التأثير الوقائى للثيموكينون على احتشاء عضلة القلب المحدث تجريبيا
"دراسة على رسم القلب والتغيرات البيوكيميائية والهستولوجية فى الجرذان"

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يهدف هذا العمل إلى تقييم التأثير الوقائى لمادة الثيموكينون على التغييرات الحادثة فى رسم القلب الكهربى، والكيمياء الحيوية وعلى أنسجة القلب أثناء مرض احتشاء عضلة القلب المحدث نتيجة لحقن مادة الايزوبرينالين فى فئران التجارب حيث قسمت ذكور الفئران إلى أربعة مجموعات، تم الاحتفاظ بالأولى كمجموعة ضابطة والمجموعة الثانية تم إعطاء فئران التجارب مادة الثيموكينون جرعة ١٠مجم/كجم/يوم عن طريق الفم لمدة ٧ أيام متتالية فقط، والمجموعة الثالثة تم استحداث مرض احتشاء عضلة القلب عن طريق حقن ٨٥ مجم /كجم مادة إيزوبرينالين تحت الجلد لمدة يومين متتاليين بفترة فاصلة ٢٤ ساعة فقط، أما المجموعة الرابعة تم فيها إعطاء فئران التجارب مادة الثيموكينون جرعة ١٠مجم/كجم/يوم عن طريق الفم لمدة ٧ أيام متتالية وكان الحقن بالايزوبرينالين فى اليومين السادس والسابع، وقد أظهرت النتائج أن المجموعة المرضيه من الفئران حدثت بها تغيير معنوى كبير فى نمط تخطيط القلب وذلك بسبب تشوهات التوصيل، وزيادة معنوية فى كلا من دلالات الموت المبرمج للخلايا وتركيز الكرياتين كيناز فى الدم وتشوه فى أنسجة القلب. أما فى المجموعة المعالجة بمادة الثيموكينون فقد حدث تحسن كبير فى الرسم الكهربى للقلب والتغيرات التشريحية المرضية وكان تركيز الكرياتين كيناز ودلالات الموت المبرمج فى الحدود الطبيعية بالمقارنة بالمجموعة الضابطة، مما يدل على أن تناول الثيموكينون يؤدى إلى حماية من مرض احتشاء عضلة القلب الناجم عن حقن مادة الايزوبرينالين.

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This finding might be a scientific support to understand the beneficial effects of thymoquinone on cardioprotection against myocardial injury, in which oxidative stress has long been known to contribute to the pathogenesis.

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ruptured. The increased levels of these enzymes are indicative of severity of cell necrosis and ISP mediated peroxidative myocyte injury. The increase in activities of these enzymes is in agreement with previous studies[25-26-27].

On the other hand, Thymoquinone pre-treatment significantly ameliorated ISP-induced CK-MB enzymes activities elevations. Furthermore, it almost preserved the normal architecture of the heartwhich in consonance with previous studies[16][28-29].

This present findings are strongly suggestive of potent antioxidant activity of TQ against ISP-induced oxidative stress in MI.

Histopathologic examination of cardiac tissues correlated with the observed biochemical and ECG changes as the protective effects of TQ on histopathological changes of myocardium were further supported by light microscopic observations. Subsequent to isoprenaline administration, significant myonecrosis, edema, and infiltration of inflammatory cells were observed in light microscopic examination of the myocardium. These histopathological perturbation in isoprenaline-intoxicated rats are attributed to a decline in oxygen supply with paramount rise in wall-stress[30-31-32].

However, TQ pretreatment to ISP-intoxicated rats has shown resistance towards necrosis, edema, and inflammation and protected cardiomyocytes from the deleterious effects of isoprenaline. Such effects can be explained by previously reported anti-oxidant and anti-inflammatory potentials of TQ[33-34-35]. Rats which received TQ per se treatment exhibited a normal myocardial histology, which is suggestive of the fact that TQ at this dose does not render any significant adverse effects on myocardium and is safe for myocardial cells.

5. Conclusion

The present study demonstrated that subcutaneous injections of isoprenaline produced myocardial infarction in rats as evident by the release of myocyte injury markers in serum. Myocardial lesions were associated with decreased antioxidant defense status in the heart electrocardiographic, histopathological changes and release of CK-MB. In addition, the present study provided experimental evidence that Thymoquinone maintained the antioxidant activity and improved cardiac performance following high-dose isoprenaline administration.

4. Discussion

Myocardial infarction induced by injection of ISP is a standardized model to study the beneficial effects of numerous drugs and antioxidants. In the present study, we found that Thymoquinone pre-treatment exerts a strong cardioprotection in ISP induced MI in rats. The ECG is considered the single most important initial clinical test for diagnosis of myocardial ischemia and infarction. Its correct interpretation is usually the basis for immediate therapeutic interventions and/or subsequent diagnosis tests. In the present study, ISP intoxication shows significant alterations in ECG patterns. The characteristic ECG findings were elevation of ST-segment and QTc interval. These alterations could be due to ISP auto oxidation and generation of free radicals which further produced oxidative stress. Increased oxidative stress causes loss of cell membrane function in injured myocardium[8][(15)]. This elevation reflects the potential difference in the boundary between ischemic and non-ischemic zones[(16-17)]. Furthermore, ST-segment elevation was observed in patient with acute myocardial ischemia and in experimental model of ISP in rats[(18-19)].

Pre-treatment with TQ in ISP-intoxicated rats significantly prevented the altered ECG pattern towards normal suggesting the cell membrane stabilizing potential of TQ which might be due to its potent antioxidant property which is in agreement with previous reports[(20-21-22)].

In the present study ISP-intoxicated rats showed MI which is evident by significant increase in heart rate. These changes in hemodynamic parameter indicated the activation of sympathetic nervous system which is in line with previous reports[(23-24)][12].

Pre-treatment with TQ significantly attenuated these changes in hemodynamic parameter which are evidenced from improvements in heart rate.

Additionally, ISP-induced myocardial injury was further manifested by the significant elevation in activities of serum CK-MB enzyme; due to leakage from tissue to blood serum as a result of damaged or destroyed cardiomyocytes, as well as, the cells damaged because of insufficient supply of oxygen and oxidative damage of myocardium which render the cell membrane fragile, porous, or

a or b: Significantly different from the control or ISP-intoxicated group respectively at $P \leq 0.05$ using ANOVA followed by Tukey as a post-hoc test.

3. 3. Effect of Thymoquinone on histopathological changes that associate isoprenaline-induced myocardial infarction in rats

To further characterize the cardiotoxicity induced by isoprenaline, histopathological examination of heart tissue was done. Hearts from control and thymoquinone treated rats showed regular cell distribution and normal myocardium architecture (Fig 4A and4B). Histological examination of hearts from ISP-intoxicated animals revealed marked myocardial degeneration (D) in the form of myofibrillar loss, cytoplasmic vacuolization (V) , inflammatory cell infiltration (I), edema (E) and congestion (Fig. 4C). Interestingly, pretreatment of ISP-intoxicated rats with TQ at dose (10 mg/kg) almost preserved the normal myocardium architecture (Fig. 4D).

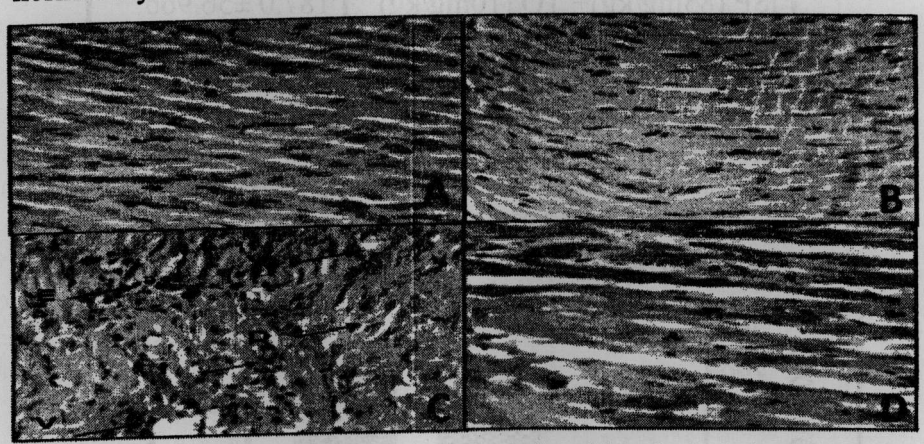

Fig. (3): Effect of TQ pre-treatment on ISP-induced histological alterations of the heart tissue (x200). Photomicrographs of haematoxylin and eosin stained sections of heart depicting (A) control group, (B) TQ (10 mg/kg) treated group, (C) ISP treated group (85mg/kg) and (D) TQ (10 mg/kg)+ISP (85 mg/kg) treated group. (A)&(B) show normal histoarchitecture of the rat heart. (C) Show ISP-induced myofibrillar loss, cytoplasmic vacuolization, inflammatory cell infiltration, edema and congestion. (D) Show that TQ pre-treatment prevented cardiomyocyte damage induced by ISP and ameliorated inflammatory cell infiltration.

3. 2. Effect of Thymoquinone on biochemical changes that associate with isoprenaline-induced myocardial infarction in rats

Isoprenaline increased serum CK-MB activity by135% as compared to the normal group. TQ pre-treatment decreased serum CK-MB activity by 32% as compared to the isoprenaline-intoxicated group (Table 2 and Fig 2).

Table (2): Cardiotoxicity marker of rats treated with TQ and/or isoprenaline (ISP).

Treated groups	CK-MB
Control	122.3±27.46
TQ (10mg/kg)	162.4±20.59
ISP (85mg/kg)	284.0 ±28.47 a
ISP (85mg/kg)+ TQ (10mg/kg)	187.0 ±56.96b

CK-MB, Creatine phosphokinase isoenzyme-MB.
Data are the mean±S.D. (n=6)
A: Significantly different from the control
B: Significantly different from ISP-intoxicated group respectively at P ≤0.05 using ANOVA followed by Tukey as a post-hoc test.

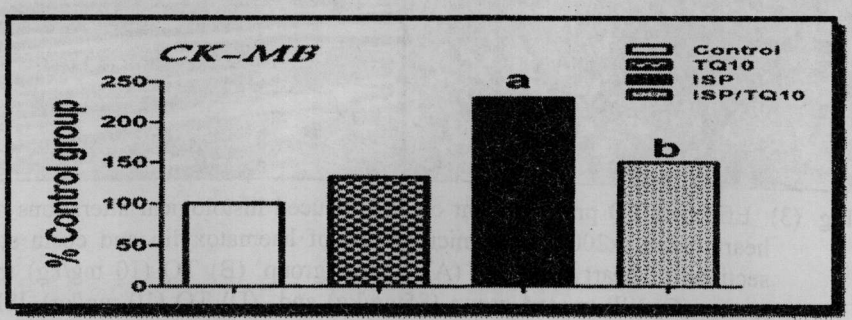

Fig. (2): Effect of *TQ* (10 mg/kg) on *CK-MB* in ISP-intoxicated group as percentage of control group.

Table (1): ECG parameters of rats treated with TQ and/or isoprenaline (ISP).

Treated groups	ST segment (mV)	QTc interval (ms)	Heart rate (beat/min)
Control	0.120±0.007	0.32 ±0.020	343.3± 11.50
TQ (10mg/kg)	0.110±0.020	0.30±0.018	319.7± 38.70
ISP (85mg/kg)	0.230 ±0.082[a]	0.46 ±0.05[a]	470.0 ± 35.59[a]
ISP(85mg/kg)+ TQ (10mg/kg)	0.15 ±0.080[b]	0.37 ±0.09[b]	434.3 ±18.74[a]

Data are the mean± S.D. (n =8).
a:Significantly different from the control
b:Significantly different from **ISP-intoxicated** group respectively at P ≤ 0.05 using ANOVA followed by Tukey as a post-hoc test.

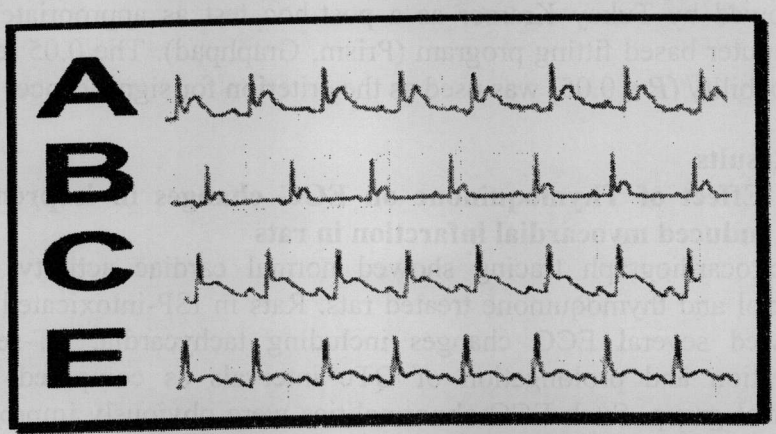

Fig.(1). Effect of TQ pre-treatment on ISP-induced alterations in ECG pattern. (A) Control group, (B) TQ (10 mg/kg) treated group, (C) ISP (85 mg/kg) intoxicated group, (D) TQ (10 mg/kg) + ISP (85 mg/kg) treated group. ECG tracing of control and TQ only treated rats normal heart rate, QTc interval. ISP-intoxicated group shows tachycardia, elevation of ST segmentand prolongation of QTc intervals. Thymoquinone supplementation at 10mg/kg dose almost normalized heart rate, ST segment and QTc intervals.

2.6. Biochemical assays

The collected serum was used for the estimation of cardiac marker enzyme creatinine kinase-MB (CK-MB), standard enzymatic kits according to method of Gerhardt &Waldenström[13].

2.7. Histopathology

After animal sacrifing, the heart specimens were fixed in 10% buffered formalin. The fixed tissues were embedded in paraffin and serial sections (5 μm thick) were cut. Each section was stained with hematoxylin and eosin (H&E) according to Banchroft&Gamble[14]. The sections were examined under the light microscope (Olympus BX10, Tokyo, Japan) for histopathological changes and photomicrographs (Olympus DP12 camera, Japan) were taken.

2.8. Statistical analysis

All the values are expressed as mean ± S.D. Statistical significance between more than two groups was tested using one-way ANOVA followed by Tukey–Kramer as a post-hoc test as appropriate using computer based fitting program (Prism, Graphpad). The 0.05 level of probability ($P \leq 0.05$) was used as the criterion for significance.

3. Results

3.1. Effect of Thymoquinone on ECG changes in isoprenaline-induced myocardial infarction in rats

Electrocardiograph tracing showed normal cardiac activity in the control and thymoquinone treated rats. Rats in ISP-intoxicated group showed several ECG changes including tachycardia, ST-segment elevation and prolongation of QTc intervals as compared to the control group. Such ECG abnormalities were obviously improved in the ISP-intoxicated animals pretreated with the Thymoquinone (10 mg/kg) as evidenced by normalization of heart rate, ST-segment and QTc intervals compared to ISP-intoxicated group (Table 1 and Fig1).

Group 2 (ISP): rats received tween 80/distilled water for 7 days. ISP (85 mg/kg, S.C.) in normal saline was injected on the 6thand 7th day at an interval of 24 h.

Group 3 (TQ): rats received distilled TQ (10 mg/kg/day, p.o.) for 7 days.

Group 4 (TQ + ISP): Animals received TQ (10 mg/kg/day, p.o.) for 7days.Rats received ISP on the 6th and 7thday at an interval of 24 h.

Twenty-four hours after the last treatment, animals were anesthetized with urethane intraperitoneal at dose (1.5 g/kg)[11] for ECG monitoring. Thereafter, blood samples were collected from the retro-orbital plexus. Serum was separated by centrifugation at 4000 rpm for 4 min and stored at -20^{0}C until analysis for the estimation of CK-MB activity. Rats were then sacrificed by decapitation and the hearts were rapidly isolated, and preserved in 10% formalin for histological examination.

2.5. Measurements of ECG changes

Electrocardiograph was recorded at the beginning of the experiment to ensure the normal ECG pattern of the rats according to method of (Mladenka, *et al*)[12]. At the end of the experiment, ECG was recorded in urethane anesthetized rats 48 h after ISP injection using Bioscience ECG recorder (Bioscience, Washington, USA). Anesthetized rats were placed in the supine position on a board and needle electrodes were inserted beneath the skin for the limb lead at position II (right forelimb to left hind limb). Every recording lasted for at least 5 min. ECG recording speed was 50 mm/s and the voltage was 1 mV/cm. Noise was minimized by a digital filter. Analysis of ECG waves was done to calculate heart rate (beats/min), ST segment (mV) and QTc interval (ms). QTc interval was calculated from Bazett's formula [QTc= QT/(square root of RR interval)]. For each parameter, measurements were done at three non-consecutive, randomly chosen points in every 5 min recording. The results are reported as mean of the three randomly selected segments.

supramaximal doses of isoprenaline, and study its therapeutic efficacy by studying the biochemical marker, electrocardiographic and histopathological changes.

2. Materials and methods

2.1. Drugs and chemicals

(±)-Isoprenaline hydrochloride and thymoquinone were purchased from Sigma Aldrich Co. St. Louis. (MO.USA). The standard enzymatic kits of CK-MB, purchased from BiodignosticCo., Egypt. The entire chemicals used in this study were of analytical grade.

2.2. Experimental animals

Male Sprague-Dawley rats (200-220 g) obtained from Nile Co. for Pharmaceutical and Chemical industries (Cairo, Egypt) were used in the present study. Rats were housed in faculty of Pharmacy, Ain-Shams University, in an air-conditioned atmosphere, at a temperature of 25 °C with alternatively 12 hour light and dark cycles. Animals were acclimated for 2 weeks before experimentation. They were kept on a standardpellet diet and water *ad libitum*.

The study was carried out according to the guidelines of the Ethics Committee, Faculty of Pharmacy, Ain-Shams University.

2.3. Induction of myocardial infarction

Myocardial infarction was induced in rats by subcutaneous injection of 85 mg/kg isoprenaline hydrochloride dissolved in saline once daily for two successive days. The selected route and dose were chosen from published literatures[9-10].

2.4. Experimental design

After acclimatization, the animals were randomly divided into the following groups consisting of 8 rats each:

Group 1 (control): rats received tween 80/distilled water (vehicle for thymoquinone) for 7 days. Normal saline (1 ml/kg) was injected subcutaneous (S.C.) on the 6^{th} and 7^{th} day.

1. Introduction

Cardiovascular disease (CVD), predominantly myocardial infarction, remains the principal cause of death in both developed and developing countries. It will be the most important cause of mortality worldwide by 2020[1]. Myocardial infarction (MI) occurs when the blood supply to a part of the heart is interrupted, causing death of heart tissue[2]. It is an acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand[3]. Inflammation is a key process involved in mediating myocardial tissue damage after an ischemic event. Neutrophils infiltrate the infarcted area where they can promote myocardial cell damage through the release of proteolytic enzymes and the production of reactive oxygen species (ROS). Inflammation may also increase the risk of recurrent ischemic events by destabilizing atherosclerotic plaques and making them prone to rupture[4]. The development of myocardial ischemia and infarction is a dynamic process with the widespread occurrence of coronary atherosclerosis and involvement of oxidative stress in the human. Among several pharmacological interventions to protect heart against oxidative stress, the use of antioxidants is most promising[5].

Isoprenaline (ISP) [1-(3,4-dihydroxyphenyl)-2-isopropylamino ethanol hydrochloride] is a synthetic β-adrenergic agonist and its subcutaneous injection induces irreversible cellular damage and ultimately myocardial infarction in rats. The acute hemodynamic and electrocardiographic changes in isoprenaline induced myocardial infarction resemble closely to those occurring in patients with myocardial infarction[6]. Therefore, the rat model of isoprenaline induced myocardial infarction offers a reliable non-invasive technique for studying the effects of various potentially cardioprotective agents[7].

It produces myocardial necrosis which caused cardiac dysfunction, increased lipid peroxidation along with an increase in the level of myocardial lipids, altered activities of thecardiac enzymes and antioxidants[8].

Therefore the present study was designed to study the effect of thymoquinone pre-treatment on the myocardial infarction induced by

Cardioprotective Effects of Thymoquinoneagainst Isoprenaline-Induced Cardiotoxicity: Electrocardiographic, Biochemical and Histopathological Evaluation

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Objective: To assess the protective effects of thymoquinone (TQ) on electrocardiographic, biochemical and histopathological changes in isoprenaline induced myocardial infarction. Methods: Myocardial infarction was induced in rats by subcutaneous (s.c) injection of isoprenaline (85mg/kg) for two consecutive days at an interval of 24 h. Rats were treated with thymoquinone orally (p.o) at dose (10 mg/kg/day) for a period of 7 days and isoprenaline (ISP) was injected on the 6th and 7th day. At the end of experiment i.e. on the 8th day electrocardiographic, biochemical and histopathological changes were monitored from control and experimental groups. Results: ISP-intoxicated rats showed a significant alteration in electrocardiograph pattern (i.e. ST, QTc&HR) due to conduction abnormalities, increased serum creatine kinase isoenzyme-MB (CK-MB), increased infarction size, myofibrillar disarrangement as indicators of oxidative stress and increase MMP9. Pre-treatment with TQ significantly prevented the ISP induced alteration in ECG, biochemical and histopathological changes. Conclusions: The present result shows that treatment of TQ in ISP-intoxicated rats significantly attenuates the induced myocardial infarction.

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