Research article

Cardioprotective effect of thymol against adrenaline-induced myocardial injury in rats

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ABSTRACT

Cardiovascular disease represents a vital global disease burden. This study aims to assess the possible cardioprotective effect of thymol against adrenaline-induced myocardial injury (MI) in rats. Furthermore the effect of thymol on cardiac function biomarkers, electrocardiogram (ECG) alterations, oxidative stress, inflammation, apoptosis and histopathological changes was assessed. MI was induced by adrenaline (2 mg/kg, s.c.) injected as a single dose for 2 consecutive days (24 h apart). Normal and control groups received the vehicle for 21 consecutive days. The other 3 groups were orally administered thymol (15, 30, 60 mg/kg) for 21 consecutive days and on day 22, adrenaline was injected as a single dose for 2 consecutive days. Then ECG examination, biochemical, histopathological, immunohistochemical analyses were carried out. Thymol reversed adrenaline-induced reduction of heart rate, prolongation of RR interval and elevation of ST interval. Thymol pretreatment significantly reduced serum aspartate dehydrogenase (AST), lactate dehydrogenase (LDH), and creatine kinase (CK) levels in MI rats. Oral pretreatment with thymol increased reduced glutathione (GSH), reduced malondialdehyde (MDA), nuclear factor-kappa B (NF-κB), and interleukin-1β (IL-1β) cardiac contents in MI rats. Additionally, thymol administration significantly decreased protein expression of caspase-3, increased Bcl-2 protein expression in cardiac tissue and ameliorated histopathological changes. This study reveals that thymol exerted cardioprotective effect against adrenaline-induced MI in rats evidenced by improving cardiac function, attenuating ECG and histopathological changes which may be partly mediated through its anti-oxidant, anti-inflammatory and anti-apoptotic effect.

1. Introduction

Cardiovascular disease (CVD) is a series of disorders affecting the heart and blood vessels, including peripheral arterial disease, coronary heart disease (CHD) and congestive heart failure (CHF) (Capo et al., 2018). World health organization (WHO) estimates that by 2020, CVD will be the primary cause of death worldwide (Bei et al., 2020).

Myocardial infarction is defined as myocardial cell death due to imbalance between myocardial oxygen demand and supply (Thygesen et al., 2018; Aydin et al., 2019). It may occur secondary to atherosclerotic plaque (type 1 myocardial infarction), or variation in myocardial oxygen supply and demand without the presence of atherosclerotic plaque (type 2 myocardial infarction), or detection of ST elevation electrocardiogram (ECG) in patients (type 3 myocardial infarction). Myocardial infarction may be associated with percutaneous coronary interventions (type 4 myocardial infarction), or coronary artery bypass surgery (type 5 myocardial infarction) (Thygesen et al., 2018).

Myocardial injury (MI) is a pre-stage for the diagnosis of MI, it is also an entity in itself. Oxidative stress in MI occurs due to excessive production of reactive oxygen species (ROS) resulting in lipid peroxidation and disruption of myocardial cell membrane (Neri et al., 2015). Excessive ROS produced in MI also results in the stimulation of inflammatory and apoptotic pathways (Neri et al., 2017; Attalla et al., 2018).

Catecholamines exert inotropic and chronotropic effects on the myocardium. Excess catecholamines cause coronary vasoconstriction, resulting in an increase in myocardial oxygen demand and a reduction in myocardial blood delivery, leading to MI (Sethi and Peiris, 2020). Catecholamines induced-MI is mediated by overproduction of ROS and depletion of cardiac anti-oxidants resulting in oxidative stress and myocardial apoptosis or necrosis (Cerretani et al., 2012; Radhiga et al., 2012).

Adrenaline is a naturally occurring catecholamine and generally considered as a hormone involved in "fight or flight" mechanism (Hara et al., 2011). Adrenaline was approved as a drug and was used first in

human cardiopulmonary resuscitation. It also possesses other therapeutic applications in the treatment of cardiac arrest, allergic reactions, glaucoma, and asthma. However, at doses exceeding physiological levels, adrenaline has been demonstrated to trigger production of ROS, reactive nitrogen species (RNS) mediated tissue damages (Diaz-Cruz et al., 2007).

Adrenaline-induced MI in rats is considered a valid experimental model which is used to investigate the cardioprotective effect of anti-oxidant agents (Mishra et al., 2016). Adrenaline was found to induce MI by causing lipid peroxidation leading to depletion of cellular antioxidants (Andreadou et al., 2009). In cardiomyocytes, adrenaline was shown to increase lipid, protein, and DNA damage with overproduction of nitrosative derivatives (Radaković et al., 2018). Since ROS play a crucial role in the pathogenesis of MI, efforts have been directed towards the use of natural products possessing anti-oxidant activity as adjuvants with few side effects and great safety in treatment of MI (Li et al., 2012).

Thymol is a naturally occurring phenolic compound and one of the primary active constituents present in the essential oil of Thymus vulgaris (Villanueva Bermejo et al., 2015). Reports have demonstrated that thymol has shown anti-oxidant, anti-microbial (Tsai et al., 2011; Wang et al., 2017), and hepatoprotective effects (Alam et al., 1999). Moreover, it has been demonstrated that thymol markedly inhibits the production of tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) in inflammation (Liang et al., 2014; Luna et al., 2017).

Although, Meeran and Prince (2012) reported the protective effect of thymol against isoproterenol-induced myocardial infarction in rats, its effect on adrenaline-induced MI has not been investigated yet. So, this study was planned to evaluate the cardioprotective effect of thymol against adrenaline-induced MI in rats. In this context, ECG examination, cardiac serum enzymes, oxidative stress biomarkers, inflammatory cytokines, anti-apoptotic and apoptotic protein expression as well as histopathological examination were carried out.

2. Material and methods

2.1. Animals

Male albino Wistar rats weighing (120–150 g) were obtained from the animal house colony of the National Research Centre (Giza, Egypt). Animals were housed under standardized laboratory conditions (12h alternating light/dark cycle and were fed standard laboratory pellets with water ad libitum). The study protocol and procedures have been implemented in compliance with the National Institutes of Health guidelines (NIH publication No. 85–23, revised 2011) and in accordance with the Ethics Committee of the National Research Centre, Egypt (registration number 18/127).

2.2. Drugs and chemicals

Adrenaline 1 mg/ml S.C. ampoules (Cid, Giza, Egypt) (commercially available, CID, Egypt). Thymol was purchased from Sigma-Aldrich (St Louis, MO, USA).

2.3. Experimental design

Rats were randomly allocated into 5 groups (8 rats/per group). Group I: received distilled water orally for 21 consecutive days. On day 22, distilled water was administered subcutaneously as a single dose for 2 consecutive days (24 h apart) (served as normal). Groups II: received distilled water orally for 21 consecutive days. On day 22, adrenaline (2 mg/kg, s.c.) was injected as a single dose for 2 consecutive days (24 h apart) (served as control MI). Groups (III–V): received Thymol (15, 30, 60 mg/kg, p.o.), respectively for 21 consecutive days. On day 22, adrenaline (2 mg/kg, s.c.) was injected as a single dose for 2 consecutive days (24 h apart). The doses of adrenaline and thymol were selected according to previous published data of Amin et al. (2016) and Asadbegi et al. (2018), respectively.

2.4. ECG examination

Rats were anesthetized with thiopental (45 mg/kg, i.p.). ECG was recorded for 1 min by inserting subcutaneous peripheral limb electrodes using ECG Powerlab module that consists of Powerlab/8sp, and animal bio-amplifier, Australia, in addition to Lab Chart7 software with ECG analyzer (Salama et al., 2018). Heart rate (HR), RR interval, and ST height were recorded.

2.5. Serum collection and tissue preparation

Blood samples were collected from the retro-orbital sinus of rats. Collected blood samples were centrifuged using a cooling centrifuge (2k15; Sigma/Laborzentrifugen) at 1538g for 10 min and the resulting serum was used for determining AST, LDH, and CK levels.

Rats were sacrificed after 24 h of adrenaline administration under light anesthesia with ether. Hearts were isolated and homogenized (MPW-120; Medical Instruments) in 20% (w/v) ice-cold phosphate buffer. Then, the homogenate was centrifuged using a cooling centrifuge (2k15; Sigma/Laborzentrifugen) at 1538g for 5 min and the resulting supernatant was used for determining the cardiac contents of malondialdhyde (MDA), reduced glutathione (GSH), nuclear factor kappa B (NF-κB), and interleukin-1beta (IL-1β).

2.6. Serum biochemical parameters

2.6.1. Serum AST level

Serum AST was measured using Reitman and Frankel (1957) method. The concentration was measured spectrophotometrically at 505 nm. The serum AST level is expressed as U/L.

2.6.2. Serum LDH level

LDH level was estimated using the enzymatic method according to manufacturer instructions (ChronoLab, France) and measured spectrophotometrically at 340nm. Serum LDH level is expressed as U/L.

2.6.3. Serum CK level

CK level was determined enzymatically using Abbot et al. (1984) method and the concentration was measured using spectrophotometer at 340 nm. Serum CK level is expressed as U/L.

2.7. Cardiac biochemical parameters

2.7.1. Determination of cardiac MDA content

Lipid peroxidation was assayed by measuring cardiac levels of MDA according to the method described by Ruiz-Larrea et al. (1994). The supernatant was read spectrophotometrically at 532 nm, and the cardiac content is expressed in nanomoles of MDA per milligram of cardiac tissue.

2.7.2. Determination of cardiac GSH content

Cardiac GSH content was determined according to the method described by Ellman (1959). Calculation of GSH concentration was based on a standard GSH curve and is expressed in micromoles of GSH per gram of cardiac tissue.

2.7.3. Determination of cardiac NF-κB content

NF-κB content was determined using a rat-specific immunoassay kit (Rat NF-κB ELISA) from Biosource, Inc., according to the manufacturer's protocol. The intensity of the colored product was directly proportional to the concentration of rat NF-κB, as determined by microplate reader (Biotech ELx800) set at 450 nm. The sample concentration was determined against a standard curve and is expressed in nanogram of NF-κB per gram of cardiac tissue.

2.7.4. Determination of cardiac IL-1β content

IL-1β content was determined using a rat-specific immunoassay kit (Rat IL-1β ELISA) from Glory Science (Del Rio,Texas, USA)., according to the manufacturer's protocol. The intensity of the colored product was directly proportional to the concentration of rat IL-1β, as determined by microplate reader (Biotech ELx800) set at 450 nm. The sample concentration was determined against a standard curve and is expressed in nanogram of IL-1β per gram of cardiac tissue.

2.8. Histopathological examination

Different sections from the normal and treated hearts were removed and fixed in 10% neutral formalin for 48h. The tissues were washed, dehydrated and embedded in paraffin. Finally, 4 μm thick sections were stained with H&E for assessment of the histopathological lesions including cardiomyocyte necrosis and leukocytic infiltration. Ten sections per group were examined. The lesions were semi quantitatively graded according to the method of Downing and Lee (1983). Score 0 = no histological abnormality, 0.5 = the lesions are focal and equivocal, 1 = the lesions are definite and scarce, 1.5 = the lesions are extensive and non-transmural and 2 = the lesions are extensive and transmural.

2.9. Immunohistochemical analysis

Immunohistochemical staining of activated caspase-3 and Bcl-2 was performed as previously described (Ibrahim et al., 2015). The heart sections were deparafinized, rehydrated and blocked in 3% hydrogen peroxide. Then, the sections were incubated with rabbit polyclonal anti-caspase-3 (1:1000 dilution; BD Biosciences, Le Pont-de-Claix, France) and mouse monoclonal anti-Bcl-2 (1:200 dilution, Santa Cruz Biotechnology) as primary antibodies. Diaminobenzidine (DAB) chromogen was added to visualize the immune reaction. Finally, the slides were counterstained in hematotoxylin and cover-slipped. Semi quantitative grading score for assessment of activated caspase-3 and Bcl-2 was carried out, in ten random high power fields, according to the percentage of immune stained cells in high power field (HPF) (40X). Score 0 = no staining; 1-positive staining in < 25% of cells/HPF; 2-positive staining in 25–50% of cells/HPF; 3-positive staining in >50% of cells/HPF.

2.10. Statistical analysis

Data are expressed by as mean ± SEM. Comparison for more than 2 groups were carried out by one way ANOVA followed by Tukey's multiple comparison test, except for histopathological and immunohistopathological analyses were carried out by one way ANOVA followed by Newman-Keuls multiple comparisons test. All analyses utilized GraphPad Prism 6.0 statistical package for Windows (GraphPad, San Diego, Calif.). Statistical significance was set at $p < 0.05$.

3. Results

3.1. Effect of thymol on HR, RR, and ST interval of adrenaline-induced MI in rats

Table 1 shows that MI-induced by single s.c. injection of adrenaline in a dose of 2 mg/kg for 2 successive days showed a significant decrease in HR by 30.69%, increase in RR interval by 60.36% and increase in ST interval by 561.54%, as compared to normal rats.

Oral pretreatment of thymol in doses of 15, 30, and 60 mg/kg showed a significant increase in HR by 57.37, 35.98, and 32.94%, respectively decrease in RR interval by 30.63, 30.26 and 26.20%, respectively, and decrease in ST interval by 32.94, 26.20, and 76.74%, respectively as compared to control (MI) rats.

Table 1. Effect of Thymol on ECG pattern.

Parameters			
Treatment	Heart rate (bpm)	RR interval (s)	ST segment
Normal	356.100@±3.727	0.169@±0.002	0.013@±0.003
Control (MI)	246.800* ± 19.590	0.271* ± 0.033	0.086* ± 0.016
Thymol (15 mg/kg)	388.400@±7.952	0.188@±0.007	0.043@±0.009
Thymol (30 mg/kg)	335.600@±33.980	0.189@±0.018	0.040@±0.010
Thymol (60 mg/kg)	328.100@±6.171	0.200 ± 0.013	0.020@±0.000

Data are presented as mean ± SEM. Statistical analysis are carried by one way ANOVA followed by Tukey's multiple comparison test.
*Significant difference from normal group at $p < 0.05$.
@Significant difference from control (MI) group at $p < 0.05$.

3.2. Effect of thymol on serum biochemical parameters

As depicted in Table 2, adrenaline-induced MI resulted in significant increase in serum AST, LDH, and CK levels by 50.96, 71.31, and 59.96%, respectively as compared to normal rats.

Oral pretreatment of thymol (15 mg/kg) showed a significant decrease in AST level by 16.32% as compared to control (MI) group and was significant from normal group. Thymol treatment (30, 60 mg/kg) significantly reduced AST level by 24.00, and 36.24%, respectively as compared to control (MI) group.

Oral administration of thymol (15 mg/kg) tended to decrease LDH level but this decrease was not significant from control (MI) group. Thymol (30 mg/kg) still showed a significant increase in LDH level to be similar to control (MI) group. At a dose of 60 mg/kg, thymol significantly reduced LDH level by 31.29% as compared to control (MI) rats.

Regarding CK level, Thymol (15 mg/kg) significantly decreased CK level by 27.63% when compared to control (MI) group and increased it by 15.77% as compared to normal group. Oral pretreatment with thymol (30 mg/kg) significantly reduced CK level by 30.35% as compared to

Table 2. Effect of Thymol on serum biochemical parameters.

Parameters			
Treatment	AST (U/L)	CK (U/L)	LDH (U/L)
Normal	414.900@±10.377	110.512@±2.091	450.64@±26.35
Control (MI)	626.333* ± 20.164	176.773* ± 3.383	771.99* ± 17.53
Thymol (15 mg/kg)	524.100*@±11.492	127.937*@±7.086	549.14 ± 49.45
Thymol (30 mg/kg)	476.000@±16.326	123.122@±2.768	795.39* ± 48.46
Thymol (60 mg/kg)	399.333@±17.740	147.196*@±3.691	530.42@±94.77

Data are expressed as mean ± SEM. Statistical analysis are carried by one way ANOVA followed by Tukey's multiple comparison test.
*Significant difference from normal group at $p < 0.05$.
@Significant difference from control (MI) group at $p < 0.05$.

Table 3. Effect of thymol on MDA and GSH contents in heart homogenate.

Parameters		
Treatment	MDA (nmol/mg)	GSH (μmol/g)
Normal	174.038@±4.591	3.937@±0.104
Control (MI)	390.064* ± 22.482	2.573* ± 0.057
Thymol (15 mg/kg)	178.098@±13.818	3.622@±0.312
Thymol (30 mg/kg)	169.381@±7.239	4.534@±0.166
Thymol (60 mg/kg)	178.893@±0.779	3.916@±0.161

Data are expressed as mean ± SEM. Statistical analysis are carried by one way ANOVA followed by Tukey's multiple comparison test.
*Significant difference from normal group at $p < 0.05$.
@Significant difference from control (MI) group at $p < 0.05$.

Table 4. Effect of thymol on heart biochemical parameters.

Parameters		
Treatment	NF-κB (ng/g)	IL-1β (ng/g)
Normal	193.833[@]±7.489	77.273[@]±2.710
Control (MI)	915.250* ± 21.578	83.394* ± 0.508
Thymol (15 mg/kg)	523.667*[@]±11.614	78.914[@]±0.762
Thymol (30 mg/kg)	359.250*[@]±5.478	78.220[@]±2.795
Thymol (60 mg/kg)	252.500*[@]±6.873	77.778[@]±2.704

Data are expressed as mean ± SEM. Statistical analysis are carried by one way ANOVA followed by Tukey's multiple comparison test.
*Significant difference from normal group at $p < 0.05$.
[@]Significant difference from control (MI) group at $p < 0.05$.

control rats. Thymol (60 mg/kg) resulted in significant decrease in CK level by 16.73% as compared to control rats and was significantly different from normal value.

3.3. Effect of thymol on cardiac oxidative stress biomarkers

As demonstrated in Table 3, adrenaline-induced MI showed a significant elevation in MDA cardiac content by 124.13% as compared to normal rats. Oral pretreatment with thymol (15, 30, and 60 mg/kg) significantly reduced cardiac MDA content by 54.34, 56.58, and 54.14%, respectively as compared to control rats.

Control (MI) rats resulted in significant reduction of cardiac GSH content by 34.65% as compared to normal rats. Thymol (15, 30, and 60 mg/kg) significantly restored cardiac GSH content by 40.77, 76.21, and 52.20%, respectively as compared to control rats (Table 3).

3.4. Effect of thymol on cardiac inflammatory mediators

Table 4 illustrates that cardiac contents of NF-κB, and IL-1β in control MI rats were significantly increased by 372.18, and 7.92%, respectively as compared to normal rats.

Significant decrease in cardiac NF-κB content was depicted after thymol pretreatment (15, 30, 60 mg/kg) in MI rats in a dose dependent manner by 42.78, 60.785, and 72.41%, respectively as compared to control rats.

Thymol (15, 30, 60 mg/kg) significantly reduced IL-1β cardiac content by 5.34, 6.20, and 6.73%, respectively as compared to control rats.

3.5. Histopathology

Heart of normal rats revealed normal cardiac muscle fibers with no definite lesions (cardiomyocytes necrosis and inflammation) (mean score, 0.00 ± 0.00) (Figure 1a). Conversely, severe MI involving the full thickness of the heart tissue was demonstrated in control (MI) group (mean score, 1.90 ± 0.06). Abundant contraction bands of necrotic cardiomyocytes with fragmentation of myofibers were frequently demonstrated in this group. The necrotic cardiomyocytes are intensely eosinophilic and diffusely
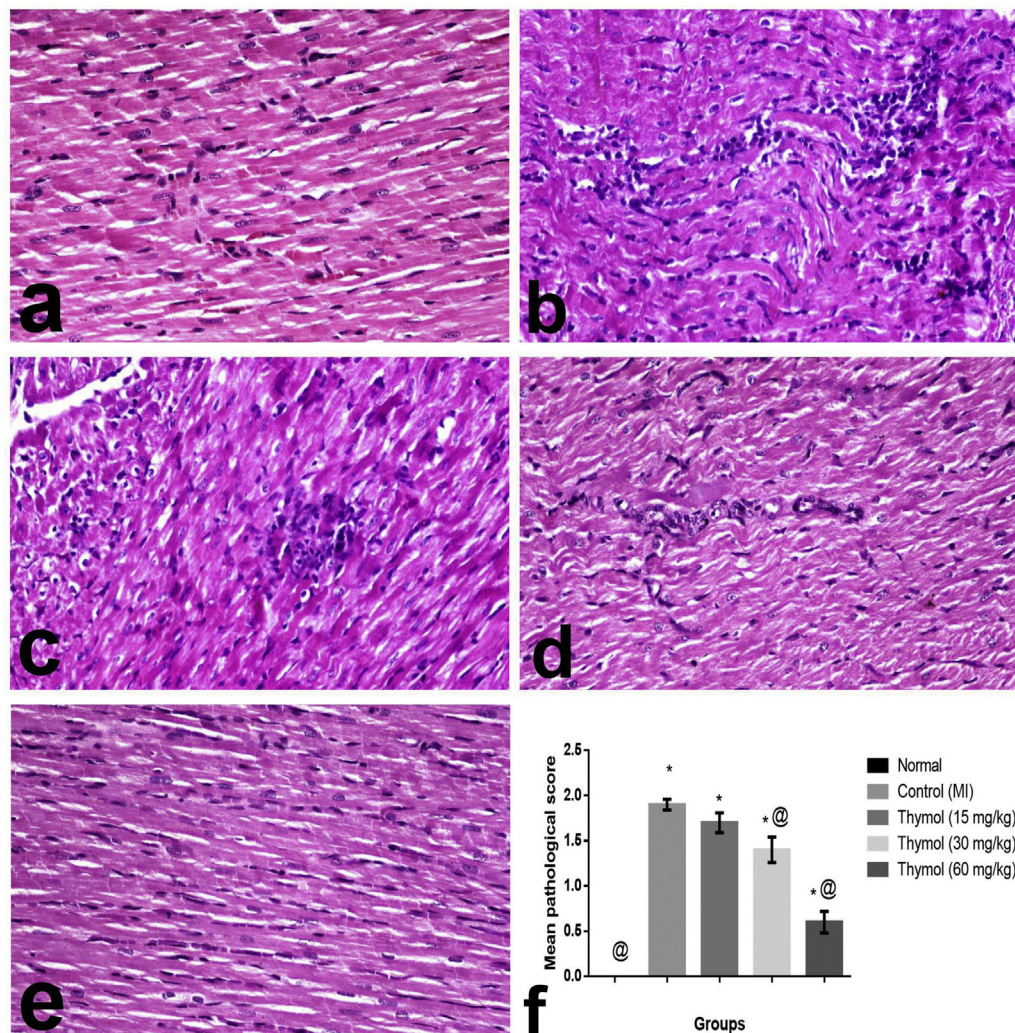

Figure 1. Photomicrograph of heart tissue stained with H&E. (a) Normal rats showing normal cardiomyocytes, (b) Control (MI) group showing contraction bands of necrotic cardiomyocytes which are diffusely infiltrated with massive cellular infiltrates, (c) Thymol (15 mg/kg) group showing focal necrosis of cardiomyocytes that are infiltrated with mononuclear cells, (d) Thymol (30 mg/kg) showing minute focal aggregation of histiocytes, and (e) Thymol (60 mg/kg) showing normal cardiomyocytes, (f) The mean pathological score recorded in the heart tissue of all groups (H&E, 40X). The data are presented as mean ± SEM. Statistical analysis was carried out by one-way ANOVA followed by Newman-Keuls multiple comparison test.*Significant difference from normal group at $p < 0.05$. [@]Significant difference from control (MI) group at $p < 0.05$.

infiltrated with massive cellular infiltrates (Figure 1b). The cellular infiltrates are mostly mononuclear cells and large histiocytes. Apoptotic cardiomyocyte with condensed chromatin was also observed. Mild attenuation of the lesions was demonstrated in thymol (15 mg/kg) (mean score, 1.70 ± 0.11) in which focal necrosis of cardiomyocytes was evident (Figure 1c). Marked regression of cardiomyocyte necrosis and inflammatory cellular infiltrates were recorded in thymol (30 mg/kg) (Figure 1d) (mean score, 1.40 ± 0.14). Remarkable amelioration was noticed in thymol (60 mg/kg) with normal cardiomyocytes in most examined sections (Figure 1e) (mean score, 0.60 ± 0.12).

3.6. Immunohistochemistry

The results of immunohistochemical analysis recorded in normal and treated hearts were illustrated in Figures 2 & 3.

3.6.1. Activated caspase-3 protein expression

No caspase-3 expression was demonstrated in normal heart (Figure 2a). While, control (MI) group revealed significant increase of caspase-3 expression that was demonstrated in the cardiomyocyte sarcoplasm and apoptotic nuclei as well as the endothelial lining of the blood vessels (Figure 2b). Additionally, the inflammatory cells surrounding the necrotic muscle fibers showed intense immune staining. Decreased expression of activated caspase-3 was recorded in thymol (15 mg/kg) pretreated group. The immune reaction was demonstrated in the endothelial lining of the blood vessels and few cardiomyocytes (Figure 2c). Significant decrease of activated caspase-3 was recorded in thymol 15, 30 and 60 mg/kg pretreated groups that was demonstrated in the cytoplasm of sparse cardiomyocytes (Figure 2d & e, respectively).

3.6.2. Bcl-2 protein expression

Bcl-2 protein expression was non-detectable in normal heart (Figure 3a). In control (MI) group, Bcl-2 protein expression was demonstrated in the necrotic area that are infiltrated by inflammatory cells, but few sparse cardiomyocytes exhibiting Bcl-2 protein immune reactivity were demonstrated in other areas (Figure 3b). Non-significant difference in Bcl-2 protein expression was recorded in thymol (15 mg/kg) pretreated group, which revealed weak expression (Figure 3c).While, significant increase in Bcl-2 protein expression was recorded in thymol 30 and 60 mg/kg pretreated groups respectively (Figure 3d & e, respectively).

4. Discussion

This study provides new evidence on the cardioprotective effect of thymol (15, 30, 60 mg/kg) against adrenaline-induced MI in rats. To the best of the authors' knowledge this is considered the first study to investigate the role of cardiac enzymes, ECG alterations, oxidative stress, inflammation, apoptosis as well as histopathological changes of thymol in adrenaline-induced MI in rats.

Subcutaneous administration of adrenaline (2 mg/kg) for 2 consecutive days in the present study resulted in abnormalities in ECG pattern
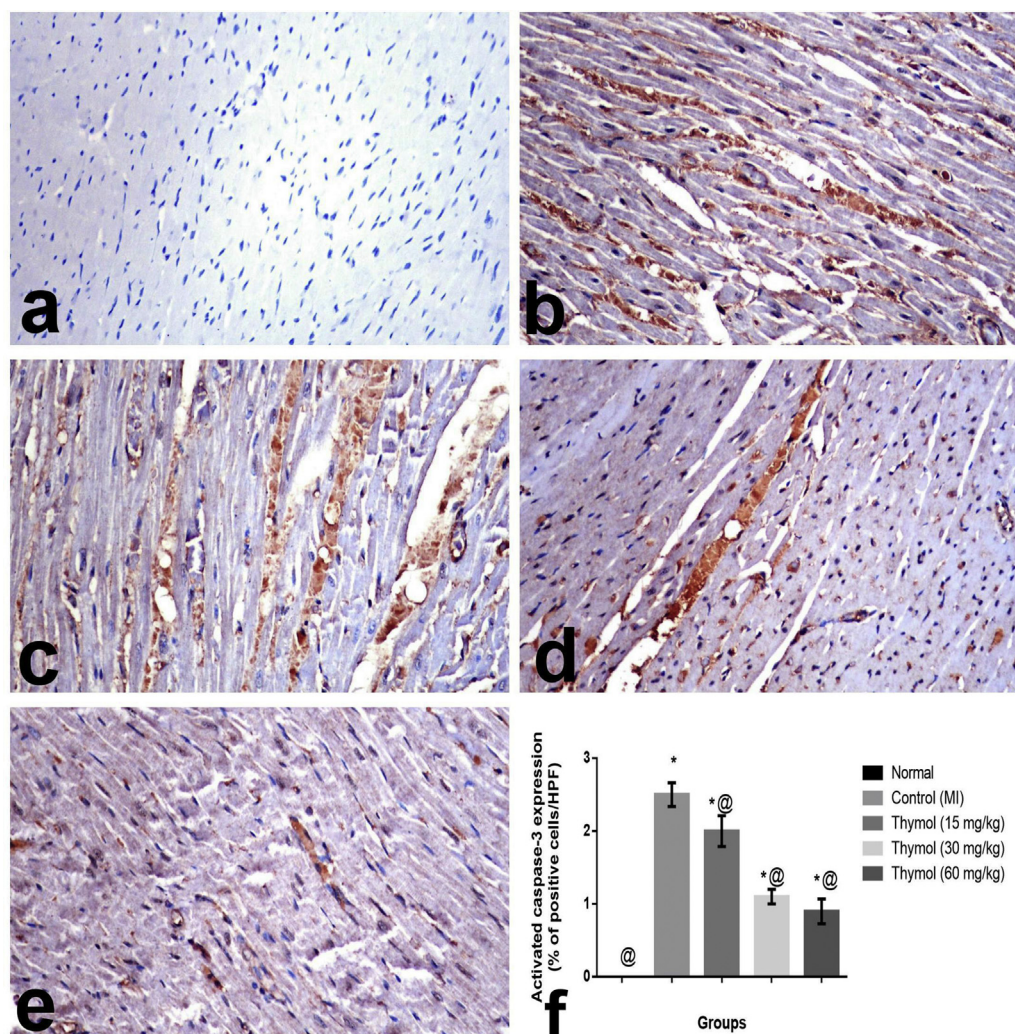

Figure 2. Photomicrograph of heart tissue, immunohistochemically stained with activated caspase-3. (a) Normal rats showing no caspase-3 expression, (b) Control (MI) group showing increased activated caspase-3 expression in the cardiomyocyte sarcoplasm and apoptotic nuclei as well as the endothelial lining of the blood vessels, (c) Thymol (15 mg/kg) group showing decreased expression of activated caspase-3 in the endothelial lining of the blood vessels and few cardiomyocytes, (d) Thymol (30 mg/kg) showing few cardiomyocytes expressing activated caspase-3, and (e) Thymol (60 mg/kg) showing decreased caspase-3 expression in the cytoplasm of sparse cardiomyocytes, (f) The % of activated caspase-3 positive cells/HPF. Statistical analysis was carried out by one-way ANOVA followed by Newman-Keuls multiple comparison test. *Significant difference from normal group at $p < 0.05$. @Significant difference from control (MI) group at $p < 0.05$ (Activated caspase-3 immunohistochemical staining, 40X).
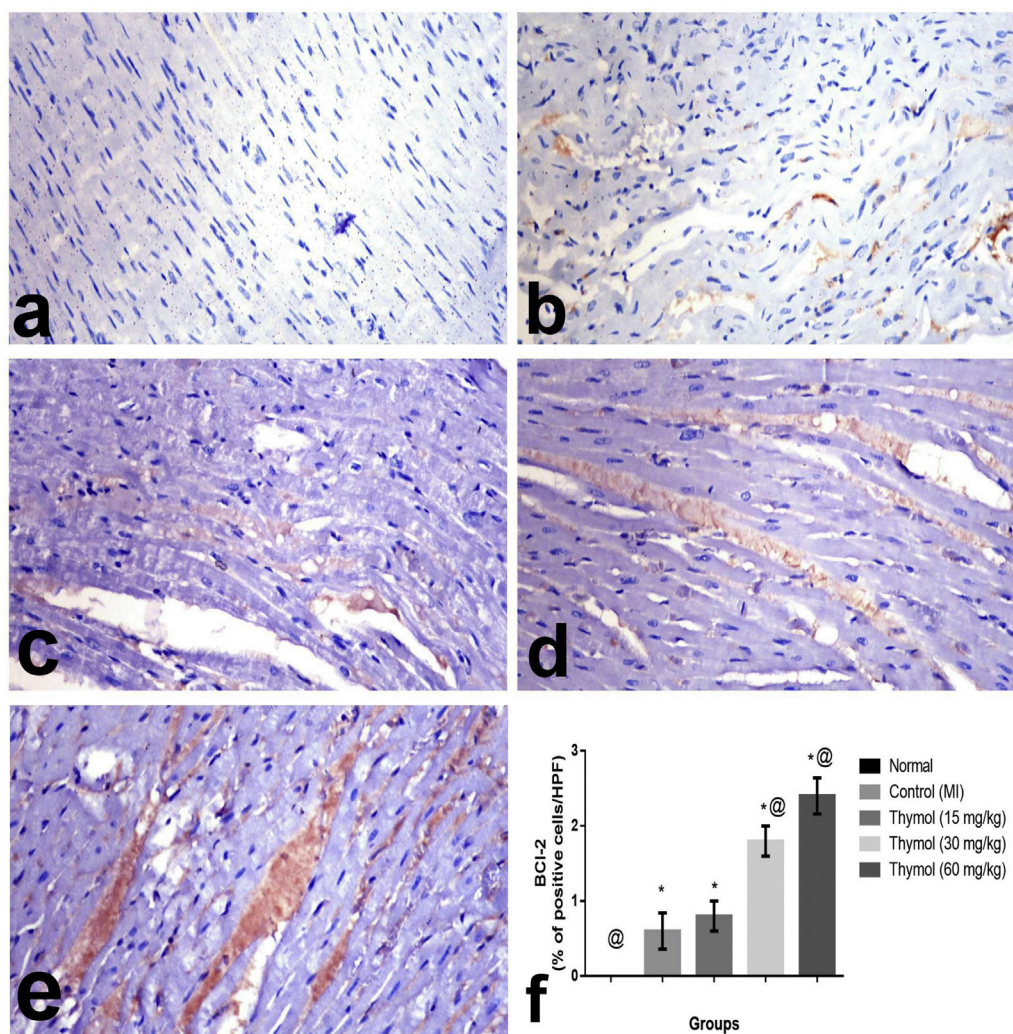
Figure 3. Photomicrograph of heart tissue, immunohistochemically stained with Bcl-2. (a) Normal rats showing non detectable Bcl-2 protein expression, (b) Control (MI) rats showing sparse cardiomyocytes exhibiting Bcl-2 protein immune reactivity, (c) Thymol (15 mg/kg) group showing weak expression of Bcl-2 protein, (d) Thymol (30 mg/kg) showing increased Bcl-2 protein expression, and (e) Thymol (60 mg/kg) showing significant increase of Bcl-2 protein expression in the cytoplasm of cardiomyocytes and (f) The % of Bcl2 positive cells/HPF. Statistical analysis was carried out by one-way ANOVA followed by Newman-Keuls multiple comparison test. *Significant difference from normal group at $p < 0.05$. @Significant difference from control (MI) group at $p < 0.05$. (Bcl-2 immunohistochemical staining, 40X).

as manifested by prolongation of RR interval, decrease in HR, and elevation in ST interval. This finding is in harmony with that of Salama et al. (2018) and indicates the induction of myocardial damage by adrenaline. Adrenaline associated prolongation of RR interval may be due to atrioventricular block (Nakamura et al., 2006) and ST segment elevation which is a sign of acute myocardial infarction (Khodeer et al., 2019).

In the current study, adrenaline administration elevated serum levels of cardiac enzymes; AST, LDH, and CK levels as described earlier (Wang et al., 2017). This reveals that adrenaline administration lead to myocardial damage and leakage of cardiac enzymes into the circulation.

Thymol pretreatment reduced the elevated RR and ST intervals and controlled HR, implicating amelioration in ECG pattern. Thymol administration reduced serum levels of AST, CK and LDH, reflecting preservation of membrane integrity and thereby cardioprotection against adrenaline-induced MI. Those findings are paralleled by improved histopathological features of cardiac lesions and are in consistence with a prior study of El-Sayed et al. (2016) who demonstrated the cardioprotective effects of thymol against doxorubicin-induced cardiotoxicity.

The cardiac cells contain a huge number of mitochondria which is the primary source of energy and ROS in mammalian cells (Dhalla et al., 1993; Marchi et al., 2012). Adrenaline administration elevated MDA (marker of lipid peroxidation) and reduced GSH (endogenous anti-oxidant) cardiac contents in rats. This finding is in accordance to prior study of (Mishra et al., 2016) and implicates that oxidative stress mediated adrenaline-induced MI and disruption of myocardial membrane.

Thymol pretreatment significantly restored cardiac MDA and GSH contents in rats. This reveals that thymol exerted its cardioprotective effect against adrenaline-induced MI through its anti-oxidant activity. This finding is in line with prior study of Meeran and Prince (2012).

Oxidative stress leads to cell inflammation (Wood, 2020). ROS activates NF-κB, a central player that regulates the development of inflammatory cardiac vascular damage via elevating inflammatory cytokines (e.g. TNF-α, IL-1β) (Gloire et al., 2006; Fiordelisi et al., 2019). In line with this notice, adrenaline significantly elevated cardiac NFκB and IL-1β contents, demonstrating that inflammation mediated adrenaline-induced MI in rats.

In this work, NF-κB and IL1β were suppressed by thymol pretreatment, indicating that thymol exerted its cardioprotective effect against adrenaline-induced MI via its anti-inflammatory effect mediated by its anti-oxidant effect. This was paralleled with improved cardiac function indices and further confirmed previous reports that showed the importance of inhibition of inflammatory cytokines in alleviation of cardiac injury (Seropian et al., 2014; Raish et al., 2019). The anti-inflammatory effect of thymol was described also in acute lung injury model via inhibition of TNF-α, IL-6 releases and NF-κB activation (Wan et al., 2018).

Oxidative stress is closely associated with cell apoptosis (Yu et al., 2015). Excessive ROS production leads to oxidative stress accompanied with intracellular macromolecules (DNA, proteins, membrane lipids) damage, thus, killing the cells either by apoptosis or necrosis (Zaki et al., 2018). In response to injurious stimuli, NF-κB is translocated to the nucleus, phosphorylated and binds to target genes including TNF-α, interleukins (e.g IL-1β and IL-6), and anti-apoptotic proteins such as Bcl2 (Maimaitiaili et al., 2018).

Persistent MI is accompanied with apoptosis and progressive loss of cells with subsequent cardiac remodeling, ventricular dilation, myocardial fibrosis, gradual decrease of cardiac function and heart failure after progression of myocardial infarction (Konstantinova et al., 2011; Rondeau et al., 2011). Bcl-2 expression blocks features of apoptosis, like plasma membrane blebbing, DNA cleavage, and nuclear condensation. Thus, it plays a crucial role in cell survival and acts as a negative regulator of cell death induced by various stimuli. Caspase-3 is a key mediator of apoptosis that catalyzes the cleavage of key cellular proteins having a protease-like activity (Porter and Janicke, 1999).

Additionally, myocardial apoptosis speeds up the development of necrosis the effect that determines the degree of MI. This was observed in the current study through histopathological examination which showed both apoptotic and necrotic cardiomyocytes upon adrenaline injection. Bcl-2 (B cell lymphoma 2), a proto-oncogene that is associated with apoptosis. It is an anti-apoptotic gene and a key downstream target gene of NF-κB (Ola et al., 2011) that is subsequently activated to regulate inflammatory responses, cell differentiation and apoptosis. In the same manner, adrenaline administration significantly reduced Bcl2 protein expression and elevated caspase-3 protein expression as compared to normal rats, showing that adrenaline-induced MI was mediated by apoptosis. This finding is inaccordance with that of Salama et al. (2018).

Thymol pre-treatment in the present work resulted in increased protein expression of Bcl2 and suppression of caspase-3 with marked regression of apoptosis and necrosis, revealing that its anti-oxidant and anti-inflammatory effect mediated its anti-apoptotic and thereby cardioprotective effect against adrenaline-induced MI. Similarly, thymol showed previous anti-apoptotic effect in carotid tissue of hypercholesterolemic rats (Bayatmakoo et al., 2017).

5. Conclusion

It can be concluded that thymol possessed cardioprotective effect against adrenaline-induced MI in rats possibly by ameliorating cardiac tissue biomarkers, attenuating ECG changes, improving histopathological changes resulting from oxidative stress, inflammation and apoptosis. More studies are warranted to study the exact molecular mechanisms that may play a role in the cardioprotective effect of thymol in MI rats.

Declarations

Author contribution statement

S.A. El-Marasy, S.A. El Awdan and H.M.I. Abdallah: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

A. Hassan: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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