Thymoquinone protects the heart against isoproterenol-induced myocardial ischemia in mice: A histopathological study

Sir,

*Nigella sativa* Linn. (*Ranunculaceae*) is commonly known as black seed or black cumin, used as herbal medicine all over the world for the treatment and prevention of asthma, diarrhea, dyslipidaemia, etc. Thymoquinone (TQ) is one of the several volatile oils that extracted from black seeds. The cardioprotective effect of TQ against doxorubicin-induced cardiotoxicity in rats is related to its antioxidant property. TQ has been shown to have cardioprotective effect against isoproterenol-induced myocardial injury in various *in vivo* and *in vitro*, suggested that TQ may quench oxidant radicals and prevent membrane lipid peroxidation in tissues, in addition to its ability in increasing the levels of antioxidant enzymes. The rational of this study is related to the previous studies that showed the induced cardiotoxicity by isoproterenol is due to the formation of free radicals and its oxidation products. Therefore, the aim of this study is to assess the cardioprotective effect of TQ against isoproterenol induced-cardiac cell necrosis in mice from the histopathological point of view at the time before, concomitant, and after the insult of myocardial cell necrosis that induced by isoproterenol.

This study conducted in the Department of Pharmacology, College of Medicine, University of Anbar in Al-Anbar Governorate-Iraq in cooperation with the Department of Medicine, Al-Mustansiriya University in Baghdad, Iraq, during 2012. The study approved by the Scientific Committee of the Institute. Thirty-six female 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. The animals were subgrouped 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 TQ (10 mg/kg body weight, i.p.) dissolved in 5% dimethylsulfoxide
- **Group III**: Treated with isoproterenol (30 mg/kg body weight, i.p.) dissolved in distilled water
- **Group IV**: Treated with TQ (10 mg/kg body weight, i.p) 24 h before the treatment with isoproterenol (30 mg/kg body weight, i.p.)
- **Group V**: Treated with concomitant injections of isoproterenol (30 mg/kg body weight, i.p) and TQ (10 mg/kg body weight, i.p), each injection in each side of the abdomen
- **Group VI**: Treated isoproterenol (30 mg/kg body weight, i.p), then after 24 h the animals received TQ treatment (10 mg/kg body weight, i.p).

Then all animals were sacrificed by cervical decapitation. The ventricles were excised and preserved in freshly prepared formalin solution (10%) and were manually processed for histopathological study. The histopathological findings were graded according to Goldspink *et al.* and Filho *et al.* studies which carried on 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): Severe infiltration of inflammatory cells around blood vessels an in 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.

Drugs

Isoproterenol and TQ were purchased from Sigma-Aldrich, UK.

Table 1 summarizes the histopathological findings. In Group I, the cardiac histopathological findings were normal myofibril texture. TQ (Group II) did not produce significant histopathological changes in the heart and the myofibril texture, which ranged from Grade 0 (three animals) and Grade 1 (three animals). Isoproterenol (Group III) induced histopathological changes related to Grade 3 (one animal) and Grade 4 (five animals). TQ protects the heart from isoproterenol-induced cardiac cell necrosis when injected before isoproterenol (Group IV) or when administered concomitantly (Group V). TQ attenuated the isoproterenol-induced cardiac cell changes when administered after isoproterenol (Group VI).

The results of this study show that TQ protects the heart against isoproterenol-induced myocardial necrosis using different time schedule of administration. I.e. TQ offers cardioprotection (when it is given before isoproterenol) obviates the cell necrosis (when it is given concomitantly with isoproterenol) and improves the cardinecrosis (when it is given after isoproterenol). In this study, the cardioprotective effect of TQ is differed with the different time schedule of administration. The cardioprotective effect of TQ in Groups IV and V may be related to its antioxidant capacity. Previous studies showed that TQ protects the heart against cyclophosphamide or doxorubicin-induced cardiotoxicity via this mechanism. Moreover, recent studies disclose the mechanism of cardioprotective effect of TQ against myocardial ischemia and cardionecrosis that was induced by isoproterenol to scavenge the free radicals and antioxidant properties. Literature review does not disclose that TQ blocks the β1-adrenoeceptor, but the possibility of β1-adrenocceptor 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.

It concludes that TQ protects the heart against isoproterenol-induced myocardial ischemia before or at the time and even after the cardiac insults that induced by isoproterenine suggesting that different mechanisms involved in cardioprotection.

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Conflicts of Interest

There are no conflicts of interest.

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References

1. Nagi MN, Mansour MA. Protective effect of thymoquinone against doxorubicin-induced cardiotoxicity in rats: A possible mechanism of protection. Pharmacol Res 2000;41:283-9.
2. Randhawa MA, Alghamdi MS, Maulik SK. Cardioprotective Effect of Thymoquinone, an Active Principle of Nigella sativa, on Isoproterenol Induced Myocardial Injury. 6th European Congress of Pharmacology (EPHAR 2012) Proceedings of the British Pharmacological Society; 2012, July 17-20: Granada, Spain; 2012. p. 232.
3. Murugesan M, Ragunath M, Prabu T, Nadanasabapathi S, Sakhthive M, Manju V. Protective role of black cumin (Nigella sativa) on isoproterenol induced myocardial infarction in rats. Int J Pharm Clin Sci 2012;1:45-53.
4. Goldspink DF, Burniston JG, Ellison GM, Clark WA, Tan LB. Catecholamine-induced apoptosis and necrosis in cardiac and skeletal myocytes of the rat in vivo: The same or separate death pathways? Exp Physiol 2004;89:407-16.
5. Filho HG, Ferreira NL, de Sousa RB, de Carvalho ER, Lobo PL, Filho JG. Experimental model of myocardial infarction by isoproterenol in rats. Braz J Cardiovasc Surg 2011;26:469-76.
6. Aydin MS, Kocarslan A, Kocarslan S, Kucuk A, Eser I, Sezen H, et al. Thymoquinone protects end organs from abdominal aorta ischemia/reperfusion injury in a rat model. Rev Bras Cir Cardiovasc 2015;30:77-83.
7. Randhawa MA, Alghamdi MS, Maulik SK. The effect of thymoquinone, an active component of Nigella sativa, on isoproterenol induced myocardial injury. Pak J Pharm Sci 2013;26:1215-9.