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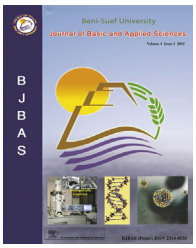

Full Length Article

Nebivolol and chrysin protect the liver against ischemia/reperfusion-induced injury in rats

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ABSTRACT

Oxidative stress plays a key role in the pathogenesis of hepatic ischemia/reperfusion (I/R)-induced injury, one of the leading causes of liver damage post-surgical intervention, trauma and transplantation. This study aimed to evaluate the protective effect of nebivolol and chrysin against I/R-induced liver injury via their vasodilator and antioxidant effects, respectively. Adult male Wister rats received nebivolol (5 mg/kg) and/or chrysin (25 mg/kg) by oral gavage daily for one week then subjected to ischemia via clamping the portal triad for 30 min then reperfusion for 30 min. Liver function enzymes, alanine transaminase (ALT) and aspartate transaminase (AST), as well as hepatic Myeloperoxidase (MPO), total nitrate (NOx), glutathione (GSH) and liver malondialdehyde (MDA) were measured at the end of the experiment. Liver tissue damage was examined by histopathology. In addition, the expression levels of nitric oxide synthase (NOS) subtypes, endothelial (eNOS) and inducible (iNOS) in liver samples were assessed by Western blotting and confirmed by immunohistochemical analysis. Both chrysin and nebivolol significantly counteracted I/R-induced oxidative stress and tissue damage biomarkers. The combination of these agents caused additive liver protective effect against I/R-induced damage via the up regulation of nitric oxide expression and the suppression of oxidative stress. Chrysin and nebivolol combination showed a promising protective effect against I/R-induced liver injury, at least in part, via decreasing oxidative stress and increasing nitric oxide levels.

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1. Introduction

Hepatic ischemia/reperfusion (I/R) injury is a major hepatic problem that arises in many situations such as liver transplantation, resection, circulatory shock, and post traumatic interruption of blood supply to liver (Kim et al., 2013). The release of reactive oxygen species (ROS) underlies the pathophysiology of the injury during the reperfusion stage which provokes inflammatory response in the form of activation of kuppfer cells, inflammatory cytokines and infiltration of leucocytes which, in turn, leads to liver cells damage (Jaeschke, 2006; Schwartz et al., 2013). The activation of Kuppfer cells leads to the release of ROS such as superoxide, hydroxyl, and hydrogen peroxide radicals which are the main causes of tissue damage especially after the release of nitric oxide (NO) from endothelium upon reperfusion forming peroxynitrite with oxygen free radicals (Taniai et al., 2004).

In normal liver, there is a delicate balance between NO vasodilating effect and endothellin (ET) vasoconstricting effect which controls the vascular rhythm in liver (Jaeschke, 2003; Nakamura et al., 1995). NO is produced in liver by two subtypes of nitric oxide synthetases; endothelial nitric oxide synthetase (eNOS) and inducible nitric oxide synthetase (iNOS) (Tian et al., 2010). eNOS is produced naturally by endothelium and it is responsible for maintaining blood supply to the liver. While the production of iNOS is induced in the endothelial, hepatocyte and other hepatic cells under special conditions such as decreased blood supply or increased vasoconstriction injury (Serracino-Inglott et al., 2001). iNOS is responsible for the vasodilatation of liver sinusoid and consequent hypotension and stroke following the hepatic injury (Serracino-Inglott et al., 2001). Thus, we hypothesized that the use of some agents with vasodilating effects before the ischemic stage would increase the amount of NO produced from eNOS and decrease the induction of iNOS during the reperfusion stage. In addition, the use of agents with antioxidant activities should be beneficial in the treatment of hepatic injury following I/R.

In the current study, we assessed the potential protective effect of an NO producing drug such as nebivolol and an antioxidant such as chrysin against I/R-induced liver injury in rats. Nebivolol is a long acting vasodilating β_1-blocker with some β_3 agonist activity (Chlopicki et al., 2002; Feng et al., 2012; Gupta and Wright, 2008; Maffei and Lembo, 2009). It acts as NO producer by activating soluble guanylate cyclase which increases cGMP. cGMP acts as a smooth muscle relaxant in endothelium and may alleviate I/R-induced injury (Parenti et al., 2000; Sobocanec et al., 2006). Chrysin is a natural flavone which is used as a dietary supplement found in many plants, honey, and propolis (Williams et al., 1997). It has many reported biological activities such as anti-oxidant (Lapidot et al., 2002; Pushpavalli et al., 2010a), anti-inflammatory (Gresa-Arribas et al., 2010), Antidiabetic (Lukačínová et al., 2008), anxiolytic properties (Wolfman et al., 1994) as well as vasorelaxant effects (Duarte et al., 2001).

In an attempt to elaborate the mechanism of the potential hepatoprotective effects of nebivolol and chrysin, we investigated its effect on the redox status of liver rats by assessing the levels of lipid peroxides, NO and protein thiols. In addition, the expression levels of nitric oxide synthetase subtypes (eNOS and iNOS) in liver samples were assessed by Western blotting.

2. Materials and methods

2.1. Animals

Adult male Wister rats weighing 220–250 gm were used for performing this experiment and were obtained from national research center (Giza, Egypt), they were housed in cages, 10 animal per each cage and maintained at room temperature and 12/12 h light–dark cycle and free access of water and food. All animal use and handling were done in accordance with protocols approved by the Faculty of Pharmacy, Beni-Suef University Animal Care and Use Committee.

2.2. Surgical procedure and experimental design

Fifty rats were used for performing this study divided into 5 groups, the first one receiving dimethyl sulphoxide (DMSO) in saline and kept as control, second group is kept as ischemic control given DMSO in saline, third group was given chrysin (25fmg/kg/day) (Pushpavalli et al., 2010b), fourth group was given nebivolol (5 mg/kg/day) (Heeba and El-Hanafy, 2012), fifth group was given combination (chrysin plus nebivolol). All rats were pretreated with drugs by oral gavage once daily for 7 consecutive days,after the 30 min of the last dose and overnight fasting animals were anaesthetized with 70 mg/kg thiopental sodium (Fouad & Jresat, 2011) and middle laparotomy was incised and portal triad (portal vein, hepatic artery, bile duct) clamped for 30 min (ischemic period) and releasing the clips for allow reperfusion for another 30 min then the animal was sacrificed and blood sample collected as well as liver was excised and median and left lobe was removed for further biochemical investigations (Dulundu et al., 2007).

2.3. Determination of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST)

Determination of ALT and AST was performed using the commercially available diagnostic kits (Randox Laboratories Ltd., UK) according to the manufacturer instructions. The assay is based on the measurement of hydrazone derivatives which are produced by the interaction of alpha oxoglutarate with alanine and aspartate respectively. The amount of hydrazone derivative is proportional to the quantity of released enzyme.

2.4. Determination of oxidative stress biomarkers

Hepatic glutathione (GSH) was measured according the method described before and expressed as μmol/gm wet tissue (Beutler and West, 1977), hepatic malondialdehyde (MDA) was measured according to the method of Mihara and Uchiyama (1978)and expressed as nmol/gm wet tissue (Mihara and Uchiyama, 1978), hepatic Myeloperoxidase (MPO) was measured according to the method of Harada et al. (1999) and expressed as U/gm wet tissue (Harada et al., 1999), and