Cardioprotective effects of metformin and alpha lipoic acid against myocardial complications induced by hypothyroidism in rats

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Research Article

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Abstract

The therapeutic effects of alpha lipoic acid (LA) and metformin (MET) against the myocardial complications associated with hypothyroidism were assessed in the current study.

Rats were divided into three groups: control, hypothyroidism model induced by propylthiouracil (PTU), hypothyroidism model treated with LA and/or MET. At the end of the experiment, the thyroid hormones (T\textsubscript{3}, T\textsubscript{4} and TSH) were measured in the serum. Lipid peroxidation (MDA), nitric oxide (NO), reduced glutathione (GSH), NrF\textsubscript{2}, BCL\textsubscript{2}, NF-κB, serotonin (5-HT), norepinephrine (NE) and dopamine (DA), acetylcholinesterase (AchE), monoamine oxidase (MAO) and Na\textsuperscript{+},K\textsuperscript{+},ATPase were measured in the cardiac tissue. The histopathological changes were also examined.

PTU significantly decreased T\textsubscript{3} and T\textsubscript{4} and significantly increased TSH. A significant increase in MDA, GSH, NrF\textsubscript{2}, BCL\textsubscript{2}, NF-κB, 5-HT, NE, DA, AchE, MAO and Na\textsuperscript{+},K\textsuperscript{+},ATPase and a significant decrease in NO were observed in the cardiac tissue of hypothyroidism model. This was associated with histopathological changes. LA alone or with MET restored T\textsubscript{3} and TSH and improved almost all the biochemical changes except the decreased NO and the increased DA in the cardiac tissue. MET restored T\textsubscript{3}, T\textsubscript{4} and TSH and the biochemical changes induced in the cardiac tissue. MET ameliorated the histopathological changes that were still observed with LA alone or in combination with MET.

The present findings indicate that MET had cardioprotective effect against hypothyroidism and its myocardial complications. This effect was less prominent with LA alone or in combination with MET. No synergistic effect was observed between LA and MET.

Introduction

The heart is considered one of the main targets of thyroid hormone action, and any alteration in the status of thyroid hormones can indirectly affect the functions of the heart [1]. The impact of thyroid hormones on the heart and cardiovascular system results in some of the most common symptoms of thyroid disorders [2]. Experiments in thyroid disorder animal models and at the cellular level have shown that thyroid hormones regulate cardiac structural, electrical, and functional remodeling. The atrial myocardium has a high sensitivity to thyroid hormones that have both endocrine and paracrine effects on atrial physiology promoting atrial fibrosis, which leads to atrial fibrillation vulnerability [3]. Patients suffering from thyroid disorders have a higher risk of developing atrial fibrillation than healthy subjects [4]. Cardiovascular diseases induced by hypertension and dyslipidemia are also associated with hypothyroidism [5, 6].

Hypothyroidism is characterized by a reduction in oxygen and substrate utilization by the major organs of the body leading to a decrease in the cardiac output demands. Moreover, hypothyroidism modifies cardiac function directly by changing myocyte-specific gene expression [7]. Reduced T3 levels also cause oxidative stress and enhance apoptotic rate, which may aggravate ventricular dysfunction [8].
Alpha lipoic acid (LA) is a natural antioxidant which plays an important role in metabolism. It is considered one of the most powerful cellular oxidation regulators [9]. It is a disulfide compound synthesized from cysteine (as a sulfur source) by lipoic acid synthase in the mitochondria of cardiomyocytes and other tissues [10, 11]. LA therapy has been suggested to be beneficial in the treatment or management of diabetic cardiomyopathy and other cardiovascular complications by activating cardiac H₂S system [12]. LA is well known to promote cellular antioxidant capacity and interact with numerous ROS [13]. It has been shown that LA can increase cellular defense systems and protect against ROS-induced myocardial damage [14].

Metformin (MET) is an adenosine monophosphate-activated protein kinase (AMPK) agonist that has been used widely for more than 50 years to treat type 2 diabetes mellitus [15]. In a follow-up study of 10.7 years, it was found that MET-treated subjects had a 30% lower risk of all macrovascular diseases and a reduced incidence of microvascular and macrovascular complications when compared to subjects treated with other drugs [16]. Kobashigawa et al. reported that the upregulation of AMPK and its downstream target molecules underlies the cardioprotective effect of MET against doxorubicin-induced toxicity [17]. MET reduces the development of heart failure after infraction by improving left ventricular ejection fraction, increases myocardial tolerance to ischemic reperfusion injury, improves the outcomes of patients with advanced systolic heart failure and decreases diabetes mellitus complications [18, 19].

MET probably suppresses the inflammatory response by inhibiting NFκB through AMPK-dependent and independent mechanisms [20]. These cardioprotective effects were mediated by the antioxidant, anti-inflammatory, and anti-apoptotic properties of MET [21, 22]. Pretreatment with MET could provide considerable cardioprotection by reducing caspase-3 and cardiac troponin I in cardiotoxicity induced in rats by doxorubicin due to AMPK activation [22].

MET can reduce reactive oxygen species (ROS) formation in animal models associated with heart failure by AMPK activation, and protect myocardial cells from oxidative stress [17].

The present study was carried out to evaluate the therapeutic effect of LA and/or MET against the myocardial complications induced by hypothyroidism in rat.

**Material and methods**

Forty Wistar adult male rats were used in the present study. Their weights ranged between 150 to 170 g. The animals were obtained from the Animal House Colony of the National Cancer Institute, Cairo University, Egypt. All animals were placed in plastic cages with stainless steel covers and had ad libitum access to standard laboratory diet and tap water. The cages were kept in a temperature-controlled (20–25°C) and artificially-illuminated (12 hrs. dark/light cycle) room free of any chemical contamination. They were left for 7 days for adaptation with the lab conditions prior to starting the experiment. All animals received animal care in compliance with the international guidelines and experiments were approved by the Ethics Committee of the National Research Centre (ethical approval number, 18183).
Chemicals

Propylthiouracil (PTU) was purchased from Amoun Pharmaceutical Company (El-Obour City, Cairo, Egypt). Metformin (MET) was obtained from Minapharm for Pharmaceuticals and Chemical Industries (Cairo, Egypt). α-lipoic acid (LA) was obtained from EVA Pharma for Pharmaceuticals and Medical Appliances (Cairo, Egypt).

Experimental design

Rats were divided into two groups; control rats (n = 8) that received daily oral administration of tap water (2 ml/kg body weight) till the end of the experiment, and rat model of hypothyroidism (n = 32) induced by daily oral administration of PTU (15 mg/kg body weight) for 45 days [23]. Starting from the 46th day the animals of the rat model of hypothyroidism were divided into four groups: rat model of hypothyroidism (n = 8) that received PTU (15 mg/kg body weight) for 30 days, rat model of hypothyroidism (n = 8) treated daily with MET orally (300 mg/kg body weight) [24] one hour after the daily oral administration of PTU (15 mg/kg body weight) for 30 days, rat model of hypothyroidism (n = 8) treated daily with LA orally (100 mg/kg body weight) [25] for 30 days one hour after the daily oral administration of PTU (15 mg/kg body weight) and rat model of hypothyroidism (n = 8) co-treated daily with MET and LA orally one hour between each treatment for 30 days. At the end of the experiment, blood samples were collected from the retro-orbital venous plexus of the animals in clean heparinized capillary tubes and then centrifuged at 3000 rpm for 15 minutes at 4°C to separate sera. The separated sera were stored at -80°C till analysis. Rats were decapitated and the heart of each rat was divided longitudinally into right and left halves. Each half was weighed and kept frozen at -80°C till performing the biochemical measurements.

Biochemical measurements

The left half of the heart was homogenized in tris-Hcl buffer (pH = 7.4) to measure the oxidative stress parameters, the levels of Nrf2, Bcl2 and NF-κB and the activity of monoamine oxidase (MAO), acetylcholinesterase (AchE) and Na⁺,K⁺,ATPase.

Thyroid hormones

Thyrotropin (TSH), total thyroxine (T₄) and total triiodothyronine (T₃) were determined in serum using immunoassay ELISA Kit supplied by AB Diagnostic Systems GmbH, Berlin (Germany).

Oxidative stress parameters

Lipid peroxidation (MDA)

Malondialdehyde (MDA), as an indicator of lipid peroxidation, was measured in the cortex and hippocampus according to Ruiz-Larrea et al. [26]. MDA was determined by measuring thiobarbituric acid reactive species (TBARS). MDA produces a red-colored complex after reacting with thiobarbituric acid. The absorbance of this complex was measured spectrophotometrically at 532 nm.

Nitric oxide (NO)
Nitric oxide (NO) level was measured using Griess reagent based on the method of Montgomery and Dymock [27]. Nitrite is used as a measure for NO due to its stability. It reacts with Griess reagent to give a deep purple colored compound whose color was read spectrophotometrically at 540 nm.

**Reduced glutathione (GSH)**

The method of Moron et al. was used to measure reduced glutathione (GSH) [28]. The –SH groups of GSH reduce Ellman’s reagent to form 2-nitro-s-mercaptobenzoic acid which has a yellow color that was read spectrophotometrically at 412 nm.

**Levels of Nrf2, Bcl2 and NF-κB**

The levels of Nrf2, Bcl2 and NF-κB were estimated in the cardiac tissue using rat ELISA Kits of Nrf2, Bcl2 and NF-κB purchased from Sunlong Biotech Co., Ltd, Zhejiang, China.

**Enzyme activities**

**Monoamine oxidase (MAO)**

The estimation of monoamine oxidase (MAO) activity in the selected brain areas was carried out using the method described by Holt et al. (1997). The principle of this method was based on the conversion of benzylamine to benzaldehyde whose absorbance was measured at 250 nm.

**Acetylcholinesterase (AchE)**

The determination of acetylcholinesterase (AchE) activity was based on the procedure of Ellman et al. (1961). The method depends on the hydrolysis of acetylthiocholine iodide by acetylcholinesterase to give thiocholine which reacts with the –SH groups of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB). DTNB is reduced to thionitrobenzoic acid whose yellow color was read spectrophotometrically at 412 nm.

**Na⁺.K⁺,ATPase**

Na⁺.K⁺,ATPase activity was determined spectrophotometrically by the method of Tsakiris et al. [31]. Na⁺.K⁺,ATPase activity was measured as the difference between total ATPase activity (Na⁺,K⁺,Mg²⁺-dependent) and Mg²⁺-dependent ATPase activity. The color developed was read at 640 nm.

**Measurement of monoamine neurotransmitters**

The cardiac level of monoamine neurotransmitters; serotonin (5-HT), norepinephrine (NE) and dopamine (DA) were measured using the spectrofluorometric method of Ciarlone (1978). The right half was homogenized in 3 ml acidified butanol and centrifuged at 5000 rpm for 5 minutes. To 2.5 ml of the supernatant, 5 ml of heptane and 1.6 ml of acetic acid (0.2 N) were added. The mixture was vortexed and centrifuged at 5000 rpm for 5 minutes. The upper organic layer was discarded and the aqueous layer was used to measure 5-HT, NE and DA. 200 µl of the aqueous layer was added to 1.2 ml of o-phthalaldehyde. 1 ml of the aqueous layer was used to measure the fluorescence of NE and DA using spectrofluorometer. The quantitative determination of serotonin (5-HT), norepinephrine (NE), and dopamine (DA) levels were
carried out using a spectrofluorometer (Jasco FP-6500, JASCO Ltd., Tokyo, Japan) with a source of xenon arc lamp 150 W (excitation slit bandwidth of excitation monochromator: 5 nm, and emission slit bandwidth of emission monochromator: 5 nm).

**Histopathological examination**

The cardiac muscle of the control and treated rats were fixed in 10% buffered formalin, dehydrated in graded ethanol, and embedded in paraffin using standard procedures. Sections of 5 µm thickness were stained with hematoxylin and eosin (H&E) \[33\] for histopathological examination using a light microscope.

**Statistical analyses**

All results were presented as mean ± SEM. Data were analyzed by ANOVA to assess the significant difference among groups. This was followed by Duncan's post hoc test to determine the significant difference between every two groups at p-value < 0.05.

**Results**

**Thyroid hormones**

Propylthiouracil (PTU) induced a significant decrease in the serum levels of T3 (P = 0.000) and T4 (P = 0.000) and a significant increase in TSH (P = 0.005) recording −48.2%, −87.46% and 118.75%, respectively as compared to control rats. When rat model of hypothyroidism was treated with LA alone or in combination with MET, control-like values in T3 and TSH and a significant increase in T4 were obtained. Treatment with MET alone restored the thyroid hormones to control-like values (Fig. 1).

**Oxidative stress**

The present study revealed a significant increase in lipid peroxidation (P = 0.001), and GSH (P = 0.005) and a significant decrease in NO (P = 0.001) in the cardiac tissue of hypothyroid rats recording 31.25%, 39.69% and −30.95%, respectively as compared to control values. Treatment of hypothyroid rats with LA prevented the increase in lipid peroxidation and GSH but didn't affect the decrease in NO that showed a significant decrease (30.95%). MET treatment restored the cardiac levels of lipid peroxidation, GSH and NO to control-like values. The combined administration of LA and MET ameliorated the increase in the cardiac levels of lipid peroxidation and GSH but failed to prevent the decreased level of NO (Fig. 2).

**Nrf2, Bcl2 and NF-κB**

In the cardiac tissue of hypothyroid rats, a significant increase in the levels of Nrf2 (98.02%, P = 0.010), Bcl2 (112.2%, P = 0.030) and NF-κB (112.2%, P = 0.036) were observed compared to the control rats. When rat model of hypothyroidism was treated with LA, the levels of Bcl2 and NF-κB were restored to control-like values whereas Nrf2 still showed a significant increase (63.59%) compared to control
animals. Treatment of hypothyroid rats with MET alone or in combination LA restored the elevated levels of Nrf2, Bcl2 and NF-κB to control-like values (Fig. 3).

**Monoamine neurotransmitters**

ANOVA analysis revealed a significant increase in the levels of 5-HT (32.32%, P = 0.000), NE (78.46%, P = 0.000) and DA (61.95%, P = 0.036) in hypothyroid rat model compared to control values. LA treatment reduced the increased levels of monoamines induced by hypothyroidism resulting in nonsignificant changes as compared to control values. In the cardiac tissue of hypothyroid rats treated with MET, the levels of NE and DA were restored to nearly control values, however, the elevated level of 5-HT was reduced but still showed a significant increase as compared to control value. The combined administration of ALA and MET restored the level of 5-HT and NE to nearly control values and attenuated the increase in DA levels (Fig. 4).

**MAO, AchE and Na⁺,K⁺,ATPase**

In the cardiac tissue of hypothyroid rats, the activity of MAO increased significantly (P = 0.000) by 73.55% more than control rats. When hypothyroid rat model was treated with LA and/or MET, the enzyme activity was restored to control-like values (Fig. 5).

The cardiac AchE activity of hypothyroid rats showed also a significant increase (P = 0.029) recording 54.61% compared to control rats. Although treatment with LA alone or in combination with MET reduced the AchE activity, the enzyme was nonsignificantly changed as compared to control or hypothyroid rats. MET reduced AchE activity to nearly control-like value (Fig. 5).

Cardiac Na⁺,K⁺,ATPase activity exhibited a significant increase (P = 0.015) by 46.67% as compared to control rats. LA alone or with MET restored the enzyme activity recording nonsignificant changes as compared to control rats. Although MET treatment reduced the activity of Na⁺,K⁺,ATPase to 28%, the enzyme activity showed nonsignificant changes as compared to control or hypothyroid rats (Fig. 5).

**Histopathological results**

Examination of cardiac muscle sections of control rats showed that the myocardium has a striated arrangement and is organized in a linear array that ramifies and branches and anatomizes in a specific pattern which appears as a sheet. The cardiac muscle fibers are linked together by intercalated discs and are separated by fine layers of connective tissue with apparent blood capillaries (Fig. 6-A). Light microscopic examination of animal model of hypothyroidism exhibited extensive degeneration, disorganization of cardiac muscle fibers, fibroblasts, scattered hyalinization fibers; focal fatty change and nuclei enclosed by cytoplasmic vacuolation. Moreover, aggregation of cellular infiltration and apoptotic cells with areas of hemorrhage and interstitial edema was observed. Also, broken myofibril, necrosis, splitting of muscle fiber and wavy appearance of myocytes were clear. In addition, some myocytes with hydropic degeneration and pyknotic nuclei were observed (Figs. 6-B&C&D). In animal model of
hypothyroidism treated with LA, the cardiac muscle still displayed pathological changes in the form of wavy myocardial fibers that appeared thinner than normal. Additionally, cytoplasmic enlargement or cell swelling, hyalinization fiber in some places and interstitial edema were obvious (Fig. 7-A). The animal model of hypothyroidism treated with MET showed an improvement in the pathological changes with absence of degeneration, interstitial edema, and inflammatory infiltrate. The cardiac muscle fiber more or/less appeared normal (Fig. 7-B). Histopathological alterations in cardiac muscle of animal model of hypothyroidism co-treated with LA and MET almost showed recovery of myocardial tissue as evidenced by normal striation of cardiac muscle and usual intercalated disc and nuclei, although focal foci of inflammatory infiltration and interstitial edema were still present (Fig. 7-C).

Discussion

The decreased serum levels of T3 and T4 and the elevated TSH recorded in the present study represent the main indicators for the development of hypothyroidism in rat by PTU. The thyroid hormones influence myocardial contraction and relaxation, synthesis of myocardial fibers, heart rate, myosin ATPase activity, peripheral vascular resistance, blood pressure, glycogenolysis and mitochondrial metabolism through their widespread cardiac receptors [34, 35]. The thyroid hormones control the expression of structural and functional genes concerned with the maintenance of cardiac function, as alpha myosin heavy chain and sarcoplasmic/endoplasmic reticulum calcium ATPase [36] (Minerath et al., 2019). T3 protects cardiomyocytes against cell death induced by oxidative stress through opening the mitochondrial ATP-dependent K+ channel which has a protective role in rescued mitochondria [37]. Due to the importance of thyroid hormones in cardiac homeostasis, thyroid dysfunction has numerous adverse effects on the heart as it decreases cardiac contractility, lowers cardiac output, reduces stroke and vascular volume and increases systemic vascular resistance [38]. In addition, diastolic dysfunction is a common abnormality reported in hypothyroidism [39]. Moreover, hypothyroidism is associated with chronic heart failure and may be associated with pericardial effusion and cardiac tamponade [40, 41].

The present findings revealed that hypothyroidism induced oxidative stress in the myocardium. This was proved by the significant increase in lipid peroxidation as a consequence of the attack of phospholipids in the cell membrane by free radicals.

The production of free radicals could be attributed to the elevated activity of MAO, the metabolizing enzyme of monoamines (5-HT, NE and DA) [42]. MAO metabolizes monoamines by oxidative deamination producing free radicals such as H2O2 [43]. The authors demonstrated that MAO is an important source of ROS in cardiac tissue. Depending on the availability of the substrate, different signaling pathways are triggered by ROS resulting in cell proliferation and hypertrophy or apoptosis [42].

In the present study, serotonin (5-HT), norepinephrine (NE) and dopamine (DA) increased significantly in the cardiac tissue of hypothyroid rats. These monoamines are the substrates of MAO. Accordingly, the present increase in cardiac MAO activity may serve to metabolize monoamines and as a consequence, free radicals are produced.
The present increase in monoamine levels in the myocardium may be a compensatory mechanism to potentiate the reduced cardiac contractility and cardiac output induced as a result of hypothyroidism. Increased plasma levels of 5-HT have been found in patients with decompensated systolic heart failure [44] or diastolic heart failure [45]. These studies suggested that the increase in 5-HT may serve as a compensatory mechanism to enhance cardiac output by raising heart rate and cardiac force [44]. Other intracellular effects of 5-HT involve the mitochondrial oxidation of 5-HT as in mouse heart and generation of free radicals. In that way, 5-HT at high concentrations can lead to apoptosis and necrosis in cardiac tissue [42]. NE is the principal neurotransmitter released from post-ganglionic sympathetic fibers [46]. It is well known that heart failure results in an increase in cardiac NE levels, which are associated with ventricular arrhythmias, cardiac mortality, and sudden cardiac death [47]. Therefore, NE can be used as a major biomarker of cardiac disease status [48]. Similarly, DA has direct effects on the mammalian heart increasing the force of contraction and heart rate, and inducing constriction of coronary arteries [49]. However, the elevated levels of these monoamines may induce adverse effects on the heart. The cardiac toxicity of catecholamines has emphasized almost exclusively on NE and epinephrine whose excess levels may result in microvascular dysfunction, inflammation, fibrosis, and cardiac hypertrophy [50] and cause myocardial damage and death in vitro and in vivo [51, 52]. Elevated DA levels also have cytotoxic effects, as they induce lipid peroxidation, oxidative stress, enhanced cell death, and a proinflammatory status of the plasma membrane. These effects may underlie the inflammation observed in patients suffering from stress-induced heart failure [53]. DA levels are also significantly elevated in stress cardiomyopathy [54]. It has been stated that the mechanisms by which hypothyroidism leads to atrial fibrillation in humans are poorly understood [3]. The present increase in monoamine levels, especially NE, in the cardiac tissue may underlie such atrial fibrillation [55].

Another mechanism of free radical production is mediated by the influence of thyroid hormones on mitochondrial functions including oxygen consumption [37], oxidative phosphorylation [56], proton leak [57] and its biogenesis [58]. Therefore, the low levels of thyroid hormones may lead to an impairment in mitochondrial function resulting in an increase in free radicals. Hypothyroidism disrupts the mitochondrial respiratory chain leading to overproduction of free radicals [59].

In the present study, a significant decrease in cardiac NO level has been observed in the hypothyroid rats. Physiologically, NO has positive effects on the cardiovascular system, as it maintains vascular tone, and controls platelet aggregation and leukocyte adhesion. Also, NO provides anti-inflammatory and antioxidant effects [60–62]. Moreover, NO is an endothelium-derived relaxing factor, which is crucial for cardiovascular homeostasis [63]. NO produced by endothelial nitric oxide synthase, also acts as a potent vasodilator [64]. The reduction of NO bioactivity and bioavailability in the vasculature is considered not only a major contributor but also a marker of endothelial dysfunction [65, 66]. Therefore, the reduced cardiac level of NO induced by hypothyroidism, in the present study, may reduce the blood flow to the heart producing ischemia which has a role in free radical production. The reduction of myocardial NO level may be due to the interaction of NO with produced free singlet oxygen producing the toxic compound peroxynitrite that may induce nitrosative stress leading to nitration and S-nitrosylation of macromolecules, as lipids, proteins, and DNA thus precipitating myocardial damage and dysfunction [67].
Studies have shown that the key role in apoptotic initiation is due to oxidative stress [68]. Reduced T3 levels also cause oxidative stress and elevate apoptotic rate, which may exacerbate ventricular dysfunction [8].

Under such conditions, the present increase in GSH may be an adaptive mechanism to neutralize the free radicals. GSH acts as a nonenzymatic antioxidant in the myocardium [69].

Moreover, the present increase in the cardiac level of Nrf2 in hypothyroid rats may serve to counteract the induced oxidative stress. Nrf2 is a transcription factor which is highly sensitive to oxidative stress. Binding of this factor to nuclear antioxidant response elements promotes the transcription of different antioxidant genes [70]. It plays an important role in opposing oxidative stress [71]. Nrf2 is activated under different stress conditions as exposure to mild oxidative or electrophilic stress [72].

Moreover, the increased Bcl2 level observed in the present study may be another mechanism to reduce the apoptotic damage of macrocytes since Bcl2 acts as an antiapoptotic protein [73]. The expression of Bcl-2 family proteins was increased during heart failure end stage and in the apex, and left and right ventricles in patients suffering from dilated cardiomyopathy [74]. The latter authors suggested that Bcl-2 upregulation may be correlated with cardiac fibrosis and apoptosis.

NF-κB activity is increased during cardiovascular disease, and its signaling is implicated in cardiac remodeling (fibrosis), hypertrophy, and heart failure [75, 76]. Thus, the present increase in NF-κB could be a consequence of the structural changes induced by hypothyroidism.

Acetylcholine slows the heart rate by its action on muscarinic receptor which opens acetylcholine-activated K+ channel to slow the sinus node firing [77]. Thus, the present increase in AchE activity in the cardiac tissue may potentiate the reduced cardiac output and cardiac contractility induced by hypothyroidism by breaking down acetylcholine and preventing its interaction with muscarinic receptors. On the other hand, the increased AchE activity may mediate the inflammatory effect induced in the myocardium as a consequence of hypothyroidism. ACh has been recognized as a heart rate modulator and a protective molecule in heart disease context [78]. ACh protection in the myocardium is partly related to its anti-inflammatory actions [78].

The impairment of Na+,K+,ATPase activity occurs in a number of diseases including atrial fibrillation [79], ischemia [80], heart failure [81], and hypo/hyper-thyroidism [82]. The present increase in Na+,K+,ATPase activity induced by hypothyroidism may be due to the promoting effect of beta-adrenergic agonists on the number of Na+,K+ ATPase channels; this is because beta-adrenergic agonists can increase the gene expression of the enzyme, leading to increased quantity and activity of the enzyme [83]. Beta-adrenergic activation is induced, in the present study, by the elevated level of NE.

As a consequence of these biochemical changes induced by hypothyroidism, histopathological alterations have been observed in the cardiac tissue that showed extensive degeneration and disorganization of cardiac muscle fiber and fibroblasts, scattered hyalinization fibers and focal fatty
change and the nuclei were enclosed by cytoplasmic vacuolation. Moreover, aggregation of cellular infiltration and apoptotic cells were noticed. In addition, interstitial edema and hemorrhagic areas were evident. Myofibrils were broken and necrosis, splitting of muscle fiber and wavy appearance of myocytes were clear. In addition, some myocytes with hydropic degeneration and pyknotic nuclei could be observed. The present histopathological changes agreed with those observed by Atmaca et al. and Hajje et al. [84, 85].

In the present study, LA and/or MET were used to ameliorate the cardiac adverse effects induced by hypothyroidism. LA restored the serum levels of T3 and TSH but could not restore the reduced level of T4. Moreover, LA potentially prevented the lipid peroxidation. This could be attributed to its antioxidant effect. As a consequence, the elevated levels of GSH and NrF2 that have roles in combating oxidative stress were improved. The ability of LA to improve the thyroid hormones together with its antioxidant activity succeeded in preventing the adverse effects of thyroid gland hypofunction on cardiac function. This was evident from the ability of LA to restore the cardiac level of NE and 5-HT and the cardiac activity of MAO and Na⁺,K⁺,ATPase. LA is essential in the metabolism of carbohydrates, lipids, and proteins, leading to energy production in the form of adenosine triphosphate (ATP), in addition, it could protect enzymes from oxidative and nitrosative stress [86–88].

Moreover, LA enhanced cholinergic activity as indicated from the improvement in AchE activity. These effects may mediate the cardioprotective effect of LA against hypothyroidism-induced impaired cardiac functions. Furthermore, the improved cardiac levels of BCL2 and NF-κB induced by LA are indicators for its ability to reduce the damaging effects induced by hypothyroidism. The inability of LA to restore the reduced NO level and the elevated level of DA induced by hypothyroidism together with the partial recovery in NrF2 and BCL2 may underlie the incomplete recovery of the histopathological changes.

On the contrary, MET restored almost all the cardiac changes induced by hypothyroidism. MET restored the thyroid hormones and prevented the oxidative stress induced in the cardiac tissue by hypothyroidism. Restoring the thyroid hormones enables the heart to regain the cardiac contractility and hence the cardiac output. As a result, the monoamine levels together with the cholinergic activity were restored as there was no need for their compensatory mechanisms. Under such conditions, MAO activity was also restored. Accordingly, the free radicals arising from the increased oxidative metabolism of monoamines was inhibited. This was reflected in the ability of MET to prevent lipid peroxidation. In addition, the control-like values of GSH and NrF2 confirm the ability of MET to produce complete recovery from the oxidative stress induced by hypothyroidism.

Moreover, the restored Na⁺,K⁺,ATPase activity induced by MET may be due to the restored adrenergic activity that has a role in the pump activation.

MET restored the depleted levels of PGC-1α and improved mitochondrial biogenesis by enhancing endothelial nitric oxide synthase phosphorylation. This increases NO and reduces vascular inflammation and myocardial injury after ischemia [89]. Thus, the restored NO level may help in regaining the
The cardioprotective effect of NO and the blood flow to the heart. MET also prevented the damaging effect induced by hypothyroidism on the myocardium. This was indicated from the ability of MET to ameliorate the cardiac histopathological changes. The restored levels of BCL2 and NF-κB are indicators of the ability of MET to prevent apoptosis and cell death. This confirmed the cardioprotective effect of MET against the structural changes induced in the myocardium. Thus, the cardioprotective effect induced by MET observed, in the present study, could be attributed to its ability to restore the thyroid hormones and thereafter their cardioprotective effects.

The present study also investigated whether there is a synergistic effect between LA and MET against the cardiovascular complications induced by hypothyroidism or not. The effects of the combined administration were nearly similar to those obtained with LA treatment, indicating an absence of synergistic effects between the two agents. The combined administration ameliorated almost all the biochemical changes except for the decreased NO level and the increased DA level induced by hypothyroidism. An advantage of the combined administration is its ability to restore the increased NrF2. Moreover, the combined administration was better than LA in improving the histopathological changes induced in the cardiac tissue by hypothyroidism. These effects were absent in case of LA treatment and could be attributed to the effect of MET.

In conclusion, the present findings indicate that MET induced cardioprotective effects against the biochemical and histopathological changes induced by hypothyroidism. However, this effect was less prominent in the case of LA especially on the histopathological changes. Although the co-administration of MET and LA induced an improvement in the biochemical and histopathological changes, their ameliorating effects were less than those observed with MET only. The incomplete therapeutic effects of LA only or in combination with MET may be due to LA's partial therapeutic effect on the thyroid hormones. Thus, no synergistic effect was observed between the two agents. Therefore, MET alone could be a potential therapeutic treatment against hypothyroidism and its cardiac complications.

Declarations

Data availability

Data will be made available on request.

References


Physiology Heart And Circulatory Physiology, 287, H1712–H1720.


Figures

Figure 1

Figure (1): Effect of LA and/or MET on the serum levels of T₃, T₄ and TSH in hypothyroidism rat model

- Control Rats
- Hypothyroidism rat model induced by PTU
- Hypothyroidism rat model treated with ALA
- Hypothyroidism rat model treated with MET
- Hypothyroidism rat model treated with ALA+MET

Different letters indicate a significant change at p-value > 0.05 and similar letters mean nonsignificant changes between groups.

Figure 1

See image above for figure legend.
Figure 2

Figure (2): Effect of LA and/or MET on the levels of lipid peroxidation (MDA), nitric oxide (NO), and reduced glutathione (GSH) in the cardiac tissue of hypothyroidism rat model

- Control Rats
- Hypothyroidism rat model induced by PTU
- Hypothyroidism rat model treated with LA
- Hypothyroidism rat model treated with MET
- Hypothyroidism rat model treated with LA+MET

Different letters indicate a significant change at p-value > 0.05 and similar letters mean nonsignificant changes between groups.

Figure 2

See image above for figure legend.
Figure (3): Effect of LA and/or MET on the levels of Nrf2, Bcl2 and NF-κB in the cardiac tissue of hypothyroidism rat model

- Control Rats
- Hypothyroidism rat model induced by PTU
- Hypothyroidism rat model treated with LA
- Hypothyroidism rat model treated with MET
- Hypothyroidism rat model treated with LA+MET

Different letters indicate a significant change at p-value $> 0.05$ and similar letters mean nonsignificant changes between groups.

**Figure 3**

See image above for figure legend.
Figure 4: Effect of LA and/or MET on the levels of serotonin (5-HT), norepinephrine (NE) and dopamine (DA) in the cardiac tissue of hypothyroidism rat model

- Control Rats
- Hypothyroidism rat model induced by PTU
- Hypothyroidism rat model treated with LA
- Hypothyroidism rat model treated with MET
- Hypothyroidism rat model treated with LA+MET

Different letters indicate a significant change at P-value > 0.05 and similar letters mean nonsignificant changes between groups.

See image above for figure legend.
Figure 5: Effect of LA and/or MET on the activities of monoamine oxidase (MAO), acetylcholinesterase (AchE) and Na,K,ATPase in the cardiac tissue of hypothyroidism rat model.

- Control Rats
- Hypothyroidism rat model induced by PTU
- Hypothyroidism rat model treated with LA
- Hypothyroidism rat model treated with MET
- Hypothyroidism rat model treated with LA+MET

Different letters indicate a significant change at p-value > 0.05 and similar letters mean nonsignificant changes between groups.

See image above for figure legend.
Figure 6

Histopathological changes induced by hypothyroidism in the cardiac tissue.

(A) A photomicrograph of cardiac muscles of control rats showing longitudinally arranged cardiac muscle fibers with regular striations which have acidophilic cytoplasm and central, vesicular and oval nuclei, intercalated discs cross some of the fibers,(B) Heart section in group of hypothyroidism rat model showing extensive degeneration of cardiac muscle fibers, fibroblasts (FB), scattered hyalinization fiber (HF), focal fatty changes (red arrow), cytoplasmic vacuolation in nuclei (black arrow), aggregation cellular infiltration (circle), some wavy myocardial fibers (W), interstitial edema (red star), hemorrhage (H), (C) Heart section of hypothyroidism rat model (another field) showing degeneration of cardiac muscle fibers (black arrow), broken myofibrils (BMF), splitting in some muscle fibers (S), marked number of apoptotic or degenerated myocytes (black arrow), deposition of connective tissue (C.T) and interstitial edema, (D) Heart section in hypothyroidism rat model (another field) showing some myocytes with hydropic degeneration (star), pyknotic nuclei (curved arrow), splitting muscle fibers (S), lost striations and loss of continuity with adjacent myocytes, and muscle fiber vacuolation (V).
Figure 7

Effect of LA and/or MET on the histopathological changes induced by hypothyroidism in the cardiac tissue.

A photomicrograph of heart section, (A) Hypothyroidism rat model treated with LA showing the cardiac muscle still suffering from changes in the form of wavy myocardial fibers (red arrow), the myofibrils are thinner than normal, cytoplasmic enlargement or cell swelling (black arrow), hyalinization fiber in some place (HF), interstitial edema (star), and vacuolation (V). (B) Hypothyroidism rat model treated with MET showing restoration of myocardial tissue as evident from almost usual intercalated discs and nuclei. (C) Hypothyroidism rat model co-treated with LA and MET showing almost recovered myocardial tissue as evident from the normal striations of cardiac muscle, and almost usual intercalated discs, although foci of inflammatory infiltration (IF) and interstitial edema (star) were still present.