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FİGEN NARİN

NAZMİ NARİN

FATMAGÜL BAŞARSLAN

ALİ BAYKAN

SADETTİN SEZER

See next page for additional authors

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The effect of L-tryptophan on the heart in rabbits via chronic hypoxia

Authors

FİGEN NARİN, NAZMİ NARİN, FATMAGÜL BAŞARSLAN, ALİ BAYKAN, SADETTİN SEZER, HÜLYA AKGÜN, AYNUR AKIN, MUSTAFA AKÇAKUŞ, and HAKAN CEYRAN

The effect of L-tryptophan on the heart in rabbits via chronic hypoxia

Figen NARİN¹, Nazmi NARİN², Fatmagül BAŞARSLAN¹, Ali BAYKAN²,
Sadettin SEZER², Hülya AKGÜN³, Aynur AKIN⁴, Mustafa AKÇAKUŞ², Hakan CEYRAN⁵

Aim: To evaluate the protective effect of tryptophan on an experimentally produced hypoxic myocardial injury via biochemical and pathological parameters.

Materials and methods: A total of 26 rabbits were divided into 3 groups. Group 1 (n = 9) was only exposed to hypoxia. Group 2 (n = 10) was exposed to hypoxia and received L-tryptophan (200 mg/kg per day, orally for 5 days). Group 3 (n = 7) was the control group.

Before the hypoxic injury and after the delivery of the medication, serum samples were taken for troponin-I, creatine kinase myocardial isoenzymes (CK-MB), lactate dehydrogenase (LDH), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), malondialdehyde (MDA), and nitric oxide (NO) analysis, and then the rabbits were sacrificed. Next, the myocardium samples were taken and the myocardial NO, MDA, SOD, and GSH-Px enzyme activity levels were studied histopathologically.

Results: In group 1, Serum GSH-Px and SOD activities were decreased. Conversely, troponin-I, CK-MB, and LDH were elevated. Severe cardiac injury was observed histopathologically. In group 2, serum troponin-I and SOD values were increased. Mild cardiac injury was demonstrated histopathologically.

When groups 1 and 2 were compared, tissue NO and MDA levels in group 1 were higher compared to group 2, but GSH-Px level was found decreased in group 1.

Conclusion: Our findings support that there is a clear effect of the free oxygen radicals and the lipid peroxidation products on hypoxic cardiac injury. In addition, L-tryptophan supplementation has a strong protective effect on hypoxic heart by antioxidant activity.

Key words: Hypoxia, myocardial injury, cardioprotection, tryptophan, oxidative stress

Kronik hipoksili tavşanlarda L-triptofanın kalp üzerine etkisi

Amaç: Bu çalışmanın amacı, deneysel olarak meydana getirilmiş hipoksik miyokardial hasara karşı triptofanın koruyucu etkisini, biyokimyasal ve patolojik parametreler kullanarak değerlendirmektir.

Yöntem ve gereç: Toplam 26 tavşan üç gruba ayrıldı. Grup 1, 9 tavşan yalnızca hipoksiye maruz kaldı. Grup 2, 10 tavşan hem hipoksiye maruz kaldı ve L-triptofan aldı. Grup 3, 7 tavşan kontrol grubu olarak seçildi.

Hipoksik hasar öncesi ve ilaç tedavisi uygulandıktan sonra troponin-I, kreatin kinaz miyokard izoenzimi (CKMB), laktat dehidrogenaz (LDH), glutatyon peroksidaz (GSH-Px), süperoksid dismutaz (SOD), malondialdehid (MDA) ve nitrik oksit (NO) için analizler yapıldı ve tavşanlar sakrifiye edildi. Daha sonra miyokardiyal numuneler alındı ve NO, MDA, SOD, GSH-Px enzim aktivite düzeyleri histopatolojik olarak değerlendirildi.

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¹ Department of Biochemistry, Faculty of Medicine, Erciyes University, Kayseri - TURKEY

² Department of Pediatrics, Faculty of Medicine, Erciyes University, Kayseri - TURKEY

³ Department of Pathology, Faculty of Medicine, Erciyes University, Kayseri - TURKEY

⁴ Department of Anesthesiology, Faculty of Medicine, Erciyes University, Kayseri - TURKEY

⁵ Department of Heart Surgery, Faculty of Medicine, Erciyes University, Kayseri - TURKEY

Correspondence: Sadettin SEZER, Department of Pediatrics, Faculty of Medicine, Erciyes University, Talas, Kayseri - TURKEY

E-mail: sadettinsezer@yahoo.com

Bulgular: Grup 1'de serum GSH-Px ve SOD aktivitesi azaldı. Aksine, troponin-I, CK-MB ve LDH arttı. Histopatolojik olarak şiddetli kardiyak hasar gözlemlendi. Grup 2'de serum troponin-I ve SOD değerleri arttı. Histopatolojik olarak hafif kardiyomyopati gösterildi.

Sonuç: Bulgularımız, hipoksik bir kardiyomyopatide serbest oksijen radikallerinin ve lipid peroksidasyon ürünlerinin belirgin bir etkisi olduğunu desteklemektedir. Aynı zamanda, L-triptofan ilavesi, hipoksik kalp üzerine antioksidan bir etki yaparak güçlü bir koruyucu etkiye sahiptir.

Anahtar sözcükler: Hipoksi, miyokardiyal hasar, kalbin korunması, triptofan, oksidatif stres

Introduction

Chronic hypoxia is associated with increased oxidative stress as evidenced by marked lipid peroxidation and induction of antioxidant enzyme response in various tissue organs (1). Especially, hypoxia and ischemia have an important role on cardiovascular diseases (2). There are studies on etiopathogenesis and prophylaxis of hypoxic and ischemic injuries. By demonstration of the role of free oxygen radicals and antioxidant enzymes in injury, antioxidant treatment modalities are favorable in these days (3).

In the early stages of myocardial ischemia/hypoxia, neutrophils and monocytes infiltrate intima and degenerate the endothelial cells. Free oxygen radicals, cytokine, and nitric oxide are released by the degeneration of the endothelial cells and cause lipid peroxidation. The products of lipid peroxidation, such as malondialdehyde (MDA), result in myocardial damage (4,5).

Creatine kinase (CK-MB), lactate dehydrogenase, and troponin-I (Tn-I) are used to determine the myocardial injury (1). Researchers are still looking for appropriate antioxidant agent to protect myocardium from the hypoxic and ischemic injuries (3).

L-tryptophan is the precursor of melatonin and has a protective effect on the free oxygen radical neutralization (6). Reiter et al. reported that N-acetylserotonin, which is a derivative of melatonin or tryptophan, also has a free oxygen radical neutralization activity. Moosmann et al. reported that membrane lipoproteins, especially in the lipid dense regions, have high concentrations of tryptophan (7,8). High levels of tryptophan protect the lipid layer from peroxidation, which indicates that tryptophan may be used as a pharmacological agent as a cytoprotective antioxidant (9). Today, all the effects of tryptophan are not clear yet, and in the literature there are a limited number of studies on this subject.

In this study, a myocardial hypoxic injury was created experimentally and the protective effect of tryptophan was evaluated by observing troponin-I and CK-MB levels. Because they are involved in tissue injuries, SOD, NO, GPX, MPO, and tryptophan interactions were also evaluated.

Materials and methods

The study was performed on 26 New-Zealand white rabbits weighing between 1300 g and 2600 g. All rabbits were male with an age range of 60-90 days. After a 15-day adaptation period, rabbits were distributed into 3 groups: group 1 (n = 9) was only exposed to hypoxia. Group 2 (n = 10) was exposed to hypoxia and received L-tryptophan. Group 3 (n = 7) was the control group and received distilled water.

A hypoxic condition was obtained using a 25 × 25 × 62 cm³ funnel with input and output ports for air. The funnel was ventilated with 10% oxygen and 90% nitrogen mixture at 5 L/min flow rate for 5 min. Oxygen-nitrogen mixture flow continued for an additional 10 min after the rabbits were placed in the funnel. The oxygen saturation and the heart rate were monitored with a pulse oximeter. The rabbits were let to breathe room air for 10 min (1,10). During this process, when bradycardia or respiratory failure had developed, the experiment was discontinued.

Hypoxia and tryptophan application: Same hypoxic condition was applied to the rabbits in group 2 for only 5 min-ventilation. Then, L-tryptophan (Sigma Chemical Company, Sigma, St. Louis, MO) was administered orally in the amount of 200mg/kg per day for 5 days.

After the adaptation period, serum samples were taken from groups 1 and 2 at the beginning of the study (day 0) and day 6 after the hypoxia for troponin-I, CK-MB, LDH, plasma GSH-Px, SOD, MDA, and NO. At the end of day 6, animals were sacrificed and

histopathologic analyses were performed. In addition, cardiac tissue samples were taken to be analyzed.

Frozen serum samples were dissolved and CK-MB and Tn-I levels were analyzed on the same day. Serum CK-MB (with 0.5 cc serum samples) analyses were performed using a Konelab 60i auto analyzer (ThermoFisher Scientific, Finland) with the reagent kits produced by Medkim corp. Serum Tn-I levels were studied using an Innotrak Aio Immunoanalyzer (Innotrac diagnostics, Turku, Finland) with Innotrak Aio TM Troponin I Analyte Pen kit (with 0.5 cc serum samples). Serum LDH levels were also studied using a Konelab auto analyzer with LDH reagent kits produced by Medkim in 0.5 cc serum samples.

Serum and myocardial GSH-Px activity determination: The activity was calculated using Paglia and Valentine's combined enzymatic method: measuring the peroxidation rate of H_2O_2 and glutathion (GSH) reaction (11). Tissue GSH-Px activity detection: myocardial homogenate centrifuged at (1/4 w/v) 13200 rpm for 30 min and obtained supernatant tamponated with a phosphate tampon (0.05 M, pH = 7.4) and diluted (1/10). For tissue GSH-Px activity detection, 0.05 mL sample was used (11).

Serum and myocardial SOD activity levels were measured by a method developed by Sun et al. (12). Xanthine oxidase was used for superoxide producer and inhibition of nitroblue tetrazolium (NBT) reduction. Tissue SOD of activity measurement: 0.05 mL supernatant obtained from myocardial homogenate was diluted (1/10) with phosphate tampon (pH: 7.4).

Detection of Serum MDA activity: Jain's method was used: measurement of colorful product of MDA and thiobarbituric acid (TBA) at 532 nm wavelength (12). Tissue MDA detection was performed using a methods developed by Okawa et al. (14).

Tissue NO detection: The 0.05 mL homogenate obtained from the supernatant was diluted (1/4 v/v) by Somogy reactant (10% $ZnSO_4$ and 0.5 N NaOH) and then deproteinized and centrifuged at 1500 rpm for 10 min at + 4°C (15). Using this supernatant, NO ($NO_2 + NO_3$) levels were measured. The nitrate (NO_3) first reduced to nitrite (NO_2) and the standard scale for nitrite was used to determining the nitrate levels (16).

To histopathological evaluation of the hematoxylin and eosin stained slides were performed using a light microscope. The scoring was achieved by the severity of the histopathological changes (17).

The severity of the cardiac injury degree was graded between zero and 3 as shown below.

0: no evident cardiac injury, 1: mild cardiac injury, 2: moderate cardiac injury, 3: severe cardiac injury.

Statistical Analysis

SPSS 10.0 was used for statistical analysis. Median (minimum-maximum) values were used for comparing the parameters. For comparing the groups, Kruskal-Wallis variance analysis and Mann-Whitney U tests were used; comparisons within the groups were carried out by Wilcoxon signed ranks test. Statistical significance was set at a level of $P < 0.05$.

Results

In group 1, significant increase in troponin-I, CK-MB, LDH, SOD, and a decrease in GSH-Px were detected on day 6 as shown in Table 1 ($P < 0.05$). Severe cardiac injury was observed histopathologically. GSH-Px and SOD activity in this group was also found decreased (Table 1).

In group 2, troponin-I and SOD activity increased significantly as shown in Table 2. ($P < 0.05$). However, increase in troponin-I was less pronounced as compared to group 1. There was no significant change in other parameters as presented in Table 2 ($P > 0.05$).

NO levels in groups 1 and 2 were statistically much higher than the control group ($P < 0.05$). In group 2, NO level was significantly lower than group 1 as shown in Table 3 ($P < 0.05$). When the tissue SOD levels were compared, all groups were very similar ($P > 0.05$). In group 1, the tissue MDA level was statistically much higher compared to groups 2 and 3 ($P < 0.05$), and also group 2 MDA level was statistically much higher compared to group 3 ($P < 0.05$, Table 3). GSH-Px level in group 2 was higher compared to group 1 ($P < 0.05$, Table 3).

When the histopathological findings were compared, the score of group 1 statistically much higher compared to the other groups as shown in Table 4 ($P < 0.05$).

Table 1. Serum CKMB, LDH and Tn-I, plasma MDA, SOD, GSH-Px and NO levels in group 1 median (min-max).

n = 9	Day 0	Day 6	P
Tn-I (ng/mL)	0.21(0.16-0.25)	0.32(0.22-0.41)	<0.05
CK-MB (U/I)	3857(939-8447)	5450(1288-7781)	<0.05
LDH (IU)	660(603-984)	1276(765-8700)	<0.05
MDA (µmol/L)	0.360(0.288-1.232)	0.616(0.384-1.648)	>0.05
SOD (U/L)	1.023(0.929-1.411)	0.882(0.770-0.964)	<0.05
GSH-PX (U/mL)	0.224(0.127-0.488)	0.122(0.096-0.154)	<0.05
NO (µmol/L)	6.04(1.64-8.56)	7.52(0.00-10.50)	>0.05

Table 2. Serum CKMB, LDH and Tn-I, plasma MDA, SOD, GSH-Px and NO levels in group 2 median (min-max).

n = 9	Day 0	Day 6	P
Tn-I (ng/mL)	0.21(0.19-0.32)	0.25(0.20-0.47)	<0.05
CK-MB (U/I)	3569(1596-5250)	4285 (2518-6219)	>0.05
LDH (IU)	702(288-2570)	689(388-1498)	>0.05
MDA (µmol/L)	0.392(0.272-1.152)	0.447(0.352-1.088)	>0.05
SOD (U/L)	0.999(0.729-1.294)	1.440(0.858-1.635)	<0.05
GSH-PX (U/mL)	0.128(0.104-0.635)	0.130(0.084-0.824)	>0.05
NO (µmol/L)	6.80(3.04-11.72)	7.36(7.10-9.44)	>0.05

Table 3. Comparison of tissue GSH-Px, NO, SOD, and MDA levels.

Group	n	GSH-Px	NO	SOD	MDA
		(mU/µg protein) Median (Min-Max)	(µmol × 10 ⁻³ /µg protein) Median (Min-Max)	(U/µg protein) Median (Min-Max)	(nmol/µg protein) Median (Min-Max)
1	9	0.0039(0.0023-0.0043)	0.10 (0.05-0.15) ^a	4.96(2.68-6.96)	0.16(0.10-0.45) ^a
2	10	0.0054(0.0022-0.0083) ^b	0.087 (0.04-0.18) ^{a,b}	6.09(1.39-11.28)	0.09(0.02-0.27) ^{a,b}
3	7	0.0046(0.022-0.0055)	0.035 (0.017-0.077)	4.97(1.33-7.26)	0.031(0.007-0.12)

^a P < 0.05, when compared with group 3

^b P < 0.05, when compared with group 1

Table 4. Comparison of histopathological cardiac injury scores.

Group	n	Cardiomyopathy scores		
		Mild (%)	Moderate (%)	Severe (%)
1 Hypoxic	9	0	33	66 ^a
2 Hypoxic + L-Tryptophan	10	40	20	40
3 Control	7	0	0	0

^a P < 0.05, when compared with group 3

Discussion

Cardiovascular diseases, especially in the developed countries, are the main cause of morbidity and mortality in the world. Early detection of hypoxia and ischemia, the main etiology of cardiovascular diseases, and early and appropriate treatment should be prevent mortality (4).

L-tryptophan should have similar effects like melatonin because it is the precursor amino acid of melatonin. There are few studies on the antioxidant activity of L-tryptophan: the way of action is preventing superoxide anion and hydrogen peroxide production. Therefore, it may be used as an antioxidant agent (18,19).

Jaffe et al. reported that Tn-I is more sensitive and specific than CK-MB in the myocardial injury (20). LDH isoenzyme activities are not specific for myocardial injury. CK-MB sensitivity is 92%, and Tn-I sensitivity is over 93% after the first few hours. Vordenwinkler et al. reported that cardiac Tn-I increased in parallel to cardiac Tn-T, CK-MB, and LDH in effluents from an isolated perfused rat hearts after hypoxia-reoxygenation-induced myocardial injury (21).

In this study, Tn-I, CKMB, and LDH levels in the hypoxic group were found significantly elevated, which is in agreement with the literature. Tn-I, CK-MB, and LDH level elevations demonstrate that significant hypoxia caused a myocardial injury. In the hypoxic group that received tryptophan, although mild Tn-I elevation was observed, there was no significant change in CK-MB and LDH. Therefore, this should signify that the tryptophan medication controlled the myocardial injury, and in minor injuries Tn-I is more reliable than CK-MB and LDH because of the minor changes in CK-MB and LDH levels. These results were also supported by the histopathologic findings.

A production of the free oxygen radicals, a lipid peroxidation and a decrease in the antioxidant enzymes are the major factors in the pathogenesis of a hypoxic myocardial injury (3). In the case of the myocardial ischemia reperfusion, Ferrari et al. advocated that the degree of an injury depended on the amount of the free oxygen radicals and the antioxidant defense systems (22). A study by Prasad et

al. showed that an increase in MDA was detected (23). Therefore, he concluded that in the ischemic conditions, an elevation in free oxygen radicals results in an inhibition or a decrease of the antioxidant defense system.

In our study, there was a statistically significant decrease in MDA in the tryptophan group than the hypoxic group, and we found the same results in the tissue. These results indicated that the antioxidant activity of tryptophan was from the inhibition of the lipid peroxidation.

Depre et al. concluded that there is an increase in the NO synthase activity after the myocardial ischemia of a rabbit heart (24). This increase causes the NO deposition and myocardial damage.

In our study, NO levels in tissues of the hypoxic group were significantly higher compared to the other groups. High NO levels in the hypoxic group supported that NO had an effect on the hypoxic myocardial injury. The myocardial NO levels in the tryptophan group were lower than the hypoxic group. These results showed that myocardium was protected by tryptophan.

The antioxidant enzymes, which protects the cardiomyocytes from the oxygen radicals, are GSH-P and SOD. Guarnieri et al. reported that there was a decrease in the GSH-Px and SOD activity in the myocardial hypoxemia/ischemia (2,25). Kihlström et al. and Hoshida et al. showed that, after 5 min ischemia, mitochondrial SOD, catalase, and GSH-Px activity increased in a dog heart (26,27).

In our study, the plasma GSH-Px and SOD level decreased significantly in the hypoxic group. In the L-tryptophan group, there was a statistically significant increase in SOD, but the increase in GSH-Px activity was not significant this group. In the tissue we found that GSH-Px activity in L-tryptophan was higher than hypoxemic group and this increase was statistically significant. SOD activity in the tissue did not change significantly. These results showed that the antioxidant activity in hypoxic injury was increased by tryptophan.

In our study, there were severe myocardial damage findings, such as a myocardial fibril swelling, interstitial edema, disorganization, and a necrosis in

the hypoxic group. In the group that received L-tryptophan, we found mild to moderate cardiomyopathy findings, such as a normal myocardium accompanied to a myocardial fibril swelling, interstitial edema, and disorganization. It was observed that L-tryptophan did not protect the heart injury completely, but regressed the hypoxia effects. Our histopathological findings supported that L-tryptophan as an antioxidant could regress the myocardial injury. Our findings were in agreement with Yuan and Llesuy (28,29).

References

1. Neckar J, Szarszoł O, Herget J, Ostadal B, Kolar F. Cardioprotective effect of chronic hypoxia is blunted by concomitant hypercapnia. *Physiol Res* 2003; 52: 171-75.
2. Haider KH, Stimson WH. Cardiac myofibrillar proteins: biochemical markers to estimate myocardial injury. *Mol Cell Biochem* 1999; 194: 31-9.
3. Dhalla NS, Elmoselhi AB, Hata T, Makino N. Status of myocardial antioxidants in ischemia-reperfusion injury. *Cardiovasc Res* 2000; 47: 446-56.
4. Das UN. Free radicals , cytokines and nitric oxide in cardiac failure and myocardial infarction. *Mol Cell Biochem* 2000; 215: 145-52.
5. Halliwell B. Free radicals and antioxidants: a personal view. *Nutr Rev* 1994; 52: 253-65.
6. Brzozowski T, Konturek PC, Konturek SJ, Pajdo R, Bielanski W, Brzozowska I et al. The role of melatonin and L-tryptophan in prevention of acute gastric lesions induced by stress, ethanol, ischemia, and aspirin. *J Pineal Res* 1997; 23: 79-89.
7. Reiter RJ, Tan DX, Cabrera J, D'Arpa D. Melatonin and tryptophan derivatives as free radical scavengers and antioxidants. *Adv Exp Med Biol* 1999; 467: 379-87.
8. Moosmann B, Behl C. Cytoprotective antioxidant function of tyrosine and tryptophan residues in transmembrane proteins. *Eur J Biochem* 2000; 267: 5687-92.
9. Narin F, Demir F, Akgün H, Baykan A, Koçer D, Üzümlü K. Doxorubicin-induced experimental cardiotoxicity and effect of L- tryptophan on cardiotoxicity. *Erciyes Med J* 2005; 27(6): 7-16.
10. Rumsey WL, Abbott B, Bertelsen D, Mallamaci M, Hagan K, Nelson D et al. Adaptation to hypoxia alters energy metabolism in rat heart. *Am J Physiol* 1999; 276: 71-80.
11. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967; 70: 158-69.
12. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. *Clin Chem* 1988; 34: 497-500.
13. Jain SK. Evidence for membrane lipid peroxidation during the in vivo aging of human erythrocytes. *Biochim Biophys Acta* 1988; 937: 205-10.
14. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochem* 1979; 95: 351-58.
15. Mashage H, Kok B, Huizenga JR, Jansen PL. Nitrite and nitrate determination in plasma, a critical evaluation. *Clin Chem* 1995; 41: 892-96.
16. Schmidt HHHW, Warner TD, Nakane M, Forstermann U, Murad F. Regulation and subcellular location of nitrogen oxide synthases in RAW264.7 macrophages. *Mol Pharmacol* 1992; 41: 615-24.
17. Saad SY, Najjar TA, Al-Rikabi AC. The preventive role of deferoxamine against acute doxorubicin-induced cardiac, renal and hepatic toxicity in rats. *Pharmacol Res.* 2001 Mar; 43 (3): 211-8.
18. Ronen N, Livne E, Gross B. Oxidative damage in rat tissue following excessive L-tryptophan and atherogenic diets. *Adv Exp Med Biol* 1999; 467: 497-505.
19. Christen S, Peterhans E, Stocker R. Antioxidant activities of some tryptophan metabolites: possible implication for inflammatory diseases. *Proc Natl Acad Sci USA* 1990; 87: 2506-2510.
20. Jaffe AS, Landt Y, Parvin CA, Abendschein DR, Geltman EM, Ladenson JH. Comparative sensitivity of cardiac troponin I and lactate dehydrogenase isoenzymes for diagnosing acute myocardial infarction. *Clin Chem* 1996; 42: 1770-76.
21. Vorderwinkler KP, Mair J, Puschendorf B, Hempel A, Schlüter KD, Piper HM. Cardiac troponin I increases in parallel to cardiac troponin T ,creatine kinase and lactate dehydrogenase in effluents from isolated perfused rat hearts after hypoxia-reoxygenation-induced myocardial injury. *Clin Chim Acta* 1996; 251: 113-17.
22. Ferrari R, Ceconi C, Curello S, Cargnoni A, Pasini E, Giuli D et al. Role of oxygen free radicals in ischemic and reperfused myocardium. *Am J Clin Nutr* 1991; 53: 215-22.

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23. Prasad K, Lee P, Mantha SV, Kalra J, Prasad M, Gupta JB. Detection of ischemia-reperfusion cardiac injury by cardiac muscle chemiluminescence. *Mol Cell Biochem* 1992; 115: 49-58.
24. Depre C, Fierain L, Hue L. Activation of nitric oxide synthase by ischaemia in the perfused heart. *Cardiovasc Res* 1997; 33: 82-87.
25. Guarnieri C, Flamigni F, Calderera CM. Role of oxygen in the cellular damage induced by re-oxygenation of hypoxic heart. *J Mol Cell Cardiol* 1980 ; 12: 797-808.
26. Kihlström M, Kainulainen H, Salminen A. Enzymatic and nonenzymatic lipid peroxidation capacities and antioxidants in hypoxic and reoxygenated rat myocardium. *Exp Mol Pathol* 1989; 50: 230-38.
27. Hoshida S, Kuzuya T, Fuji H, Yamashita N, Oe H, Hori M et al. Sublethal ischemia alters myocardial antioxidant activity in canine heart. *Am J Physiol* 1993; 264: 33-39.
28. Yuan SM. Cytochemistry and ultrastructure of canine myocardium undergoing global ischemia and reperfusion injury. *Kaohsiung J Med Sci* 1999; 15(1) :1-7.
29. Llesuy S, Milei J, Picone V, Gonzalez Flecha B, Beigelman R, Boveris A. Effect of vitamins A and E on ischemia-reperfusion damage in rabbit heart. *Mol Cell Biochem* 1995; 145(1): 45-51.