Thymoquinone Protects the Heart against Isoprenaline-Induced Myocardial Ischemia in Mice: A Histopathological Study

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Abstract

Background: Thymoquinone (TQ) is the most bioactive volatile oil that extracted from *Nigella sativa* seeds. It protects the tissue against ischemia-reperfusion injury including skeletal muscle, kidney, testes, liver and brain
Objectives: This study aimed to investigate the cardio-protective effect against isoprenaline-induced myocardial cell necrosis in mice
Materials and methods: Thirty six female albino mice were sub-grouped into six groups, each of six animals to receive intraperitoneal injection of dimethylsufoxide (5%W/v), TQ (10 mg/kg) or isoprenaline (30 mg/kg) as the following: Group I: dimethylsulfoxide, Group II: TQ , Group III: isoprenaline, Group IV: TQ twenty four hours prior to the treatment with isoprenaline, Group V:concomitant injections of isoprenaline and TQ and Group VI: treated with isoprenaline twenty four hours before treatment with TQ. After twenty four hours of treatment, the animals were sacrificed by cervical decapitation, the heart is rapidly removed and the left ventricle fixed in formalin solution (10%) for histopathological processing.
Results: Isoprenaline induced inflammatory cells infiltration around the blood vessels and through the cardiac tissue with focal cardiac cell necrosis. Thymoquinone protects the heart against isoprenaline-induced changes. In Groups IV and V mild inflammatory cell infiltration were observed whereas in Group VI, the histopathological findings included normal cardiac cell texture, attenuation of inflammatory cells infiltration and limited focal necrosis.
Conclusions: Thymoquinone protects the heart against isoprenaline-induced myocardial ischemia before or at the time and even after the cardiac insults that induced by isoprenaline suggesting that different mechanisms involved in cardio-protection.
Key words: Cardiac protection, Isoprenaline, Thymoquinone.

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Introduction

Nigella sativa Linn. (Ranunculaceae) is commonly known as black seed or black cumin, is used as herbal medicine all over the world for the treatment and prevention of asthma, diarrhea, dyslipidaemia…etc. Thymoquinone (TQ) is one of several volatile oils that extracted from black seeds. It exerts *in vitro* and *in vivo* anticancer, anti-inflammatory, and anti-oxidant activities (Gali-Muhtasib *et al.*, 2006). Thymoquinone acts as a superoxide anion radical scavenger and preserves the activity of anti-oxidant enzymes; catalase, glutathione peroxidase and glutathione-S-transferase (Woo *et al.*, 2012). The antioxidant property of TQ explained the cardio-protective effect against doxorubicin-induced cardiotoxicity in rats (Nagi and Mansour, 2000). Previous studies showed that TQ protects the tissue against ischemia-reperfusion injury including skeletal muscle, kidney, testes, liver and brain (Hosseinzadeh *et al.*, 2012; Awad *et al.*, 2011; Gökçe *et al.*, 2010; Abd El-Ghany *et al.*, 2009). In experimental transient global cerebral ischemia using four-vessel-occlusion method for 20 min in rats, TQ protect brain tissue against cerebral ischemia – reperfusion injury via reducing the activity of lipid peroxidation (Hosseinzadeh *et al.*, 2007). In experimental animal model using N(omega)-nitro-l-arginine methyl esters (l-NAME)-induced hypertension, TQ reduced the increase in systolic blood pressure in a dose dependent manner and decreased the elevated creatinine as a result of l-NAME-induced nephrotoxicity (Khattab and Nagi, 2007). Thymoquinone has been shown to have cardioprotective effect against isoproterenol-induced myocardial injury in various *in vivo* studies in rats (Randhawa *et al.*, 2012) and *in vitro*, suggested that thymoquinone may quench oxidant radicals and prevent membrane lipid peroxidation in tissues, in addition to its ability in increasing the levels of antioxidant enzymes (Murugesan *et al.*, 2012). The induced cardiotoxicity by isoproterenol is due to the formation of free radicals and its oxidation products (Murugesan *et al.*, 2012; Vaiyapuri *et al.*, 2011). Therefore, the aim of this study is to assess the cardio-protective effect of TQ against isoprenaline induced-cardiac cell necrosis in mice from the histopathological point of view.

Materials and Methods

This study conducted in Department of Pharmacology, College of Medicine, the University of Anbar in Al-Anbar Governorate, Iraq during 2012. The study approved by the scientific committee of the institute. Thirty six female white albino mice purchased from the animal house of The National Centre of Drug Control and Research in Baghdad, Iraq. The animals were allowed *ad libitum* access to food and tap water. Then they were sub-grouped into six groups, each of six animals to receive the following treatments:

Group I: served as control and treated with an equal volume of the dimethylsulfoxide (5% w/v), i.p.

Group II: treated with thymoquinone (10 mg/kg body weight, i.p.) dissolved in 5% diemethylsulfoxide

Group III: treated with isoprenaline (30mg/kg, body weight, i.p) dissolved in distilled water

Group IV: treated with thymoquinone (10mg/ kg body weight, i.p) twenty four hours prior to the treatment with isoprenaline (30mg/ kg body weight, i.p).

Group V: treated with concomitant injections of isoprenaline (30 mg/kg body weight, i.p) and thymoquinone (10 mg/kg body weight, i.p), each injection in each side of abdomen.

Group VI: treated isoprenaline (30mg/ kg body weight, i.p), then after twenty four hours the animals received thymoquinone treatment (10 mg/kg body weight, i.p).

Then all animals were sacrificed by cervical decapitation. The heart is rapidly removed and dissected. The atria were removed and the right ventricle (RV), left ventricle (LV) and septum were separated. RV, septum and piece of LV were preserved in freshly prepared formalin solution (10%) and were manually processed in different alcoholic and organic solutions, molded and dissected into (5µm) thickness slices to be fixed onto glass slides. The prepared slides then stained by eosin and hematoxylin stains.

The microscopical examination involved the whole sections onto the prepared slides.

The histopathological findings were graded according to Goldspink *et al.* (2004) and Lobo filho *et al.*, (2011) study which used isoprenaline-induced cardiac ischemia:

Grade 0 (normal or no significant changes): very little infiltration of inflammatory cells around blood vessels.

Grade 1(mild changes): mild infiltration of inflammatory cells near blood vessels and dilated blood vessels.

Grade 2 (moderate changes): moderate infiltration of inflammatory cells near the dilated or thickened wall blood vessels, limited focal necrotized muscle fibers.

Grade 3 (moderate to severe changes): high infiltration of inflammatory cells around blood vessels and between muscle fibers, and limited focal necrotized muscle fibers.

Grade 4 (severe changes): highly infiltration of inflammatory cells between muscle fibers (macrophage detected) and fibroblast, dilation or thickening in the wall of blood vessels and significant distracted and necrotic muscle fibers.

Results

In Group I the cardiac histopathological findings were normal myofibril texture ranged between grade 0 (4 animals) and grade 1 (2 animals) (Figure 1A).

Thymoquinone in Group II did not produce significant histopathological changes in the heart and the myofibril texture ranged from Grade 0 (3 animals) and Grade 1 (3 animals) (Figure 1B).

Isoprenaline in Group III induced massive infiltration of inflammatory cells around the blood vessels and through the muscle fibers with focal cardiac cell necrosis. The histopathological grading in isoprenaline treated group ranged between Grade 3 (one animal) and Grade 4 (five animal) (Figure 1C). Thymoquinone protects the heart from isoprenaline-induced cardiac cell necrosis when injected before isoprenaline (Group IV).

The histopathological were ranged from normal myofibril (two animals), very little inflammatory cell infiltration (one animal) to mild inflammatory cell infiltration (three animals) without evidence of focal cardiac cell necrosis (Figure 2A). Thymoquinone also protects the heart against isoprenaline when administered concomitantly (Group V).

Normal myofibril was observed in two animals; little inflammatory cell infiltration (two animals) and; mild inflammatory cell infiltration with dilated blood vessels (two animals) (Figure 2B). Thymoquinone attenuated the isoprenaline-induced cardiac cell changes when administered after isoprenaline (Group VI). Thymoquinone abolished the cardiac effect of isoprenaline in two animals; prevented the cardiac cell necrosis in one animal and; attenuated the inflammatory cells infiltration to moderate degree with limited focal necrosis in three animals (Figure 2C). Table (1) below summarized the above findings by a simple manner:

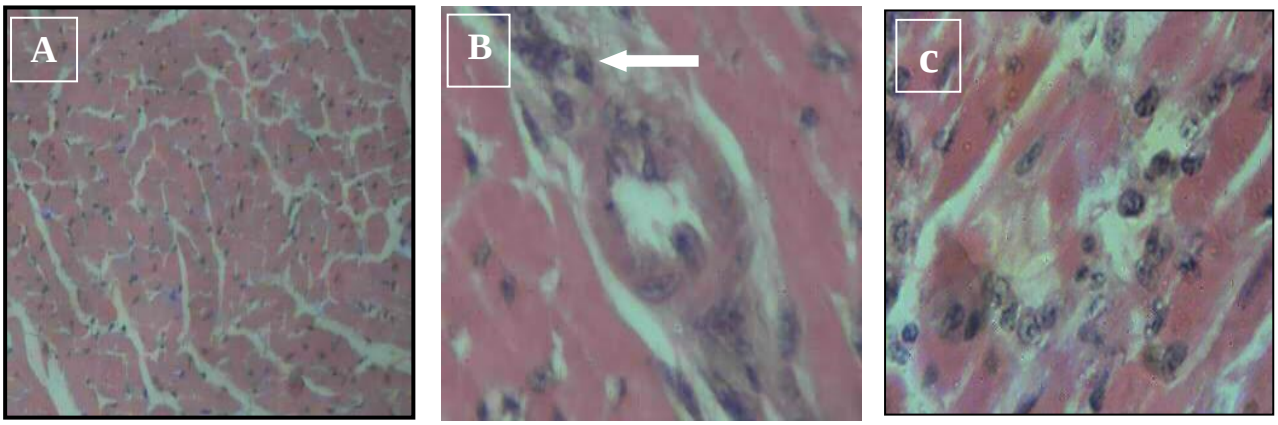

Figure (1)

The effect of dimethylsulfoxide [A], thymoquinone [B] and isoprenaline [C] on the myocardium. In which, [A, B] show normal tissue texture with very little infiltration of inflammatory cells near the blood vessels in [B, *arrow*]. Picture [C] shows high infiltration of inflammatory cells between the distructed myocardial fibers.

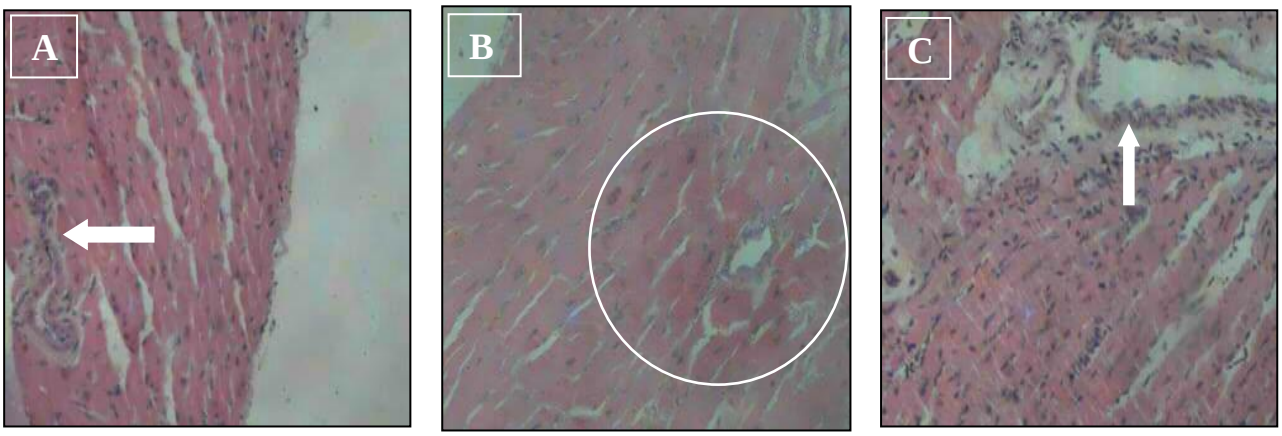

Figure (2)

Effect of thymoquinone on isoprenaline induced cardiac cell necrosis when thymoquinone administered before [A], concomitant [B] and after [C] isoprenaline. In which: [A], a model of treatment, show aproximatly normal tissue texture with moderate infiltration of inflammatory cell almost near the blood vessels (A, *arrow*). At the [B] model of treatment, there is a relatively normal tissue texture with mild infiltration of inflammatory cells near the blood vessels and between the myocardial fibers. A very limited focal necrotized muscle fibers and patchy slightly higher eosinophilic areas have been detected (B, *circles*). At the [C] model of treatment, the changes demonstrated a mild – moderate - severe myocardial changes. These are ranged from mild and high infiltration of inflammatory cells (*arrow*) and extend to show limited focal necrotized muscle fibers.

Table (1) : Show the distribution of the grouped animals among the designed grades of myocardial changes in response to different treatment modalities, (♦): represent the number of animals undergone the corresponding grade of changes.

Grade of change / Animal's grp.	Grade 0 normal	Grade 1 mild	Grade 2 moderate	Grade 3 Moderate - severe	Grade 4 severe
Group 1 (control)	♦♦♦♦	♦♦			
Group 2 (TQ)	♦♦♦	♦♦♦			
Group 3 (ISO)				♦	♦♦♦ ♦♦
Group 4 (TQ → ISO)	♦♦	♦	♦♦♦		
Group 5 (TQ ↔ ISO)	♦♦	♦♦	♦♦		
Group 6 (ISO → TQ)	♦♦		♦	♦♦♦	

Discussion

The results of this study show that TQ protect the heart against isoprenaline-induced myocardial cell necrosis using different time schedule of administration i.e. prior, concomitant or even after administration of isoprenaline. Myocardial ischemia as a result of activation β1-adrenoceptor by isoprenaline is responsible for cardiac cell necrosis and inflammatory cell infiltration. TQ is reported as anti-ischemic agents in different organs and tissue. In the gut, the mechanisms of gastro-protective effect of TQ related to the inhibition proton pump; acid secretion and neutrophil infiltration and enhancing mucin secretion, and nitric oxide production (Magdy *et al*., 2012).

The protective effect of TQ against the injury of skeletal muscle tissue caused by lower limb ischemia-reperfusion is related to its antioxidant capacity (Hosseinzadeh *et al*., 2012). In hepatic ischemia-reperfusion injury, the anti-apoptotic effect of TQ is through attenuating oxidative stress and inhibiting TNF-α induced NF-κB activation (Abd El-Ghany *et al*., 2009). In this study the cardio-protective effect of TQ is differed with the different time schedule of administration. The cardio-protection effect of TQ in Groups IV and V may be related to its antioxidant capacity. Previous studies showed that TQ protect the heart against cyclophosphamide or doxorubicin-

induced cardiotoxicity *via* this mechanism (Nagi *et al*., 2011; Nagi and Mansour, 2000).

The other possibility of cardio-protection is related to decrease cardiac demand as a result of negative inotropic effect of TQ (El-Tahir *et al*., 1993), and this mechanism explains the cardioprotection in Groups V and VI. Moreover, the possibility of β1-adrenoceptor blocking activity should be not excluded and through this mechanism TQ reduces the oxygen demand and explained the cardio-protection in Groups IV, V and VI. Al-Majed *et al* (2001) reported relaxation of guinea pig trachea preparation challenged with histamine. Al-Hariri *et al.*, (2009) found that *Nigella sativa* supplement for two months resulted in normal heart responsiveness to isoprenaline in isolated heart preparation. Further study found that the hearts of Nigella-treated rats developed a significant cardiac hypertrophy associated with an increase in the baseline cardiac inotropic properties upon progressive cardiac stress by isoproterenol infusion Yar *et al.*, 2008). It concludes that TQ protects the heart against isoprenaline-induced myocardial ischemia before or at the time and even after the cardiac insults that induced by isoprenaline suggesting that different mechanisms involved in cardio protection.

Acknowledgement

My thanks go to the histopathologist: Dr. Najem-Al-Dean Abdullah, Department of Oral Histopathology, College of Dentistry, Anbar University, for his efforts in analyzing of histopathological findings.

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