and E on oxidative stress and pancreatic injury in experimental ischemia/reperfusion-induced pancreatitis.

MATERIAL and METHOD

Animals

Experiments were performed on 40 adult male Sprague-Dawley rats weighing 250-352 g. All animal procedures were completed in accordance with National Institutes of Health Guidelines on the care and use of laboratory animals for research purposes. Animal rights were protected. The protocol was approved by the ethical committee of Süleyman Demirel University School of Medicine.

Experimental procedures

Animals were divided into four experimental groups (10 rats in each group) as follows: control; ischemia/reperfusion; erdosteine administration before ischemia/reperfusion; vitamin C and E administration before ischemia/reperfusion. In the erdosteine group, animals received erdosteine per orally at a daily dose of 150 mg/kg for 3 days before ischemia/reperfusion.[23] In the vitamin C and E group, animals received intraperitoneal vitamin C 200 mg/kg/d and intramuscular vitamin E 150 mg/kg/d for 3 days before ischemia/reperfusion.[23] Animals in the control and ischemia/reperfusion groups received saline solution intraperitoneally for 3 days.

Animals were anesthetized with ketamine/xylazine (100/10 mg/kg) intramuscularly. Ischemia/reperfusion was carried out by occlusion of both hind limbs (proximal to trochanter major) of the animals using tourniquets. After 60 min ischemia, the tourniquets were removed to allow blood reperfusion for 120 min. Midline laparotomy was performed in all animals at the end of the reperfusion period. The entire pancreas was rapidly excised and sectioned into two pieces along the longitudinal axis for histological analysis and biochemical assays. The pancreas samples were rinsed in cold phosphate-buffered saline (pH 7.4) and stored in a deepfreeze (–20°C) until biochemical analyses.

Measurement of oxidative stress status

Tissue samples were homogenized in four volumes of ice-cold Tris-HCl buffer (50 mM, pH 7.4) in a teflon homogenizer (Ultra Turrax IKA T18 Basic, USA) for 2 min at 5,000*g* at 4°C. Then the levels of malondialdehyde (MDA) and protein were measured. The homogenate was then centrifuged at 5,000*g* at 4°C for 60 min. The supernatant was collected for each assay of protein levels, glutathione peroxidase (GSH-Px) and catalase (CAT) activities. An equal volume of an ethanol/chloroform mixture (5:3, v/v) was added to the homogenate and the supernatant solution was extracted. This was clarified by centrifugation at 5,000*g* at 4°C for 30 min. The clear upper layer was collected and assayed for superoxide dismutase (SOD) activity and protein level.

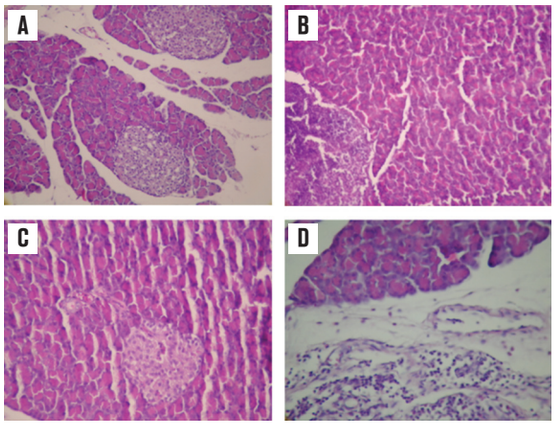

Figure 1. A: Animals from control group presented no histological alterations, **B-D:** The pancreatic specimens from other three groups showed vacuolization, PMN cell infiltration, edema and necrosis.

MDA level was measured by using the method of Draper and Hadley.[25] The principle of the method is the spectrophotometric measurement of the color produced during the reaction to thiobarbituric acid (TBA) with MDA. For this purpose, 2.5 ml of 100 g/l trichloroacetic acid solution was added to 0.5 ml of serum in a centrifuge tube and placed in a boiling water bath for 15 min. After cooling in tap water, the mixture was centrifuged at 1,000×g for 10 min, and 2 ml of the supernatant was added to 1 ml of 6.7 g/l TBA solution in a test tube and placed in a boiling water bath for 15 min. The solution was then cooled in tap water, and its absorbance was measured using a spectrophotometer (UV-1601, Shimadzu, Japan) at 532 nm. The results were expressed as nanomole per gram protein in pancreas tissue.

The method for the measurement of SOD as described by Woolliams et al. was based on the principle in which xanthine reacts with xanthine oxidase to generate superoxide radicals that react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazon dye.[26] The SOD activity is then measured by the degree of inhibition of this reaction. Superoxide dismutase activity was expressed as units per gram protein.

CAT activity was quantified in pancreas tissue by the method of Aebi.[27] The principle of the method is based on the determination of the rate constant (s^{-1}, k) for H_2O_2 decomposition at 240 nm. Results were expressed as k (rate constant) per gram protein. →

Table 1: Parameters of oxidative stress in ischemia/reperfusion induced pancreatic injury in rats*

Groups	SOD (U/g protein)	CAT (k/g protein)	GSH-Px (U/g protein)	GSH (nmol/mg protein)	MDA (nmol/g protein)
1- Control (n=10)	395.1± 36.6	0.47±0.07	14.28±1.28	43.69±4.89	1.96±0.27[f]
2- I/R (n=10)	296.6±34.2	1.22±0.24[ac]	10.38±1.47[d]	53.03±4.46	3.14±0.27
3- Erdosteine+I/R (n=10)	374.4±42.7	0.64±0.11[b]	16.63±1.91	44.09±6.49	2.03±0.19[g]
4- Vitamins C and E+I/R (n=10)	378.1±34.3	0.53 ± 0.09	16.15±2.04[e]	52.89±4.72	2.18±0.21[h]
P values					
Group 1 - Group 2	NS	0.001	NS	NS	0.002
Group 1 - Group 3	NS	NS	NS	NS	NS
Group 1 - Group 4	NS	NS	NS	NS	NS
Group 2 - Group 3	NS	0.008	0.014	NS	0.002
Group 2 - Group 4	NS	0.002	0.022	NS	0.007

I/R; ischemia/reperfusion, MDA; malondialdehyde, SOD; superoxide dismutase, CAT; catalase, GSH-Px; glutathione peroxidase, GSH; reduced glutathione, NS; not significant, *All parameters expressed as mean ± SE and analyzed by one-way ANOVA, a: p=0.001, When Control group was compared with I/R group, b: p=0.008, When I/R group was compared with Erdosteine + I/R group, c: p=0.002, When I/R group was compared with Vitamin C and E + I/R group, d: p=0.014, When I/R group was compared with Erdosteine + I/R group, e: p=0.022, When I/R group was compared with Vitamin C and E + I/R group, f: p=0.002, When Control group was compared with I/R group, g: p=0.002, When I/R group was compared with Erdosteine + I/R group, h: p=0.007, When I/R group was compared with vitamin C and E + I/R group

GSH-Px activity was quantified in pancreas tissue by the method of Paglia and Valentine.[28] The principle of the method was as follows: GSH-Px catalyzes the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance of NADPH was measured by a spectrophotometer at 340 nm. The activity was expressed as units per gram protein.

Reduced glutathione (GSH) levels were measured in pancreas according to the method described by Beutler.[29] The amount of total GSH was determined from a standard curve obtained with known amounts of GSH standards. GSH levels were expressed as nanomole/milligram protein. Protein was assayed by using the method of Lowry et al.[30]

Histological examination

The injury of the pancreas was evaluated by histological examination of tissue sections (5 μm) fixed in 10% buffered formalin and stained with hematoxylin and eosin (H&E). One pathologist experienced in pancreatic pathology performed the histopathological evaluation blindly. Edema, vacuolization, polymorphonuclear neutrophil (PMN) infiltration and necrosis were assessed in detail and the changes were represented as follows; 0=normal, 1=low grade, 2=moderate, and 3=severe.[3]

Statistical analysis

Data were analyzed using the SPSS® for Windows computing program. All results were expressed as mean±SE. One-way analysis of variance (ANOVA) and post-hoc multiple comparison tests (LSD) were performed on the data of biochemical variables to examine the difference among groups. P values <0.05 were regarded as statistically significant.

RESULTS

The results of the biochemical analyses of pancreatic tissue were shown in Table 1. Ischemia/reperfusion resulted a significant increase in MDA levels (p=0.002) and CAT activities (p=0.001) in pancreatic tissue when compared with control group. Administration of erdosteine and vitamin C and E together before ischemia/reperfusion injury prevented the increase in MDA levels (p=0.002 and p=0.007, respectively) and CAT activities (p=0.008 and p=0.002, respectively). Ischemia/reperfusion injury resulted a decrease in GSH-Px activity in pancreatic tissue when compared with control group. Administration of erdosteine and vitamins C and E before ischemia/reperfusion injury prevented a significant decrease in GSH-Px activities (respectively, p=0.014 and p=0.022). Nevertheless, GSH levels and SOD activity were not significantly different among groups.

The results of the histological analysis of pancreatic tissue were shown in Table 2. The histological analysis showed that edema (p=0.0001), vacuolization (p=0.0001), polymorphonuclear neutrophil (PMN) infiltration (p=0.0001) and necrosis (p=0.004) in ischemia/reperfusion group compared with control group. Administration of erdosteine and vitamins C and E before ischemia/reperfusion injury prevented a significant decrease in edema (p=0.0001 and p=0.048, respectively). Administration of erdosteine before ischemia/reperfusion injury prevented a significant decrease in PMNs (p=0.013).

Animals from control group presented no histological alterations (Figure 1A). The pancreatic specimens from other three groups showed vacuolization, PMN cell infiltration, edema and necrosis (Figure 1B-D).

DISCUSSION

We studied the protective effects of erdosteine and vitamin C and E combination during ischemia/reperfusion induced pancreatic injury in rats. The effect of these compounds on the levels of GSH and MDA and enzyme activities of SOD, CAT, and GSH-Px were investigated. Also, histological changes were examined→

by light microscopy. In the present study, ischemia/reperfusion enhanced oxidative stress and pancreatic injury whereas erdosteine and vitamin C and E combination reduced these effects. In this study, MDA levels in the ischemia/reperfusion group significantly increased when compared with the control group.

Previous studies show that ischemia/reperfusion induce MDA levels in animal models.[22,23,31,32] On the other hand, in the erdosteine and vitamins C and E groups the MDA levels significantly decreased when compared with the ischemia/reperfusion group. In experimental studies the antioxidant activity of erdosteine demonstrated in various organs other than pancreas.[20-23] These studies have shown that the administration of erdosteine prevents lipid peroxidation induced by ischemia/reperfusion. We also found that erdosteine prevented the increase of MDA concentration, which is the measure of lipid peroxidation in the pancreatic tissue during ischemia/reperfusion. This may be related to the free radical scavenging properties of erdosteine, which contains two blocked sulfhydryl groups accounting for its antioxidant activity.[33] Several studies have demonstrated the antioxidant activities of vitamins C and E in various organs including pancreas.[12,13,24,34] Lu et al. showed that ascorbic acid treatment markedly decreases MDA concentrations in pancreatic tissue.[13] We hypothesized that the vitamin C and E may effectively protect pancreas by their antioxidant effects on ischemia/reperfusion induced pancreatic injury.

Studies investigating the antioxidant effect of erdosteine have shown varying activities of endogenous antioxidant enzymes including SOD, CAT and GSH-Px after ischemia/reperfusion injury.[20,22,23] While SOD activity has increased in renal, spinal cord and lung tissues, the activity of CAT has decreased in spinal cord but has increased in lung tissue. GSH-Px activity has exhibited a decrease in spinal cord tissue but has no change in lung tissue after ischemia/reperfusion injury. This may be due to interorgan differences in the antioxidant activity and susceptibility to oxidant injury. CAT and SOD are the main enzymes of the enzymatic antioxidant defence system responsible for protection against the increase in production of reactive oxygen species.[35] Hydrogen peroxide formed by the catalytic reaction of SOD is both a reactive form of oxygen and a normal cellular metabolite, and it is further detoxified by GSH-Px and CAT.[35] In this study, CAT activities in pancreatic tissue were increased in the ischemia/reperfusion group. On the other hand, the increased activities of CAT could be due to its depletion or inhibition as a result of the increased production of free radicals by GSH-Px. In agreement with this hypothesis, SOD and GSH-Px activities are tended to decrease in ischemia/reperfusion group, despite this depletion did not reach to significance. Nevertheless, erdosteine has restored the activities of these enzymes by attenuation of oxidative stress. In the present study, GSH levels were not significantly different among groups.

Table 2: Parameters of histopathological evaluation in ischemia/reperfusion induced pancreatic injury in rats*

Groups	Edema	Vacuolization	PMN	Necrosis
1- Control (n=10)	0.00±0.0	0.00±0.0	0.00±0.0	0.00±0.0
2- I/R (n=10)	1.70±0.2[a]	1.40±0.2[d]	1.10±0.2[d,h]	0.90±0.2[j]
3- Erdosteine+I/R (n=10)	0.50±0.1[b]	0.80±0.2[e]	0.40±0.1	0.50±0.2
4- Vitamins C and E+I/R (n=10)	1.10±0.2[c]	1.10±0.2[f]	0.80±0.2[g]	0.70±0.2[j]
P values				
Group 1 - Group 2	0.0001	0.0001	0.0001	0.004
Group 1 - Group 3	NS	0.018	NS	NS
Group 1 - Group 4	0.001	0.002	0.005	0.022
Group 2 - Group 3	0.0001	NS	0.013	NS
Group 2 - Group 4	0.048	NS	NS	NS

NS: not significant. PMN: polymorphonuclear, I/R: ischemia/reperfusion, *All parameters expressed as mean ± SE and analyzed by one-way ANOVA, a: p=0.0001, When I/R group was compared with control and Erdosteine + I/R group b: p=0.001, When control group was compared with vitamin C and E + I/R group, c: p=0.048, When I/R group was compared with vitamin C and E + I/R group, d: p=0.0001, When control group was compared with I/R group e: p=0.018, When control group was compared with Erdosteine + I/R group, f: p=0.002,When control group was compared with vitamin C and E + I/R group, g: p=0.005, When control group was compared with vitamin Cand E + I/R group, h: p=0.013, When I/R group was compared with Erdosteine + I/R group, i: p=0.004 , When control group was compared with I/R group, j: p=0.022, When control group was compared with vitamin C and E + I/R group

Among other factors, the specific type of pancreatitis, severity of pancreatic injury and the time length of ischemia and reperfusion represent the oxidative stress response.[36-38] In the present study, we used a rat hind limb model to induce ischemia and reperfusion. It was demonstrated that ischemia and reperfusion of lower extremities could lead to oxidative injury of lung as a remote organ.[23] On the contrary to the models of in situ ischemia/reperfusion of the pancreas ischemia is induced by clamping the main supplying vessels, we induced damage to pancreatic tissue by in situ hind limb ischemia as a simple and reproducible model.

Histologically, pancreatic ischemia/reperfusion injury is characterized by interstitial edema, focal hemorrhage, and granulocyte infiltration.[38] Our histological parameters support biochemical parameters. The tissues of the ischemia/reperfusion group showed significantly histopathological changes including edema, vacuolization, polymorphonuclear neutrophil (PMN) infiltration and necrosis. On the other hand, in erdosteine group the edema and PMN infiltration found significantly decreased when compared with the ischemia/reperfusion group. In the vitamin C and E combination group, the edema significantly decreased when compared with the ischemia/reperfusion group. In erdosteine group, the damage was less severe than in the ischemia/reperfusion and vitamin C and E groups. Erdosteine seems to have more protective effect on ischemia/reperfusion injury of pancreas.→

CONCLUSION

In conclusion, the administration of erdosteine and vitamins C and E before ischemia/reperfusion they may reduce oxidative stress and histological damages in pancreatic tissue. Although further studies are needed, erdosteine and vitamins C and E may also have a positive effect on prevention for the conversion of pancreatitis from edematous to hemorrhagic state.

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REFERENCES

1. Sanfey H, Bulkley GB, Cameron JL. The role of oxygen-derived free radicals in the pathogenesis of acute pancreatitis. Ann Surg 1984; 200: 405.
2. Kyogoku T, Manabe T, Tobe T. Role of ischemia in acute pancreatitis. Hemorrhagic shock converts edematous pancreatitis to hemorrhagic pancreatitis in rats. Dig Dis Sci 1992; 37: 1409.
3. Perez A, Ito H, Farivar RS, et al. Risk factors and outcomes of pancreatitis after open heart surgery. Am J Surg 2005; 190: 401.
4. Isenmann R, Henne-Bruns D, Adler G. Gastrointestinal disorders of the critically ill. Shock and acute pancreatitis. Best Pract Res Clin Gastroenterol 2003; 17: 345.
5. Obermaier R, Benz S, Kortmann B, et al. Ischemia/reperfusion-induced pancreatitis in rats: a new model of complete normothermic in situ ischemia of a pancreatic tail-segment. Clin Exp Med 2001; 1: 51.
6. Dembiński A, Warzecha Z, Ceranowicz P, et al. Pancreatic damage and regeneration in the course of ischemia-reperfusion induced pancreatitis in rats. J Physiol Pharmacol 2001; 52: 221.
7. Benz S, Bergt S, Obermaier R, et al. Impairment of microcirculation in the early reperfusion period predicts the degree of graft pancreatitis in clinical pancreas transplantation. Transplantation 2001; 71: 759.
8. Balén EM, Ferrer JV, Zazpe C, et al. Assessment of oxidative stress in a pancreatic warm ischemia-reperfusion model. Transplant Proc 1998; 30: 569.
9. Bonham MJ, Abu-Zidan FM, Simovic MO, et al. Early ascorbic acid depletion is related to the severity of acute pancreatitis. Br J Surg 1999; 86: 1296.
10. Turkyilmaz S, Alhan E, Ercin C, et al. Effects of caffeic acid phenethyl ester on pancreatitis in rats. J Surg Res 2008; 145: 19.
11. Muñoz-Casares FC, Padillo FJ, Briceño J, et al. Melatonin reduces apoptosis and necrosis induced by ischemia/reperfusion injury of the pancreas. J Pineal Res 2006; 40: 195.
12. Eşrefoğlu M, Gül M, Ates B, et al. Antioxidative effect of melatonin, ascorbic acid and N-acetylcysteine on caerulein-induced pancreatitis and associated liver injury in rats. World J Gastroenterol 2006; 12: 259.
13. Lu XL, Song YH, Fu YB, et al. Ascorbic Acid Alleviates Pancreatic Damage Induced by Dibutyltin Dichloride (DBTC) in Rats. Yonsei Med J 2007; 48: 1028.
14. Dembinski A, Warzecha Z, Konturek SJ, et al. Extract of grapefruit-seed reduces acute pancreatitis induced by ischemia/reperfusion in rats: possible implication of tissue antioxidants. J Physiol Pharmacol 2004; 55: 811.
15. Moretti M, Marchioni CF. An overview of erdosteine antioxidant activity in experimental research. Pharmacol Res 2007; 55: 249.
16. Yilmaz HR, Iraz M, Sogut S, et al. The effects of erdosteine on the activities of some metabolic enzymes during cisplatin-induced nephrotoxicity in rats. Pharmacol Res 2004; 50: 287.
17. Selcoki Y, Uz E, Bayrak R, et al. The protective effect of erdosteine against cyclosporine A-induced cardiotoxicity in rats. Toxicology 2007; 239: 53.
18. Yagmurca M, Bas O, Mollaoglu H, et al. Protective effects of erdosteine on doxorubicin-induced hepatotoxicity in rats. Arch Med Res 2007; 38: 380.
19. Oktem F, Arslan MK, Ozguner F, et al. In vivo evidences suggesting the role of oxidative stress in pathogenesis of vancomycin-induced nephrotoxicity: protection by erdosteine. Toxicology 2005; 215: 227.
20. Ege E, Ilhan A, Gurel A, et al. Erdosteine ameliorates neurological outcome and oxidative stress due to ischemia/reperfusion injury in rabbit spinal cord. Eur J Vasc Endovasc Surg 2004; 28: 379.
21. Kurtoglu T, Sacar M, Inan BK, et al. Erdosteine ameliorates lung injury induced by transient aortic occlusion in rats. Cardiovasc J Afr 2007; 18: 367.
22. Erdogan H, Fadillioglu E, Yagmurca M, et al. Protein oxidation and lipid peroxidation after renal ischemia-reperfusion injury: protective effects of erdosteine and N-acetylcysteine. Urol Res 2006; 34: 41.
23. Sirmali M, Uz E, Sirmali R, et al. Protective effects of erdosteine and vitamins C and E combination on ischemia-reperfusion-induced lung oxidative stress and plasma copper and zinc levels in a rat hind limb model. Biol Trace Elem Res 2007; 118: 43.
24. Altuntas I, Delibas N, Demirci M, et al. The effects of methidathion on lipid peroxidation and some liver enzymes: role of vitamins E and C. Arch Toxicol 2002; 76: 470.
25. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol 1990; 186: 421.
26. Woolliams JA, Wiener G, Anderson PH, et al. Variation in the activities of glutathione peroxidase and superoxide dismutase and in the concentration of copper in the blood in various breed crosses of sheep. Res Vet Sci 1983; 34: 253.
27. Aebi H. Catalase in vitro. Methods Enzymol 1984; 105: 121.
28. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70: 158.
29. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61: 882.
30. Lowry OH, Rosebrough NJ, Farr AL, et al. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265.
31. Obermaier R, Von Dobschuetz E, Benthues A, et al. Exogenous and endogenous nitric oxide donors improve post-ischemic tissue oxygenation in early pancreatic ischemia/reperfusion injury in the rat. Eur Surg Res 2004; 36: 219.
32. Irmak MK, Koltuksuz U, Kutlu NO, et al. The effect of caffeic acid phenethyl ester on ischemia-reperfusion injury in comparison with alpha-tocopherol in rat kidneys. Urol Res 2001; 29: 190.
33. Dechant KL, Noble S. Erdosteine. Drugs 1996; 52: 875.
34. Gómez JA, Molero X, Vaquero E, et al. Vitamin E attenuates biochemical and morphological features associated with development of chronic pancreatitis. Am J Physiol Gastrointest Liver Physiol 2004; 287: 162.
35. Nazıroğlu M. Molecular Mechanisms of vitamin E on intracellular signaling pathways in brain. In: Goth L (ed). Reactive Oxygen Species and Diseases. Research Signpost Press Kerala, India 2007; 239.
36. Sajewicz W, Milnerowicz S, Nabzdyk S. Blood plasma antioxidant defense in patients with pancreatitis. Pancreas 2006; 32: 139.
37. Simovic MO, Bonham MJ, Abu-Zidan FM, et al. Manganese superoxide dismutase: a marker of ischemia-reperfusion injury in acute pancreatitis? Pancreas 1997; 15: 78.
38. Hoffmann TF, Leiderer R, Harris AG, et al. Ischemia and reperfusion in pancreas. Microsc Res Tech 1997; 37: 557.