Original article

The protective effects of erythropoietin on photoreceptor damage by formaldehyde

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Abstract

Introduction: The photoreceptor layer of retina has important role for sight. The previous study showed that accidental formaldehyde injection caused ischemia and damage in the retina. On the other hand the erythropoietin prevents neuronal injury of ischemic damage. **Objectives:** The aim of this study was to survey the effect of erythropoietin on retro bulbar formaldehyde injected photoreceptor layer of rat retina. **Materials and methods:** 30 adult rats were used and divided into three groups: 1- control group, 2- formaldehyde group (1 ml retro bulbar injected by 10% formaldehyde solution), 3- erythropoietin group (5000 units/kg immediately intraperitoneally injected by erythropoietin after formaldehyde injection for 7 days). The photoreceptor layer of retina studied using a transmission electron microscope. **Results:** Our observation showed that disorganization and vacuolization in outer segment and inner segment, pyknotic and karyolysis in outer nuclear layer were seen in formaldehyde group. But the minor sign of pathology such as lightly vacuolization in inner segment were obvious in erythropoietin group. **Conclusion:** We concluded that formaldehyde caused damage in photoreceptor layer and erythropoietin was improvement this injury.

Keywords: Erythropoietin, Formaldehyde, Photoreceptor layer, Rat

Introduction

The retina is a light-sensitive layer of tissue, lining the inner surface of the eye. The retina consists of several layers of neurons interconnected by synapses. The only layer that is directly sensitive to light is the photoreceptor layer. The photoreceptor layer has an important role in sight and contains rod and cone cells. Formaldehyde is commonly used as a fixative in medical laboratories. Ocular exposure to formaldehyde produces irritation and

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lacrimation. Depending on the concentration, formaldehyde solutions may cause irritation or corneal opacification and loss of vision (Witek et al, 1987). Accidental formaldehyde injection is paranormal and occurs when formaldehyde is confused with lidocaine in surgery room (Soltan & Hashemi, 2004). Formaldehyde causes ischemia (Soltansanjari & hashemi, 2004). Previous research indicated that the formaldehyde has toxic effects such as: oxidative stress in tissue reacts with glutathione and formate, chromosome damage and cellular apoptosis (Matsuka et al, 2010; Just et al, 2011; Anderson et al, 2010; Tang et al, 2011). On the other hand, erythropoietin stimulates the bone marrow to produce more red blood cells. The resulting rise in red blood cells increases the oxygen-carrying capacity of the blood (Koury et al, 2002). Also, erythropoietin decreases neuronal injury caused by ischemic damage (Zhang et al, 2008; Mc Vicar et al, 2011). Recent studies showed the physiological role of erythropoietin with central nervous system. There is an activation of erythropoietin of many intracellular pathways such as mitogen-activated protein kinase (MAPK), which is associated with cell survival and inhibits the apoptosis of erythroid cells (Jelkmann & Wagner, 2004; Ratajczak et al, 2001). Additionally, erythropoietin caused proliferation and maturation of erythroid precursor cells (Fisher, 2003). Also, erythropoietin produced hematopoietic cytokine by the kidney in hypoxia. So, treatment with erythropoietin protects cultured neurons from hypoxia and therapeutic strategies retinal or central nervous system regions ischemic injury (Lewczuk, 2000). Thus, in this research we evaluated neuroprotective effect of erythropoietin on ischemic damage of formaldehyde injection by transmission electron microscope.

Materials and methods

Experimental design Thirty male Wistar rats were maintained on a 12h light/12dark light cycle and temperature (22-24º). Rats aged four months and 250-300gr body weight were used. All experiments conformed to the CALAM standards of veterinary care (Patricia, 2008). The animals were divided into three groups: 1-control group, 2-formaldehyde group (0.2 ml retro bulbar injection of 10% formaldehyde solution for one dose in one eye) 3-erythropoietin group (erythropoietin at 5000 units/kg was injected intraperitoneally immediately after formaldehyde injection for 7 days) (Schwartzenberg et al, 2006). The animals were anesthetized with an intraperitoneal injection of ketamine (30 mg/kg) and xylazine (2.5 mg/kg). Then, the eyes were enucleated and fixed in 4% Gluteraldehyde in sodium cocodylate buffer for 4 hand transferred to 1% osmium tetroxide and dehydrated through a graded ethanol series. The specimens were embedded in resin. Semi thin and ultrathin sections of the retina were stained with toluidine blue forsemi thin sections and lead citrate and uranyl acetate for ultrathin sections. The morphometric study was examined by micrometrical technique in light microscope and ultrathin sections were evaluated by transmission electron microscope (Philips CM-10, Eindhoven, Netherlands). The statistical analyses were performed by using one way Anova. $P<0.05$ was considered statistically significant.

Ethical approval

All procedures and care of the animals were conducted following protocols approved by the ethical committee (Iranian Society for the Prevention of Cruelty to Animal, and Iranian Veterinary Organization).

Results

Clinical animadversion showed that scarring and edema were seen in eyelid after formaldehyde injection. The ultra-structural study of photoreceptor layer in control group showed that outer segments contain bimembranous discs like a ladder. The inner

segments included mitochondria,endoplasmic reticulum. The mitochondria near the outer segments and oval to rounded shape were observed (Figure 1). The cristae of mitochondria were sheet-like invaginations of the inner membrane into the matrix (Figure 1). The outer nuclear layer consisted of rod and cone nuclei with heterochromatin (Figure 2). The major pathological signs were seen in the photoreceptor layer in formaldehyde group. Disorganized and vacuolated outer segments, highly vacuolated inner segment, loss of cristae in mitochondria, pyknotic and karyolysis nuclei were evident after the use of retro bulbar injection of formaldehyde (Figures 3, 4). But the photoreceptor layer of erythropoietin injected group has obviously changed from the formaldehyde group. Minor signs of pathology appeared in erythropoietin group,with evidence of lightly vacuolated inner segment (Figure 5). The normal and organized outer segments, normal outer nuclear layer and normal mitochondria were evident in this group (Figures 5, 6). Morphometric study of the photoreceptor layer indicated that the mean thickness of photoreceptor layer in control, formaldehyde and erythropoietin groups was 83.3 ± 0.83 micrometer, 73.24 ± 0.82 micrometer and 82 ± 1.58 micrometer, respectively. The morphometric of photoreceptor layer showed that the thickness of this layer decreased in formaldehyde group with significant difference compared with control group ($p \leq 0.05$) and increased in erythropoietin group with no significant difference compared with control group ($p \geq 0.05$).

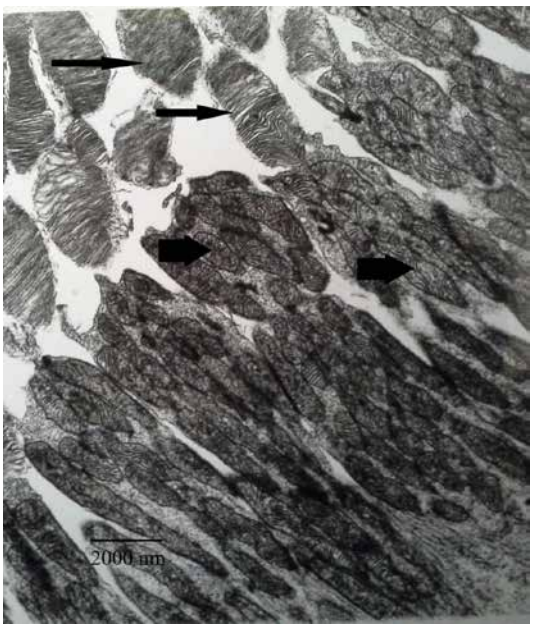

Figure 1: Electro micrograph of the photoreceptor layer in control group. The outer segment (arrows), the mitochondria in inner segment (thick arrows) (staining with lead citrate and uranyl acetate) (× 6600).

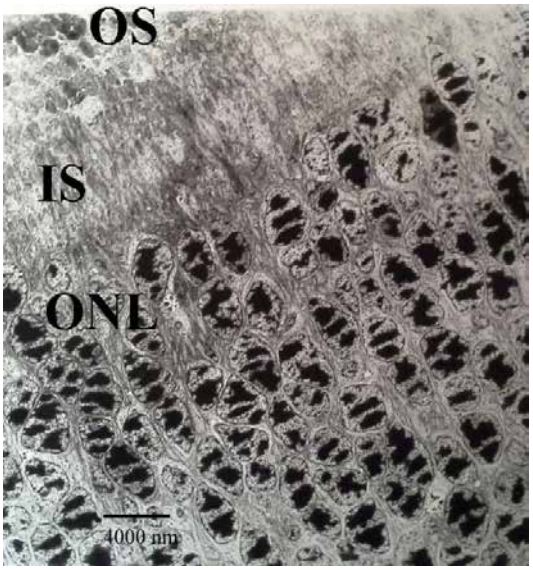

Figure 2: Electro micrograph of the photoreceptor layer in control group. The outer segment (OS), the inner segment (IS), the outer nuclear layer (ONL). (Staining with lead citrate and uranyl acetate) (× 2950).

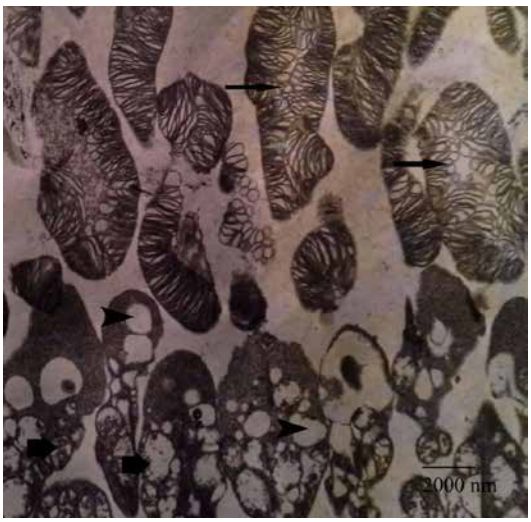

Figure 3: Electro micrograph of the photoreceptor layer in formaldehyde group. The vacuolated and disorganized outer segment (arrows), the vacuoles in inner segment (arrowheads), the destroyed mitochondria without cristae (thick arrows). (Staining with lead citrate and uranyl acetate) (× 6600).

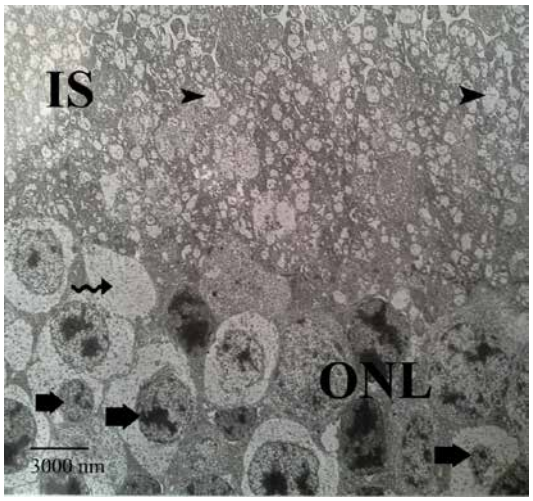

Figure 4: Electro micrograph of the photoreceptor layer in formaldehyde group. The highly vacuolated inner segment (IS) the vacuoles (arrowheads), the outer nuclear layer (ONL), pyknotic nuclei (thick arrows) and karyolysis (wave arrow). (Staining with lead citrate and uranyl acetate) (× 4500).

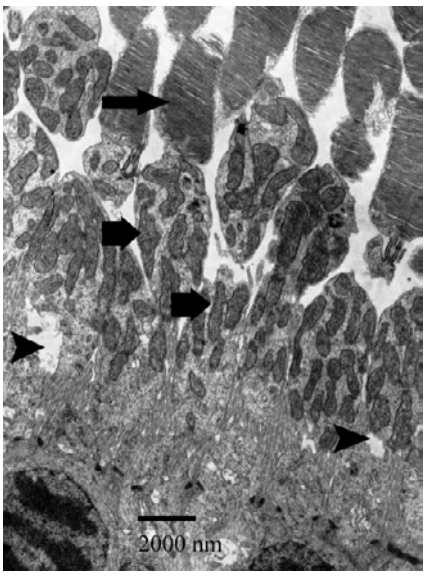

Figure 5: Electro micrograph of the photoreceptor layer in erythropoietin group. The normal outer segment (arrow), the normal mitochondria in the organized inner segment (thick arrows), vacuoles in inner segment (arrowheads) (Staining with lead citrate and uranyl acetate) (× 6600).

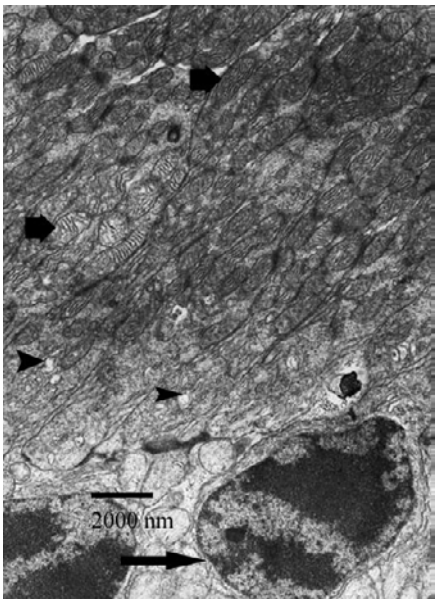

Figure 6: Electro micrograph of the photoreceptor layer in erythropoietin group. The normal mitochondria in the organized inner segment (thick arrows), vacuoles in inner segment (arrowheads), the normal nuclei in outer nuclear layer (arrow) (Staining with lead citrate and uranyl acetate) (× 6600).

Discussion

The photoreceptor layer of the rat is similar to that ofother animals (Esfandiari et al, 2009; Goodarzi et al, 2014; Garcia & Dejuan, 1999; Haacke et al, 2001).In this research,the injury produced by formaldehyde injection and improvement by erythropoietin injection were evaluated by transmission electron microscope. The rats injected with formaldehyde showed disorganization and vacuolization of the outer segment, high vacuolization of the inner segment, disappearance of cristae of mitochondria, pyknotic and karyolysis in outer nuclear layer. These histopathological changes were seen in formaldehyde group whereas the formaldehyde caused ischemia due to central retinal and ophthalmic arteries occlusion(Soltansanjari & hashemi, 2004). In addition, the formaldehyde enhanced reactive oxygen species as a result of inflammation (Turkoglu et al, 2008).The cell injury and damage occurred as a result of increasing reactive oxygen species. On the other hand, ischemia increases oxygen free radical, nitric oxide and glutamate levels (George & Cioffi, 2005). However, these damages and injuries of the photoreceptor layer corroborated prior research, showing that retinal ischemia increased apoptosis (Matsuka et al, 2010; George & Cioffi, 2005; Wyllie, 1997). By comparison, the normal outer segment, inner segment with light vacuolization and the normal outer nuclear layer were seen in erythropoietin group. Increasing erythropoietin for survival may limit neuronal damage in cerebral and retinal ischemia (Siren et al 2001; Bernaudin et al, 1999; Junk et al, 2002; Kawakami et al, 2001). Exogenous erythropoietin has been shown to decrease damage of ischemia in brain injury and inflammation (Siren et al, 2001; Bernaudin et al, 1999). Our finding corroborated the previous studies (Siren et al, 2001; Bernaudin et al, 1999; Junk et al, 2002; Kawakami et al 2001). Rats given intraperitoneal erythropoietin immediately after the formaldehyde injection for 7 days reduced photoreceptor damage. This finding demonstrates that post treatment with erythropoietin leads to improvement of neuronal damage of ischemia. The mechanisms may explain how erythropoietin improves neuronal injury. The erythropoietin has an antiapoptic role due to reduction of TUNEL- positive cells in the cerebral ischemia (Siren et al, 2001). Also, erythropoietin inhibited apoptosis by depriving growth factor (Siren et al, 2001; Bernaudin et al, 1999). In addition, the erythropoietin inhibited the release of glutamate from neuron (Kawakami et al, 2001). On the other hand, ischemia increased glutamate (George &Cioffi, 2005) but erythropoietin decreased glutamate (Kawakami et al 2001) and attenuates neuronal damage after exposure to the glutamate (Sinor& Greenberg, 2000). Our observationis confirmed by prior research showing that erythropoietin can decrease neuronal injury (Brines et al 2000; Coleman et al 2006). Also, the erythropoietin can be delivered to a damaged tissue; this treatment can affect increases in hematocrit with associated vascular occlusion (Brines et al, 2000; Coleman et al, 2006). Our finding in the outer nuclear layer of the formaldehyde group suggests that there are pyknotic and karyolysis nuclei. But erythropoietin can inhibit neuronal cell apoptosis (Grimm et al, 2002), and clean oxygen radicals of ischemia (Marti et al, 2000)against oxidative stress after ischemia (Wang et al, 2010).Our findings are consistent with the results of previous studies (Grimm et al, 2002; Marti et al, 2000; Wang et al, 2010).On the other hand, reduction of ATP produced and cessation of oxidative phosphorylation occurred in hypoxia(Macario & Conway de Macario, 2000). Depletion of ATP with decreasing of PH, increased influx of Na and Ca ions occurred in ischemia and anaerobic metabolism (Uysal et al, 2014; Thomaz Neto et al, 2013). In our research, morphological changes and loss of cristae

of mitochondria in inner segment of retina have seen and confirmed by previous study indicating that ATP reduction and increase mitochondrial permeability transition pore in ischemia (Uysal et al, 2014; Thomaz Neto et al, 2013). Also, efflux K ion and influx Na ion with water caused cellular edema in hypoxia (Chambless et al, 2003). The edema caused damage the cells and cytoplasmic organelles such as mitochondria (Esfandiari et al, 2009). These ultra-structural observations confirmed that the improvement effect of erythropoietin in damaged photoreceptor cell after ischemic induced by formaldehyde exposure. The proposed study will be the intracellular pathway associated with cell survival and apoptosis under the effect of erythropoietin and formaldehyde.

Conclusions

The results of our research concluded that the erythropoietin could increase the recovery of neuronal function after ischemia damage. Also, our observation demonstrates that erythropoietin inhibit apoptosis after ischemia and may use a therapeutic agent for retinal ischemia disease such as glaucoma or vascular occlusion.

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References

Andersen ME, Clewell HJ, Bermundez E, Dodd DE, Willson GA, Campbell JL, Thomas RS (2010). Formaldehyde: integrating dosimetry, cytotoxicity, and genomics to understand dose-dependent transitions for an endogenous compound. ToxicolSci; 118: 716–731. Doi: 10.1093/toxsci/kfq303. Epub 2010 Sep 30.

Bernaudin M, Marti HH, Roussel S, Divoux D, Nouvelot A, MacKenzie ET, Petit E (1999). A potential role for erythropoietin in focal permanent cerebral ischemia in mice.J Cereb Blood Flow Metab; 19: 643–651. PMID: 10366194.

Brines ML, Ghezzi P, Keenan S, Agnello D, de Lanerolle NC, Cerami C, Itri LM, Cerami A (2000). Erythropoietin crosses the blood-brain barrier to protect against experimental brain injury.ProcNatlAcadSci USA; 97(19):10526-10531. PMID: 10984541.

Chambless LE, Folsom AR, Sharrett AR, Sorlie P, Couper D, Szklo M, Nieto FJ (2003). Coronary heart diseaseriskprediction in the Atherosclerosis Risk in Communities (ARIC) study. J ClinEpidemiol; 56(9): 880-90.

Coleman TR, Westenfelder C, Tögel FE, Yang Y, Hu Z, Swenson L, Leuvenink HG, Ploeg RJ, d'Uscio LV, Katusic ZS, Ghezzi P, Zanetti A, Kaushansky K, Fox NE, Cerami A, Brines M (2006). Cytoprotective doses of erythropoietin or carbamylated erythropoietin have markedly different procoagulant and vasoactive activities. ProcNatlAcadSci USA; 103:5965–5970. PMID: 16585502.

Esfandiari A, Yousofi AR, Dehghan A, Safavi A (2009). Effect of intermittent light on the photoreceptor cells of the retina in rabbits. Jpn J Ophthalmol; 53: 635-639.doi:10.1007/s10384-009-0721-4

Fisher JW (2003). Erythropoietin: physiology and pharmacology update. ExpBiol Med; 228: 1-14.

Garcia M, Dejuan J (1999). Fine structure of the retina of blackbassmicropterussalmoides. HistolHistopathol; 14(4): 1053-1065. PMID:10506921

George A, Cioffi MD (2005). Ischemic model of optic nerve injury. Trans Am Ophthalmol Soc; 103: 592–613. PMCID: PMC1447590.

GoodarziA, EsfandiariA, Dehghansheibani A (2014). Ultrastructural study on the photoreceptor layer in streptozotocin-induced diabetic rats. Comp Clin Path; 23: 1293-1297. doi: 10.1007/s00580-013-1777-6

Grimm C, Wenzel A, Groszer M, Mayser H, Seeliger M, Samardzija M, Bauer C, Gassmann M, Remé CE (2002). HIF-1-induced erythropoietin in the hypoxic retina protects against light-induced retinal degeneration. Nat Med; 8:718–724. PMID: 12068288. 12.

Haacke C, Hess M, Melzer RR, Gebhart H, Smola U (2001). Fine structure and development of the retina of the grenadier anchovy coilianasus. Morphol; 248(1):41-55. PMID: 11268057

Jelkmann W, Wagner K (2004). Beneficial and aminous aspects of the pleiotropic action of erythropoietin. Ann Hematol; 83: 673-86.

Junk AK, Mammis A, Savitz SI, Singh M, Roth S, Malhotra S, Rosenbaum PS, Cerami A, Brines M, Rosenbaum DM (2002). Erythropoietin administration protects retinal neurons from acute ischemia-reperfusion injury. ProcNatlAcadSci USA; 99(16):10659-64. PMID: 12130665.

Just W, Zeller J, Riegert C, Speit G (2011). Genetic polymorphisms in the formaldehyde dehydrogenase gene and their biological Significance. Toxicol Lett; 207:121-127. Doi:10.1016/j.toxlet.2011.08.025. Epub 2011 Sep 6.

Kawakami M, Sekiguchi M, Sato K, Kozaki S, Takahashi M (2001). Erythropoietin Receptor-mediated Inhibition of Exocytotic Glutamate Release Confers Neuroprotection during Chemical Ischemia. J BiolChem;276:39469-39475.Doi: 10.1074/jbc.M105832200.

Koury MJ, Sawyer ST, Brandt SJ (2002). New insights into erythropoiesis. Curr Opin Hematol; 9(2): 93–100.PMID: 11844990.

Lewczuk P, Hasselblatt M, Kamrowski-Kruck H, Heyer A, Unzicker C, Sirén AL, Ehrenreich H (2000). Survival of hippocampal neurons in culture upon hypoxia: effect of erythropoietin. Neuroreport; 11(16): 3485-8.

Macario AJ, Conway de Macario (2000). Stress and molecular chaperones in disease. Int J Clin Lab Res; 30(2): 49-66.

Marti HJ, Bernaudin M, Bellail A, Schoch H, Euler M, Petit E, Risau W (2000). Hypoxia-induced vascular endothelial growth factor expression precedes neovascularization after cerebral ischemia. Am J Pathol; 156: 965–976. PMID: 10702412

Matsuoka T, Takaki A, Ohtaki H, Shioda S (2010). Early changes to oxidative stress levels following exposure to formaldehyde in ICR mice. J ToxicolSci; 35: 721-730. PMID: 20930466

McVicar CM, Hamilton R, Colhoun LM, Gardiner TA, Brines M, Cerami A, Stitt AW (2011). Intervention with an erythropoietin-derived peptide protects against neuroglial and vascular degeneration during diabetic retinopathy.Diabetes; 60(11): 2995-3005. Doi: 10.2337/db11-0026. Epub 2011 Sep

Patricia VT (2008) The CALAM/ACMAL Standards of Veterinary Care and laboratory animal welfare.Can Vet J Jan; 49: 86–88. PMCID: PMC2147705.

Ratajczak J, Majka M, Kijowski J, Baj M, Pan ZK, Marquez LA, Janowska-Wieczorek A, Ratajczak MZ (2001). Biological significance of MARK, AKT and JAK-STAT protein activation by various erythropoietin factors in normal human early erythroid cells. Br J Haematol; 115: 195-204.

Schwartzenberg S, Keren G, George J (2006). Erythropoietin as a protective agent in myocardial ischemia.Harefuah; 145(5): 380-3.

Sinor AD, Greenberg DA (2000). Erythropoietin protects cultured cortical

neurons, but not astroglia, from hypoxia and AMPA toxicity. NeurosciLett; 290: 213-215.

Siren AL, Fratelli M, Brines M, Goemans C, Casagrande S, Lewczuk P, Keenan S, Gleiter C, Pasquali C, Capobianco A, Mennini T, Heumann R, Cerami A, Ehrenreich H, Ghezzi P (2001). Erythropoietin prevents neuronal apoptosis after cerebral ischemia and metabolic stress.ProcNatlAcadSci U S A; 98(7): 4044-9. PMCID: PMC31176.

Soltan Sanjari M, Hashemi M (2004). Formalin toxicity after inadvertent retro bulbar and eyelid injection: a case report. RJMS; 11(42): 559-563.

Tang XQ, Ren YK, Chen RQ, Zhuang YY, Fang HR, Xu JH, Wang CY, Hu B (2011). Formaldehyde induces neurotoxicity to PC12 cells involving inhibition of paraoxonase-1 expression and activity. Clin&ExpPharmacol&Physiol; 38: 208–214. Doi: 10.1111/j.1440-1681.2011.05485.x.

Thomaz Neto FJ, Koike MK, AbrahãoMde S, CarilloNeto F, Pereira RK, Machado JL, Montero EF (2013). Ischemic preconditioning attenuates remote pulmonary inflammatoryinfiltration of diabetic rats with an intestinal and hepatic ischemia-reperfusion injury. Acta Cir Bras; 28(3): 174-8.

Türkoğlu AO, Sarsılmaz M, Çolakoğlu N, Zararsız I, Kuloğlu T, Pekmez H, Taş U (2008). Formaldehyde-induced damage in lungs and effects of caffeic acid phenethyl ester: a light microscopic study. Eur J Gen Med; 5(3): 152-156.

Uysal AI, Ocmen E, Akan M, Ozkardesler S, Ergur BU, Guneli E, Kume T, Koca U, UnalTogrul B (2014). The effects of remote ischemic preconditioning and N-acetylcysteine with remote ischemic preconditioning in rat hepatic ischemia reperfusion injury model. Biomed Res Int;2014: 892704. Doi: 10.1155/2014/892704. Epub2014 Jan 8.

Wang Q, Pfister F, Dorn-Beineke A, vom Hagen F, Lin J, Feng Y, Hammes HP (2010). Low-dose erythropoietin inhibits oxidative stress and early vascular changes in the experimental diabetic retina. Diabetologia; 53:1227–1238. Doi: 10.1007/s00125-010-1727-7. Epub 2010 Mar26.

Witek TJ, Schachter EN, Tosun T, Beck GI, Leaderer BP (1987). An evaluation of respiratory effects following exposure to 2.0 ppm formaldehyde in asthmatics: lung function, symptoms, and airway reactivity. Arch Env Health; 42: 230-237. PMID: 3310924

Wyllie AH (1997). Apoptosis: an overview. Br Med Bull;53: 451–465.

Zhang J, Wu Y, Jin Y, Ji F, Sinclair SH, Luo Y, Xu G, Lu L, Dai W, Yanoff M, Li W, Xu GT (2008). Intravitrealinjection of erythropoietinprotects both retinalvascular and neuronalcells in earlydiabetes. Invest Ophthalmol Vis Sci; 49(2): 732-42. Doi: 10.1167/iovs.07-0721.

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