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Protective Effects of Telmisartan and Trimetazidine in Isoproterenol-Induced Myocardial Hypertrophy in Rats: Role of Autophagy

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Abstract

Background: **Myocardial hypertrophy, when pathological and sustained, is one of the predictors of cardiovascular morbidity. Telmisartan (TEL) and trimetazidine (TMZ) are drugs commonly used in cardiovascular diseases, including those leading to myocardial hypertrophy. It would be adventitious if their combination also protected against myocardial hypertrophy.** ***Aim:*** **This work compared the effect of TEL, TMZ, and their combination in preventing myocardial hypertrophy in rats.** ***Subjects and Methods:*** **82 male albino rats were randomized into 10 groups. Groups included untreated, vehicle, TEL, and TMZ control groups. The other 6 groups received daily oral gavage for 35 days as follows: TEL (10 mg/kg), TMZ at two doses (10 & 20 mg/kg), and two combination groups of TEL + TMZ (10 or 20 mg/kg). On days 29th -35th rats received IP ISP (4 mg/kg/day) for a week to induce myocardial hypertrophy.** ***Results:*** **TEL, TMZ (20mg/Kg), and their combination significantly protected against ISP-induced myocardial hypertrophy as denoted by decreased heart weight (HW), HW/body weight (BW) ratio, decreased left ventricular wall thickness, fibrosis area, TGF-β1, and β-MHC. Treated groups had improved ECG, better myocardial contractility, and significantly decreased autophagy markers; LC3-I, LC3-II, and LC3-II/LC3-I ratio than that of ISP group.** ***Conclusion:*** **TEL and TMZ have comparable favorable effects against experimental myocardial hypertrophy that involve inhibition of myocardial autophagy. Further experimental in vivo studies are needed to explore the effects of individual and combined therapeutic regimens on different key regulators of autophagy in ISP-induced myocardial hypertrophy.**

Keywords: Myocardial hypertrophy, telmisartan, trimetazidine, isoproterenol, autophagy, Isoproterenol-Induced Myocardial hypertrophy.

Introduction

Myocardial hypertrophy is defined by the augmentation of ventricular mass due to increased cardiomyocyte size. It represents a crucial cellular response to hemodynamic overload, mechanical stress, neurohormonal stimulation, or myocardial injury. Myocardial hypertrophy may be compensatory for an increased workload as in pregnancy,

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or in pathological conditions as hypertension, endocrine imbalance, myocardial infarction, and congenital heart defects[1,2]. Pathological cardiac hypertrophy has deleterious effects with maladaptive remodeling, contractile dysfunction, and eventually cell death. Cell death occurs by three modes: necrosis, apoptosis, and autophagy leading eventually to heart failure (HF)[1]. Heart failure impacts more than 26 million people worldwide with a 50% mortality rate within 4 years[3]. Telmisartan (TEL) is a distinctive selective blocker of angiotensin II receptor subtype 1 (AT1) with the highest affinity and longest duration of action among its class. It has an anti-inflammatory effect and acts as a partial agonist at the peroxisome proliferator-activated receptor gamma (PPAR-γ). Telmisartan is widely used in the treatment of hypertension, congestive HF, and diabetic nephropathy[4]. Chronic high-level stimulation of angiotensin II receptors contributes to fibroblast proliferation, myocyte hypertrophy, and autophagy activation[5,6]. Studies found that AT1 receptor antagonism decreases cardiomyocyte autophagy and alleviates pathological cardiac hypertrophy[7]. Trimetazidine (TMZ) is used as an adjuvant anti-anginal drug. It is a metabolic modifier acting to partially inhibit fatty acid oxidation in the heart with a shift in energy production to glucose oxidation rather than oxidation of fatty acids[8]. Studies found that TMZ attenuates cardiac remodeling, limits the inflammatory response, inhibits apoptosis, and decreases reactive oxygen species production[9]. Moreover, pretreatment with TMZ could modulate autophagy in the heart[10]. Autophagy is an endogenous cellular process that is highly regulated primarily aiming for the degradation of dysfunctional cellular proteins and organelles. Under normal conditions, its basal level contributes to cellular homeostasis but when excessive it aggravates tissue damage[11]. Interestingly, autophagy seems to play a dual role in myocardial hypertrophy depending on the extent of stimulation[12]. Some reports have revealed that a high level of autophagy plays a beneficial role in myocardial hypertrophy[13], while other studies showed the contrasting functions of autophagy in myocardial hypertrophy. In the clinical setting measuring cardiac autophagy is not feasible[14] and experimental studies is a surrogate to assess the effect of various disease conditions and drug treatment. In this work we utilized the well-established model of experimental myocardial hypertrophy induced by isoproterenol (ISP)[15]. This model is associated with myocardial fibrosis and upregulation of autophagy[16]. Targeting cardiac hypertrophy and remodeling using different therapeutic modalities could offer a more efficacious way to prevent them. The objective of the study is to assess the protective effect of TEL and TMZ separately versus their combination in ISP-induced myocardial hypertrophy and secondarily to examine if autophagy is a possible mechanism for these effects.

Materials and Methods

Animals

The study utilized eighty-two adult male albino rats purchased from the National Research Center, Cairo, Egypt. Rats with weights from 150 to 200 g were kept in the animal house with controlled conditions; 12-hour alternating light: dark cycle and a temperature 25 ± 2° C. They were kept in clean plastic polyethylene cages with water and food available *ad libitum*. Before starting the experiment, the proposal was endorsed by the Faculty of Medicine, Suez Canal University's research ethics committee of (Ref. No. 3845#).

Drugs used in the study

Isoproterenol was purchased as a white powder from ACROS organics part of

Thermo Fisher Scientific, USA, imported via Top quality for Lab instruments and Research Chemicals, Ismailia, Egypt. The drug was dissolved in saline. Telmisartan and TMZ were purchased from Sigma Aldrich chemical company (St. Louis, MO, USA). Telmisartan was dissolved in saline while TMZ dihydrochloride was suspended in 1% carboxymethyl cellulose.

The design of the experiment

Rats were randomly categorized into 10 groups (n=8, except for ISP group n=10). The first day of drug treatment was Day one of the study. Figure 1 provides an illustration of the study design.

- Group 1 (normal control group): rats did not receive any medications.
- Group 2 (vehicle group): rats received normal saline and 1% carboxymethyl cellulose by oral gavage for 5 weeks. In the last week of the study, rats also received saline by IP injection (1ml /Kg/day) till the end of the study.
- Groups 3 & 4 (TEL & TMZ control): rats received TEL in a daily dose of 10 mg/kg (5) or TMZ in a daily dose of 20 mg/kg by oral gavage for a duration of 5 weeks [10].
- Group 5 (ISP-induced myocardial hypertrophy): rats received ISP in a dose of 4 mg/kg daily by IP injection from day 29th for 7 days.
- Groups 6-10: rats received the designated treatment for 5 weeks. Myocardial hypertrophy was induced by giving ISP via IP injection (4 mg/kg/day) for 7 consecutive days starting from the 29th day [17]. These groups were treated as follows:
- Group 6 (TEL treated group): rats received daily in a dose of TEL 10 mg/kg by oral gavage.
- Group 7 (10 mg TMZ treated group): rats were given a daily dose of TMZ 10 mg/kg by oral gavage.
- Group 8 (20 mg TMZ treated group): rats were given a daily dose of TMZ 20 mg/kg by oral gavage.
- Group 9 (TEL & 10 mg TMZ treated group; combination 1 treated group): Rats were given TEL (10 mg/kg/day) plus TMZ (10 mg/kg /day) by oral gavage.
- Group 10 (TEL & 20 mg TMZ treated group; combination 2 treated group): Rats were given by TEL (10 mg/kg/day) plus TMZ (20 mg/kg /day) by oral gavage.
- Body weight was measured on day 0 before starting the experiment, then every week for dose adjustment and follow up, and a final measurement was taken just before animal scarification.

Hemodynamic studies: Blood pressure and electrocardiography (ECG)

Blood pressure, ECG assessment, and cardiac contractility were done 24 h after the last ISP administration[18] using the research Biopac data acquisition mp150 machine ECG 100 (BIOPAC Systems, Inc.; USA). Recording of blood pressure was done via a noninvasive rat tail technique following the recommendations of the manufacturer. Before placing each rat on a board with its legs fastened by adhesive tapes, the rats were anesthetized with IP thiopental sodium (20 mg/kg body weight)[19]. The ground electrode was placed on the right leg, the V+ electrode on the left leg, and the V- electrode on the right arm of the rat using EL 405 needle electrodes. Lead II ECG was recorded for a minimum of 2 minutes[20]. The ECG parameters determined for each ECG tracing were heart rate (HR) in beats per minute, PR interval (ms), RR interval in milliseconds (ms), QRS duration (ms), QT interval (ms), QTc interval (corrected QTc interval) (ms). For QT correction, standard Bazett's formula was used "QTc = QT interval / square root of RR interval"[21], amplitude of R wave (millivolt), ST segment changes, T, Q and S wave changes and the presence of fragmented QRS (fQRS). After completing the ECG recording for each anesthetized

rat, cardiac contractility (g) was assessed by invasive technique. The pectoral muscle was separated after taking a 1 cm skin incision in the chest wall over the heart area. The strongest pulsating part was connected via a gauge to a thread attached to a lever conveying to a force transducer. The BIOPAC software recorded the heart contractility (g) as previously reported[22].

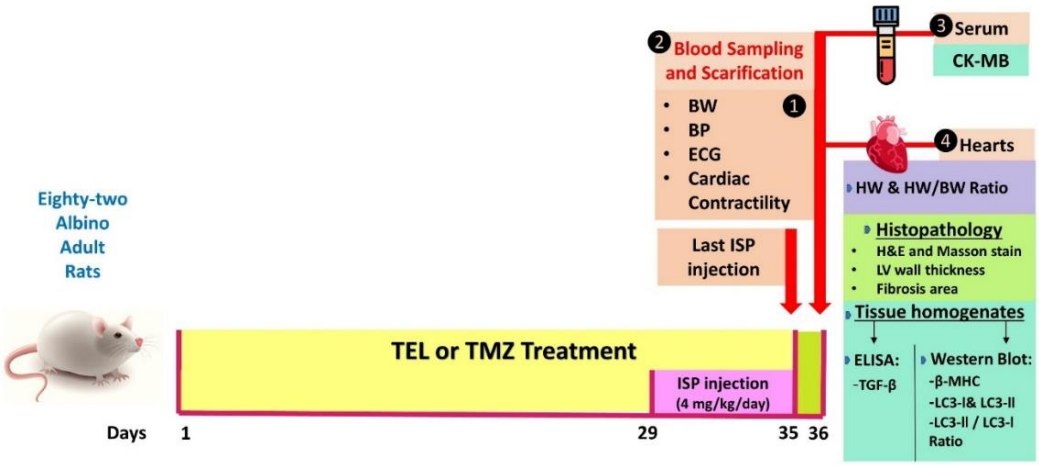

Figure 1. Schematic representation of the study protocol.

After acclimatization for one week, the first day of the study was marked as day 1. Oral telmisartan (TEL) 10 mg/kg once daily, trimetazidine (TMZ) 10 and 20 mg/kg once daily or their combinations were administered for five weeks. Starting from the day 29th and for 7 days, intraperitoneal injection of isoproterenol (ISP) 4 mg/kg once daily was administered to rats to induce myocardial hypertrophy. After 24 hours from the last ISP injection (the last day of the study), body weight (BW) of the rats was assessed followed by blood pressure (BP), electrocardiography (ECG) and myocardial contractility power assessment then blood samples were collected, and sera were assessed for the cardiac enzyme CK-MB then the rats were sacrificed, and the hearts were excised for Hematoxylin and Eosin (H&E) and Masson's Trichrome staining and histopathology. Hearts weight (HW), heart-to-body weight ratio (HW/BW), left ventricular (LV) wall thickness and the percentage of fibrosis area were assessed. Homogenized cardiac tissue was assessed for TGF-β protein expression by ELISA and β-MHC, LC3-I and LC3-II protein expression by western blotting. LC3-II/LC3-I ratio was assessed.

Serum creatine kinase-MB level (CK-MB)

After finishing the hemodynamic studies, a clean sterile capillary tube was used to collect blood samples from each rat's retro-orbital plexus. Blood samples were left to clot, and separation of sera was done by centrifugation at 3000 rpm for 10 minutes. The activity of the cardiac injury marker CK-MB was measured by immunoinhibition method using a fully automated analyzer Cobas-Integra 400 (Roche Diagnostic, Indianapolis, Germany).

Assessment of heart weight and calculation of heart-to-body (HW/BW) ratio

The heart of each rat was immediately excised after scarification, perfused with ice-cold phosphate-buffered saline, dried on filter paper, and weighted. Using the final body weight along with the weight of the heart the HW/BW ratio was calculated as HW/BW (mg/gram) and used to estimate cardiac hypertrophy. After that, a part of the heart was fixed in 10% formalin for histopathology while another part was kept in RIPA buffer at -80°C to be homogenized later for ELISA and western blotting.

Enzyme-linked immunosorbent assay (ELISA) for TGF-beta 1

First, tissues were homogenized, while on ice, using a Teflon pestle homogenizer (Glas Col homogenizer system, Vernon hills, USA) and then the material was centrifuged at 8500 rpm, at 2°C for 10 min. The attained supernatant was aliquoted and stored at -20°C

until measuring TGF-β1 using Rat TGF-β1 PicoKine ™ ELISA Kit. All the steps were done according to the manufacturer's instructions[23].

Determination of β-MHC, LC3 II, and LC3 I level by Western blotting

Cardiac tissues were homogenized in a buffer for radioimmunoprecipitation, then centrifugated to separate the protein (14,000 g for 20 min & 4°C). Quantification of protein in each obtained sample was done via Bradford Protein Assay (Bio-Rad Quick StartTM kit). Samples, in similar protein concentrations, were run on sodium dodecyl sulfate-polyacrylamide gel. Then proteins were transferred to the nitrocellulose membrane. Blocking of membranes was done by immersing in 5% non-fat milk for one hour. Membranes were then incubated overnight at 4°C with primary antibodies against β-MHC, LC3-II, LC3-I, and beta Actin (ThermoFisher, USA). After washing with Tris-Buffer Saline, membranes were exposed to the secondary antibody for one hour at room temperature. Enhanced chemiluminescence ECL Advance TM Western blotting detection kit was utilized to develop the protein bands (Amersham BioSciences, Buckinghamshire, UK). Quantification of protein bands' immunoreactivity was done using ImageJ software (NIH). The obtained bands were normalized against the housekeeping protein beta-actin.

Histopathology

Excised hearts were bisected transversely at the mid-ventricular level, fixed in 10% buffered formalin at 4°C for 24 hrs. Hematoxylin and Eosin stain and Masson's Trichrome stain were used to stain five-µm-thick slices. Slides were coded and examined by a histopathologist blinded to the treatment groups under the light microscope. Eight equidistant measurements of LV-free-wall thickness were taken at a fixed magnification to quantify the left ventricular wall thickness in each heart. Image analysis was used to calculate the overall mean thickness[24]. The fibrosis area was calculated by measuring the percentage of area-stained blue in Masson trichrome stain using ImageJ software (Version 1.5, NIH, Bethesda, Maryland, USA)[25].

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) program version 20 (Chicago, IL, USA) was used to analyze the data, and the results were expressed as mean± SD. Data normality was tested with Shapiro test. Normally distributed variables were tested via one-way analysis of variance (ANOVA) followed by the Bonferroni Post hoc multiple comparison test. Given that the "Fibrosis area" variable was not normally distributed; the Kruskal-Wallis test was used to test for the significance of differences between the normal, vehicle, and control groups against the Model group (1st set of pairwise comparisons), as well as all treated groups against the Model group (2nd set of pairwise comparisons). *P*-values were adjusted by Bonferroni correction for multiple comparisons. When *P* was <0.05, the results were considered statistically significant.

Results

Control groups that received vehicle, TEL, and TMZ without ISP showed non-significant differences in comparison to the normal rat group in all tested parameters.

Telmisartan, Trimetazidine, and their combination attenuated ISP-induced mortality: Isoproterenol-induced mortality of 30% as compared to control groups (0%). In ISP-induced cardiac hypertrophy, pretreatment with TMZ 10 mg showed a mortality of 12.5% while pretreatment with TEL, TMZ 20 mg, or their combinations exhibited no mortality.

Telmisartan, Trimetazidine, and their combination attenuated ISP-induced changes in heart weight and heart-to-body weight ratio in rats: ISP model rats showed no statistically significant change in BW as compared to the normal control group. Only TMZ (20 mg) and combination 1 treated groups showed a statistically significant increase in BW compared to normal rats and TEL-treated rats. No other treated groups demonstrated a significant change in BW compared to normal rats. Both HW and HW/BW ratios in ISP-induced myocardial hypertrophic rats were significantly higher than those of the normal group. HW and HW/BW ratios in all treated rats were significantly decreased compared with the ISP-induced myocardial hypertrophic group (Table 1).

Table 1: Telmisartan, Trimetazidine or their combination attenuated isoproterenol-induced changes in heart weight and heart-to-body weight ratio in rats

Group	BW (gram)	HW (mg)	HW/BW (mg/gram)
Normal	210.5 ±15.0	653.8 ±79.8	3.1 ±0.29
Vehicle	201.4 ±6.4	641.3 ±71.0	3.2 ±0.34
TEL Control	219.6 ±14.4	612.5 ±112.6	2.8 ±0.40
TMZ Control	215.3 ±42.0	587.5 ±172.7	2.7 ±0.41
ISP Control	238.6 ±16.5	1100.0 ±210.2 [a]	4.6 ±0.79 [a]
ISP +TEL	205.8 ±29.4	727.5 ±103.1[b]	3.6 ±0.48 [b]
ISP + TMZ 10	231.6 ±20.8	771.4±75.6 [b]	3.3 ±0.30 [b]
ISP + TMZ 20	251.8 ±27.2 [a,c]	756.3 ±111.6 [b]	3.04 ±0.6 [b]
ISP +C1	255.6 ±15.9 [a,c]	800.0 ±53.5 [b]	3.13 ± 0.20 [b]
ISP + C2	238.5 ±22.9	675.0 ±70.7 [b]	2.8 ±0.31 [b]

ISP, Isoproterenol; TEL, Telmisartan; TMZ 10, Trimetazidine 10 mg; TMZ 20, Trimetazidine 20 mg; C1, combination 1 TEL+TMZ (10 mg); C2, combination 2 TEL+TMZ (20 mg); BW, Body Weight; HW, Heart weight. Data are expressed as mean ± SD, n = 8 for all groups, except for ISP control and ISP +TMZ 10; n=7. [a] p<0.05 in comparison to the normal control group, [b] p<0.05 in comparison to ISP-control group, [c] p<0.05 in comparison to TEL-treated group using one-way ANOVA followed by Bonferroni Post hoc multiple comparison test.

Telmisartan, Trimetazidine, and their combination attenuated ISP-induced increase in serum level of the myocardial injury marker enzyme CK-MB in rats: In Figure 2, Isoproterenol-induced myocardial hypertrophic rats had a statistically significant increase in CK-MB concentration as compared to normal rats. All treated groups demonstrated a statistically significant reduction in CK-MB concentration as compared with the ISP-induced myocardial hypertrophic group. In addition, the group treated with TEL + TMZ 20 mg had statistically significantly lower levels of CK-MB concentration compared with monotherapy of TMZ in both doses.

Telmisartan, Trimetazidine, and their combination attenuated ISP-induced hemodynamic and ECG changes in rats: In Table 2, ISP-induced myocardial hypertrophic rats had significantly higher blood pressure than normal rats; both systolic blood pressure (SBP) and diastolic blood pressure. TEL treated group demonstrated a statistically significant reduction in SBP as compared with ISP-induced myocardial hypertrophic group and TMZ-treated groups in both doses. The rats

treated with TMZ 20 mg had a statistically significant reduction in SBP as compared to the ISP-induced myocardial hypertrophic group and TMZ 10 mg treated rats. There was a statistically significant decrease in SBP in the two combination-treated groups as compared to ISP-induced myocardial hypertrophic group and TMZ 10mg treated rats. In addition, the group treated with TEL + TMZ 20mg had a significant decrease in SBP compared with monotherapy of TMZ in 20 mg dose. Both combination groups demonstrated a statistically significant decrease in DBP as compared to the ISP-induced myocardial hypertrophic group while all other treated groups showed no statistically significant difference in DBP compared to normal rats.

Table 2: Effect of telmisartan, trimetazidine, or their combinations on blood pressure and electrocardiographic (ECG) patterns in Isoproterenol -induced cardiac hypertrophy in rats

Group	SBP (mm/Hg)	DBP (mm/Hg)	HR (beat/min)	R wave amplitude (mV)	QRS duration (ms)	QT interval (ms)	QTc interval (ms)	PR interval (ms)	RR interval (ms)
Normal	**113.5 ± 10.0**	**71.6 ±6.3**	**407.3 ±19.9**	**0.52 ±0.08**	**27.4 ±1.3**	**67.5 ±1.8**	**176.3 ±2.4**	**27.5 ±2.0**	**146.8 ±8.7**
Vehicle	120.0 ± 12.8	75.0 ±6.6	430.8 ±41.4	0.46 ±0.05	31.1 ±3.6	69.5 ±4.1	186.1 ±15.9	27.1 ±2.9	140.5 ±13.6
TEL Control	119.4 ± 10.7	75.8 ± 7.1	400.1 ±36.2	0.38 ±0.08	29.6 ±4.0	67.9 ±3.8	176.1 ±14.1	28.0 ±2.6	149.4 ±12.3
TMZ Control	127.8 ± 10.4	75.0 ±4.0	398.9 ±16.8	0.50 ±0.09	29.4 ±1.4	66.5 ±3.2	172.1 ±8.1	30.1 ±4.5	149.5 ±6.6
ISP Control	222.6 ±20.9 [a]	91.0 ±4.0 [a]	469.4 ±76.4	0.89 ±0.26 [a]	59.7 ±5.7 [a]	79.7 ±3.6 [a]	222.5 ±12.2 [a]	44.0 ±3.9[a]	131.4 ±27.3
ISP + TEL	130.3 ± 7.7 [b,d,e]	80.0 ±6.1	366.1 ±59.2 [b]	0.52 ±0.16 [b]	46.4 ±3.1 [a,b]	72.0 ±3.1[b]	177.5 ±20.2 [b]	25.8 ±4.9 [b]	168.1 ±28.5 [b]
ISP + TMZ 10	191.3 ± 14.7 [a,b]	82.1 ±11.1	374.1 ±70.0 [b]	0.53 ±0.14 [b]	48.4 ±7.6 [a,b]	75.4 ±4.6[a]	188.3 ±19.7 [b]	28.0 ±3.9 [b]	163.6 ±27.3
ISP + TMZ 20	153.3 ± 12.1 [a,b,d]	78.4 ±12.2	368.8 ±46.8 [b]	0.54 ±0.08 [b]	46.3 ±6.5 [a,b]	74.3 ±5.2[a]	183.6 ±13.9 [b]	29.4 ±2.9 [b]	165.0 ±22.6
ISP + C1	142.8 ± 20.0 [b,d]	76.4 ±8.7[b]	399.8 ±28.8	0.52 ±0.11 [b]	39.6 ±6.6 [a,b]	71.9 ±2.7 [b]	185.9 ±14.0 [b]	27.6 ±3.2 [b]	150.8 ±15.6
ISP + C2	125.4 ± 10.4 [b,d,e]	72.6 ±7.8 [b]	405.5 ±60.0	0.41 ±0.14 [b]	37.4 ±6.9 [a,b,c,d,e]	72.0 ±5.6[b]	186.9 ±20.7 [b]	24.0 ±4.1 [b]	150.9 ±24.4

ISP, Isoproterenol; TEL, Telmisartan; TMZ 10, Trimetazidine 10 mg; TMZ 20, Trimetazidine 20 mg; C1, combination 1 TEL+TMZ (10 mg); C2, combination 2 TEL+TMZ (20 mg); HR, Heart Rate; QTc, corrected QT interval. Data are expressed as mean ± SD, n = 8 for all groups, except for ISP control and ISP +TMZ 10; n=7. [a] p<0.05 in comparison to the normal-control group, [b] p<0.05 in comparison to ISP-control group, [c] p<0.05 in comparison to TEL-treated group, [d] p<0.05 in comparison to TMZ (10mg)-treated group, [e] p<0.05 in comparison to TMZ (20mg)-treated group using one-way ANOVA followed by Bonferroni Post hoc multiple comparison tests.

Table 2 and Figure 3 show that the ISP group showed an increase in HR as compared to normal control groups which were not statistically significant. The groups treated with TEL and the two used doses of TMZ showed a statistically significant decrease in HR as compared with ISP-induced myocardial hypertrophic rats, while coadministration of TEL and TMZ showed a statistically non-significant decrease in HR compared with ISP-

induced myocardial hypertrophic rats. In table 2 and Figure 3, ECG from the ISP-model group was associated with a significant increase in R wave amplitude, QRS duration, PR interval, and QTc interval when compared with normal rats. All treated groups demonstrated a significant decrease in those parameters compared with the ISP-induced myocardial hypertrophic group.

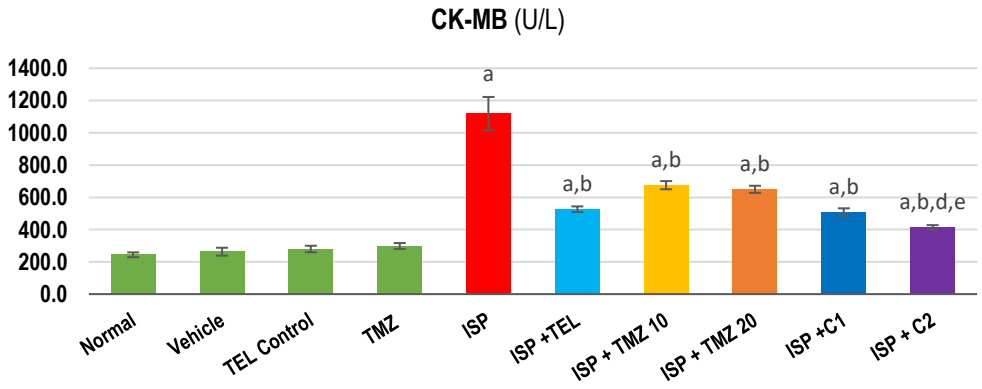

Figure 2. Effect of telmisartan and trimetazidine monotherapy and their combinations on serum levels of CK-MB in Isoproterenol-induced cardiac hypertrophy in rats.

ISP, Isoproterenol; TEL, Telmisartan; TMZ 10, Trimetazidine 10 mg; TMZ 20, Trimetazidine 20 mg; C1, combination 1 TEL+TMZ (10 mg); C2, combination 2 TEL+TMZ (20 mg). [a] $p<0.05$ in comparison to the normal-control group, [b] $p<0.05$ in comparison to ISP-control group, [d] $p<0.05$ in comparison to TMZ (10mg)-treated group, [e] $p<0.05$ in comparison to TMZ (20mg)-treated group. Data are expressed as mean ± SEM, analyzed using one-way ANOVA followed by Bonferroni Post hoc multiple comparison test, n = 8 for all groups, except for ISP control and ISP +TMZ 10; n=7.

Combination 2 group rats demonstrated a significant decrease in QRS duration compared with monotherapy. Then again, TEL treated group and combination-treated groups demonstrated a significant decrease in QT interval as compared to the ISP-induced myocardial hypertrophic group which was not statistically different from the normal control group. TMZ-treated groups in both doses showed no statistically significant decrease in QT interval as compared with the ISP-induced myocardial hypertrophic group. ISP-induced myocardial hypertrophic rats had an RR interval that was not statistically different from that of the normal control group. TEL treated group had a statistically significant increase in RR interval as compared to ISP-induced myocardial hypertrophic rats. Also, the RR interval in TMZ-treated groups in both doses and the two combination groups was not statistically different from with ISP-induced myocardial hypertrophic group.

Frequency distribution of ECG changes in rats treated with telmisartan and/or TMZ in ISP-induced cardiac hypertrophy in rats: ECG of the normal control group showed no changes in the S wave. On the other hand, a prominent S wave was observed in 57.2% of rats in the ISP group and 42.9% of rats in the TMZ 10mg/kg treated group, the percentage was 12.5% in TEL treated and TMZ 20 mg/kg treated groups. Normal control and combination-treated groups showed no rats with prominent S wave (Fig. 4-A, B). ECG of the normal control group did not show any notable changes in the Q wave. In ISP-induced myocardial hypertrophic and TMZ 10 mg/kg treated groups, two rats of each group (28.6% for each group) showed deep Q wave. However, no deep Q wave was found in all other treated groups (Fig. 4-A, B). Regarding ST segment shift, in the normal control group ST segment was Isoelectric in all rats in the group. In the ISP group,14.3% of rats (one rat) showed ST segment elevation

and 14.3% of rats (one rat) showed ST segment depression. In TMZ 10 mg/kg treated group only one rat showed ST-segment elevation. No ST segment shift was found in all other groups (Figure 4-A, B). ECG of normal control rats showed no fragmentation of QRS. Two rats of each group of ISP-induced myocardial hypertrophic group and TMZ 10 mg/kg treated group (28.6% for each group) showed fragmented QRS. However, no fQRS was found in all other groups (Figure 4-A, B). Two rats (28.6%) in the ISP group showed T wave inversion while only one rat (14.3%) showed T wave inversion in TMZ 10 mg/kg treated group. No T wave inversion was found in all other groups (Figure 4-A, B).

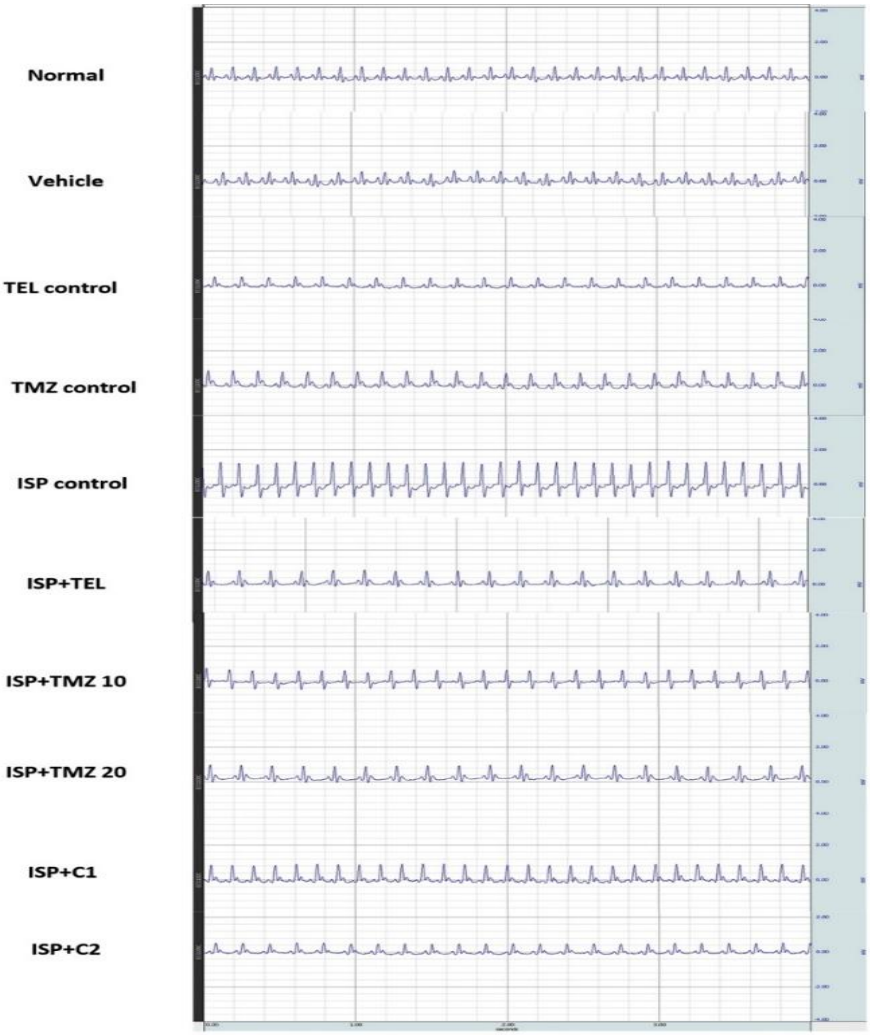

Figure 3. Effect of telmisartan, trimetazidine and their combinations on the electrocardiographic (ECG) patterns in isoproterenol-induced cardiac hypertrophy in rats of different study groups. [ISP, Isoproterenol; TEL, Telmisartan; TMZ 10, Trimetazidine 10 mg; TMZ 20, Trimetazidine 20 mg; C1, combination 1 TEL+TMZ (10 mg); C2, combination 2 TEL+TMZ (20 mg)].

Telmisartan, Trimetazidine, and their combination restored the power of myocardial contractility weakened by ISP in rats: In ISP-induced myocardial hypertrophic rats, the power of myocardial contractility was significantly weaker than in the normal control group. TEL-treated rats had higher power of myocardial contractility as compared to the ISP-induced myocardial hypertrophic group. The power of myocardial contractility in

TMZ 10 mg/kg treated rats was lower than the normal control group with no difference as compared to the ISP group while TMZ 20mg/kg treated group demonstrated a statistically significant increase in the power of myocardial contractility as compared to ISP-induced myocardial hypertrophic group. Also, the power of myocardial contractility in rats treated with the two combinations was significantly increased as compared to the ISP-induced myocardial hypertrophic group (Figure 5-A, B).

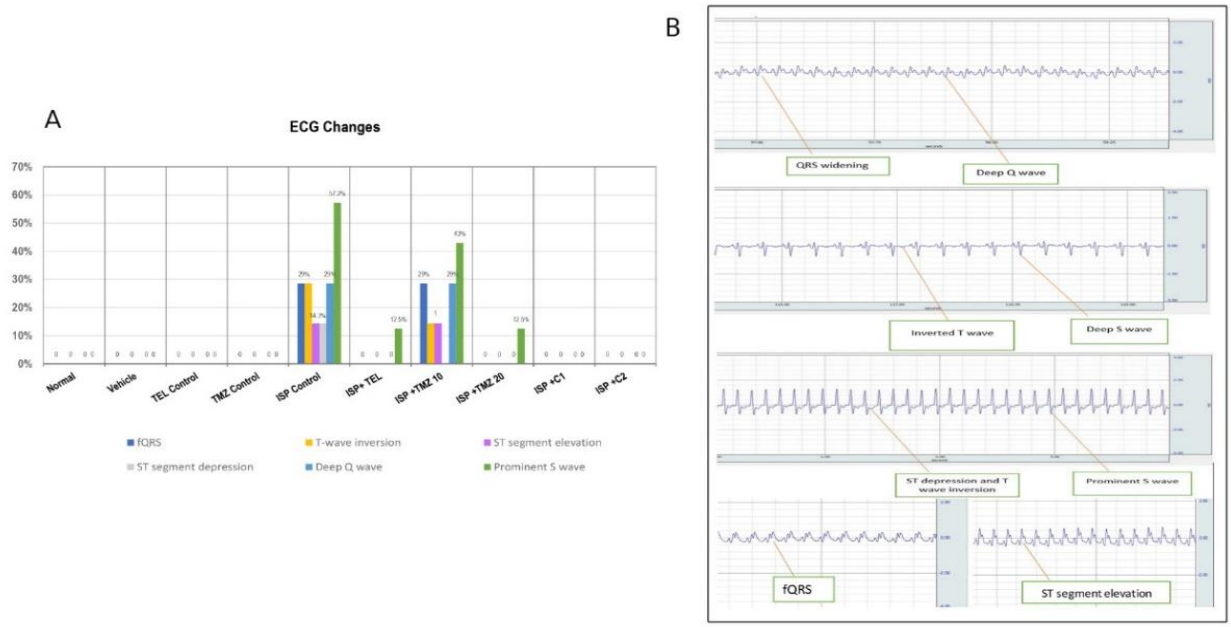

Figure 4. A. Frequency distribution of ECG changes in telmisartan and trimetazidine monotherapy versus their combinations in isoproterenol-induced cardiac hypertrophy in rats. ISP, Isoproterenol; TEL, Telmisartan; TMZ 10, Trimetazidine 10 mg; TMZ 20, Trimetazidine 20 mg; C1, combination 1 TEL+TMZ (10 mg); C2, combination 2 TEL+TMZ (20 mg); fQRS, fragmented QRS. **B. Representative electrocardiographic recordings showing different waveform changes induced by Isoproterenol in rats.**

TEL, TMZ, and their combination attenuated ISP-induced myocardial hypertrophy and fibrosis evident in histopathology: The mean LV wall thickness in the normal rat group was 1.35 ±0.15 mm which was not different from TEL or TMZ control groups (Figure 6-I (A-D), II). ISP-induced myocardial hypertrophic rats demonstrated a significant increase in LV wall thickness compared with the normal control group (Figure 6-I (E), II). On the other hand, TEL treated group showed a significant decrease in LV wall thickness than the ISP-induced myocardial hypertrophic group and TMZ 10 mg/kg treated rats (Figure 6-I (F), II). LV wall thickness was higher in TMZ 10 mg/kg treated rats than in normal control and different treated groups (Figure 6-I (G), II) while TMZ 20 mg/kg treated group demonstrated a significant decrease in LV wall thickness as compared with ISP-induced myocardial hypertrophic group and TMZ 10 mg/kg treated rats (Figure 6-I (H), II). The LV wall thickness was decreased in both groups treated with combinations as compared to ISP-induced myocardial hypertrophic group and TMZ 10 mg/kg treated rats (Fig. 6-I (I, J), II). Sections from the hearts of the normal control rats stained with H&E showed regular organization of muscle fibers into fascicles of myocytes, with cytoplasm that is eosinophilic, rich in myofibrils, and oval vesicular blunt-ended nuclei. The interstitial tissue had sparse connective tissue and vessels which had thin walls (Fig. 7-A-D, 8-A-D). In the ISP-control group, H&E staining of cardiac

sections revealed disruption of muscle bundle arrangement with moderate loss of fascicular pattern, a wide area of degenerated muscle cells having larger nuclei, and scant deeply stained cytoplasm with moderate cytoplasmic haphazardly arranged vacuolation. Scattered muscle cells showed loss of myofibrils and pale stained cytoplasm. There was focal infiltration by inflammatory cells in the form of scattered lymphocytes, other areas showed moderate edema (Fig. 7-E, 8-E). Treatment with TEL and/or TMZ showed improvements in pathological changes (Fig. 7-F-J, 8-F-J). Table 3 shows that the mean fibrosis area calculated from sections of hearts obtained from the normal rat group stained with Masson's Trichrome was 0.19 ±0.05 % with TEL and TMZ control fibrotic areas not markedly different from this group (Fig. 9-A-D). Masson's Trichrome stained heart sections from the ISP group showed marked disruption of muscle bundles with a wide area of fibrous tissue deposition highlighted by blue staining (Fig. 9-E). Table 3 shows that this group showed a statistically significant increase in fibrosis area compared with the normal control group. Masson's Trichrome staining of sections of hearts obtained from TEL-treated rats showed minimal fibrosis as highlighted by focal minimal staining (Fig. 9-F). This group showed a significant decrease in fibrosis area compared with the ISP-induced myocardial hypertrophic group. In heart sections obtained from TMZ 10mg group, there was a moderate disruption of muscle bundles with moderate fibrous tissue deposition highlighted by blue staining (Fig. 9-G). The fibrosis area in this group was significantly higher than TMZ 20mg treated group, but it was not significantly less than that of ISP-induced myocardial hypertrophic rats.

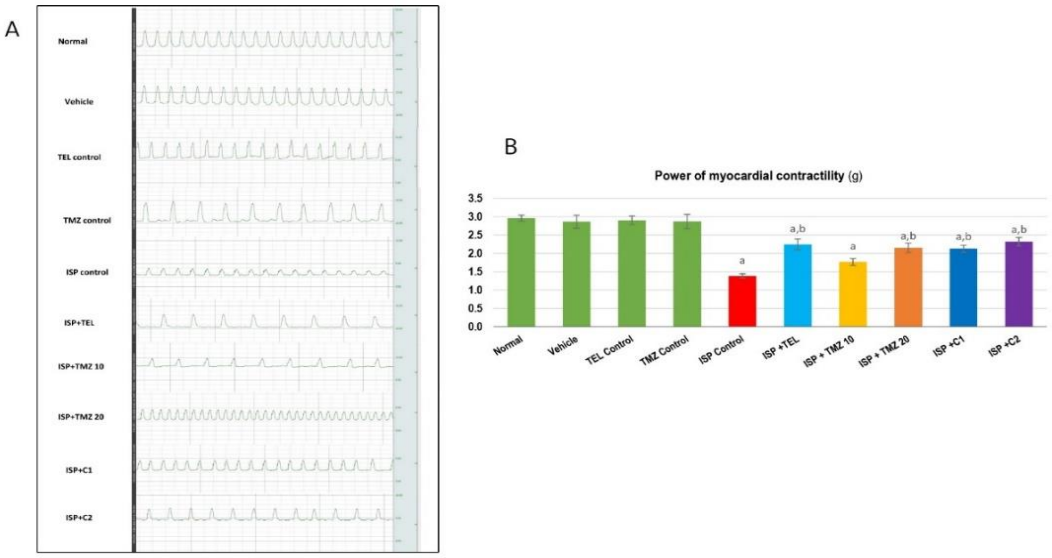

Figure 5. A. Representative Biopac output of the effect of telmisartan and/or trimetazidine or their combination on the power of myocardial contractility. B. Distribution of the power of myocardial contractility in different study groups in isoproterenol-induced cardiac hypertrophy in rats. ISP, Isoproterenol; TEL, Telmisartan; TMZ 10, Trimetazidine 10 mg; TMZ 20, Trimetazidine 20 mg; C1, combination 1 TEL+TMZ (10 mg); C2, combination 2 TEL+TMZ (20 mg). Data are expressed as mean ± SEM, analyzed, n = 8 for all groups, except for ISP control and ISP +TMZ 10; n=7. [a] $p<0.05$ in comparison to the normal control group, [b] $p<0.05$ in comparison to ISP-control group using one-way ANOVA followed by Bonferroni Post hoc multiple comparison test.

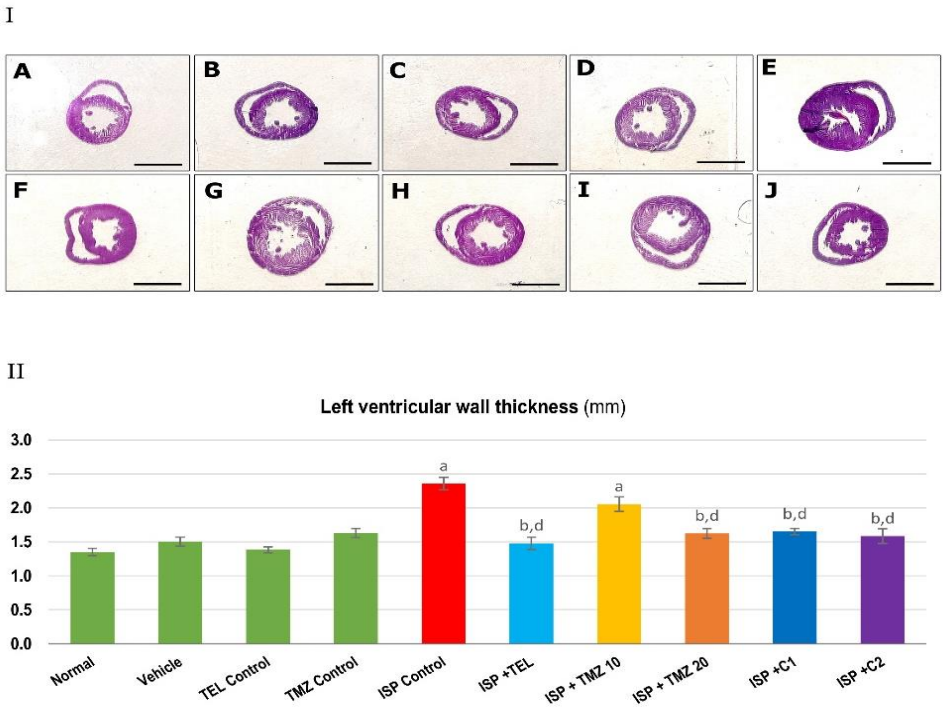

Figure 6. I. Scanning view of representative slides of different groups to show the difference in cross-section of heart tissue. A; normal-control group, B; vehicle group, C; Telmisartan (TEL) control, D; Trimetazidine (TMZ) control, E; Isoproterenol (ISP) control group, F; TEL treated group, G; 10 mg TMZ treated group, H; 20 mg TMZ treated group, I; combination 1 treated group (TEL & 10 mg TMZ treated group), J; combination 2-treated group (TEL & 20 mg TMZ treated group), Scale bar =5mm. **II. Effect of telmisartan and trimetazidine monotherapy and their combinations on the Left ventricular wall thickness in isoproterenol-induced cardiac hypertrophy.** ISP, Isoproterenol; TEL, Telmisartan; TMZ 10, Trimetazidine 10 mg; TMZ 20, Trimetazidine 20 mg; C1, combination 1 TEL+TMZ (10 mg); C2, combination 2 TEL+TMZ (20 mg). Data are expressed as mean ± SEM, n = 8 all groups, except for ISP control and ISP +TMZ 10; n=7. [a] $p<0.05$ in comparison to the normal control group, [b] $p<0.05$ in comparison to the ISP-control group, [d] $p<0.05$ in comparison to TMZ (10mg)-treated group using one-way ANOVA followed by Bonferroni Post hoc multiple comparison test.

On Masson's Trichrome staining of sections of hearts obtained from the TMZ 20 mg treated group and both combinations treated groups, there was a regular arrangement of muscle fibers into fascicles without fibrosis (Figure 9-H-J). Those groups showed a significant decrease in fibrosis area compared to the ISP-induced myocardial hypertrophic group.

TEL, TMZ, and their combination reduced cardiac TGF-β1 in ISP-induced cardiac hypertrophy: Table 3 shows that ISP-induced myocardial hypertrophic rats showed a statistically significant increase in TGF-β1 protein expression as compared with the normal control group. All treated groups demonstrated a statistically significant decrease in TGF-β1 protein as compared to the ISP-induced myocardial hypertrophic group. Co-administration of TEL and TMZ in 20 mg/kg dose led to a statistically significant decrease in TGF-β1 protein as compared to monotherapy with either TEL or TMZ 10mg, while coadministration of TEL and TMZ in 10 mg/kg dose led to a significant decrease in TGF-β1 protein as compared to monotherapy with TEL but it was not statistically different from treatments with TMZ.

TEL, TMZ, and their combination attenuated high β-MHC protein expression in cardiac tissue in ISP-induced cardiac hypertrophy: In ISP-induced myocardial hypertrophic rats, β-MHC protein expression in cardiac tissue was higher compared with normal rats.

Protein expression of β-MHC in hearts from all treated rats was significantly less than that of the ISP-induced myocardial hypertrophic group, (Figure 10-A, E).

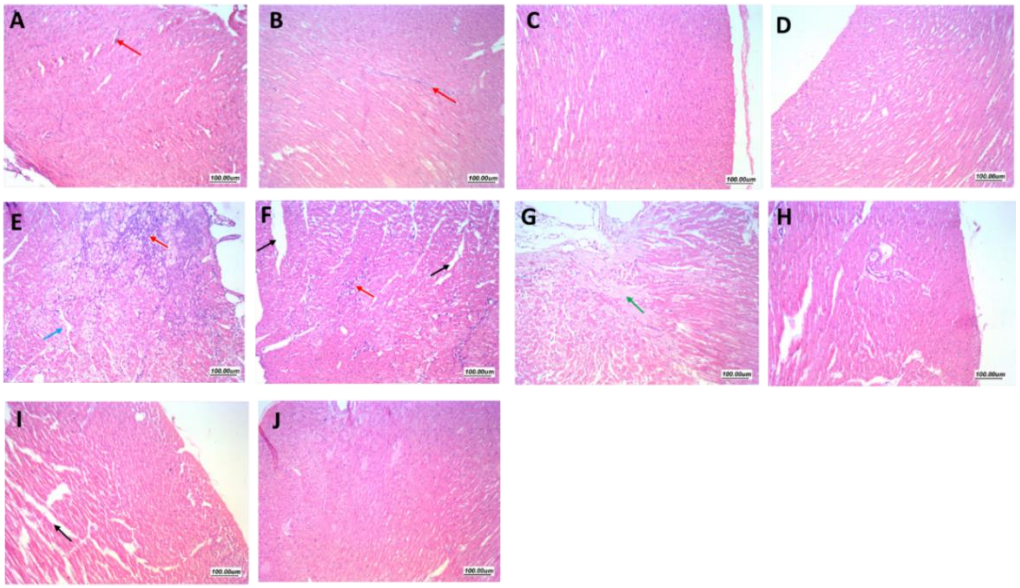

Figure 7. H& E transverse sections of the ventricles of different study groups (X10). A; normal-control group, B; vehicle group, C; Telmisartan (TEL) control, D; Trimetazidine (TMZ) control, E; Isoproterenol (ISP) control group, F; TEL treated group, G; 10 mg TMZ treated group, H; 20 mg TMZ treated group, I; combination 1 treated group (TEL & 10 mg TMZ treated group), J; combination 2-treated group (TEL & 20 mg TMZ treated group).The arrows point to; black (F,I: focal edema), red (A,B: blood vessels, E,F: degenerated myocytes), blue (E: edema), green (G: fibrosis).

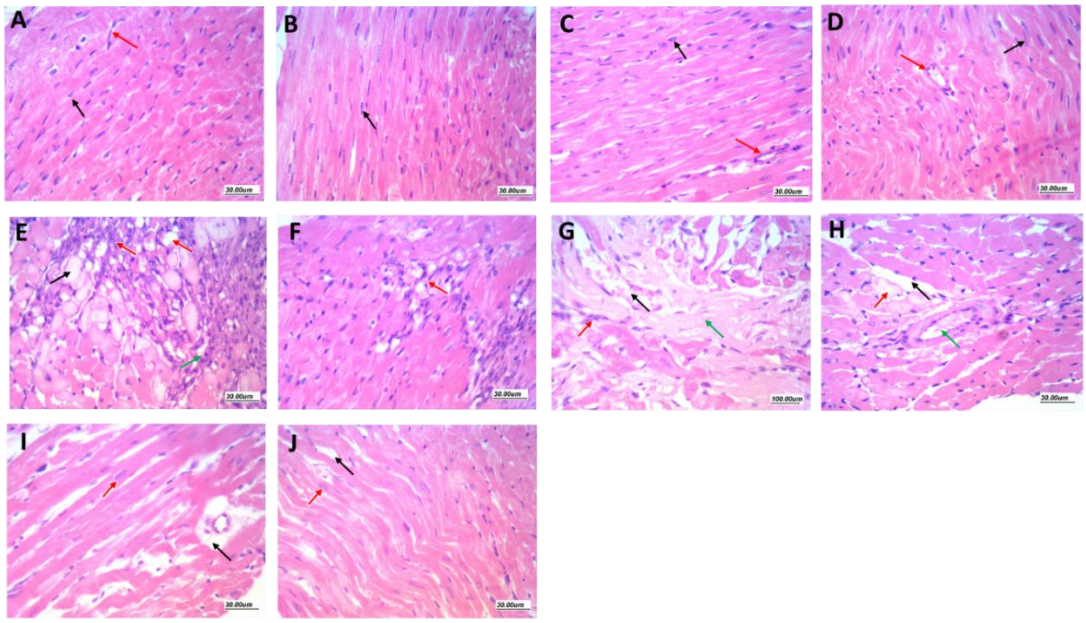

Figure 8. H& E transverse sections of the ventricles of different study groups (X40). A; normal-control group, B; vehicle group, C; Telmisartan (TEL) control, D; Trimetazidine (TMZ) control, E; Isoproterenol (ISP) control group, F; TEL treated group, G; 10 mg TMZ treated group, H; 20 mg TMZ treated group, I; combination 1 treated group (TEL & 10 mg TMZ treated group), J; combination 2-treated group (TEL & 20 mg TMZ treated group).The arrows point to; black (A-D: myocytes, E: myocytes showing loss of myofibrils, G: vascular congestion), H-J: focal edema), red (A, C, D: blood vessels, E-G: degenerated myocytes, H: myocytes showing loss of myofibrils, I, J: normal myocytes), green (E: inflammatory cells, G: fibrosis, H: vascular congestion).

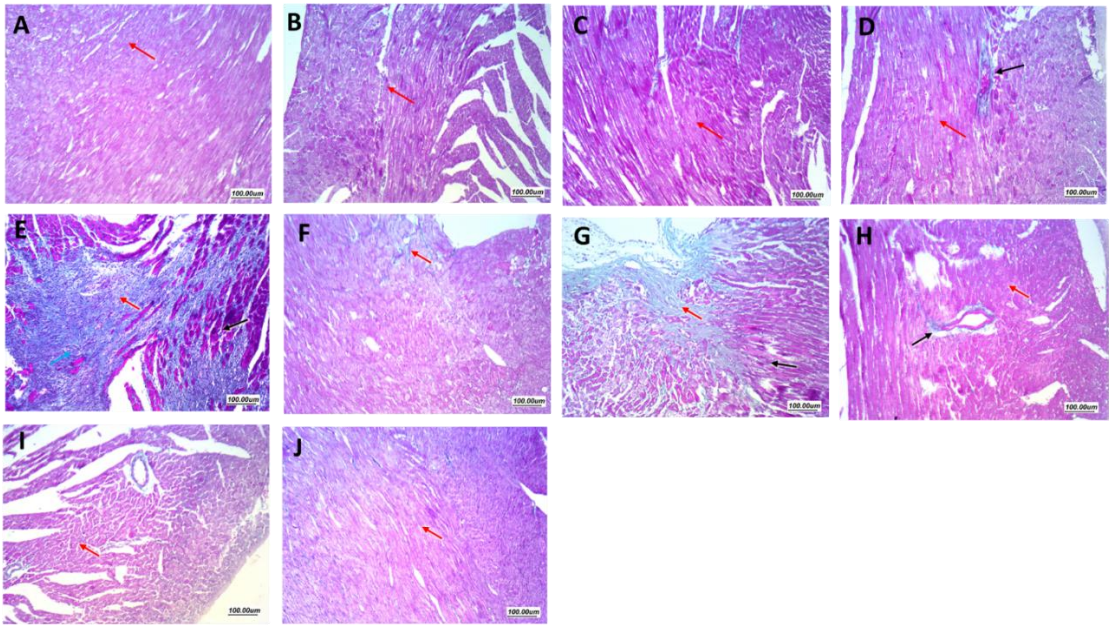

Figure 9. Masson's Trichrome transverse sections of the ventricles of different study groups (X10). A; normal-control group, B; vehicle group, C; Telmisartan (TEL) control, D; Trimetazidine (TMZ) control, E; Isoproterenol (ISP) control group, F; TEL treated group, G; 10 mg TMZ treated group, H; 20 mg TMZ treated group, I; combination 1 treated group (TEL & 10 mg TMZ treated group), J; combination 2-treated group (TEL & 20 mg TMZ treated group). The arrows point to; red (A-D, H, I, J: myocytes, E, F, G: fibrosis), and black (D, H: blood vessels, E, G: myocytes). Fibrous tissue deposition is highlighted by blue staining.

Table 3: Effect of Telmisartan, Trimetazidine or their combinations on TGF-β1 and fibrosis area in Isoproterenol-induced cardiac hypertrophy in rats

Group	TGF-β1 (pg/mg protein)	Fibrosis area (%)
Normal	104.5 ±10.1	0.19 ±0.05
Vehicle	95.4 ±11.0	0.35 ±0.10
TEL Control	98.3 ±12.2	0.55 ±0.43
TMZ Control	90.7 ±12.4	0.71 ±0.56
ISP Control	241.7 ±40.7[a]	17.19 ±5.27 [a]
ISP +TEL	156.8 ±22.2 [a,b]	0.96 ±0.54 [b]
ISP + TMZ 10	152.0 ±19.6 [a,b]	6.47 ±1.66[e]
ISP + TMZ 20	136.9 ±13.8 [b]	0.70 ±0.44 [b,d]
ISP +C1	123.2 ±21.6 [b,c]	1.04 ±0.39 [b]
ISP +C2	114.8 ±12.5 [b,c,d]	0.96 ±0.30 [b]

ISP, Isoproterenol; TEL, Telmisartan; TMZ 10, Trimetazidine 10 mg; TMZ 20, Trimetazidine 20 mg; C1, combination 1 TEL+TMZ (10 mg); C2, combination 2 TEL+TMZ (20 mg). Data are expressed as mean ± SD, n = 8 for all groups, except for ISP control and ISP +TMZ 10; n=7. [a] p<0.05 in comparison to the normal-control group, [b] p<0.05 in comparison to ISP-control group, [d] p<0.05 in comparison to TMZ (10mg)-treated group, [e] p<0.05 in comparison to TMZ (20mg)-treated group using one-way ANOVA followed by Bonferroni Post hoc multiple comparison test for TGF-β1 and Kruskal-Wallis followed by Bonferroni correction for multiple comparisons for fibrosis area.

Telmisartan, Trimetazidine and their combination decreased high protein expression of autophagy markers in cardiac tissue in ISP-induced cardiac hypertrophy in rats: LC3-I protein expression in cardiac tissue of ISP-induced myocardial hypertrophic rats was increased compared with normal rats. LC3-I protein expression in hearts obtained from all treated rats was significantly decreased compared with the ISP-induced myocardial hypertrophic group. LC3-I expression in cardiac tissue in TMZ 10 mg group was significantly decreased compared with TEL and combination 1 treated groups (Figure 10-B, E). ISP-induced myocardial hypertrophic rats expressed more LC3-II and LC3-II/LC3-I ratio compared with normal rats while LC3-II expression in hearts of all treated rats (apart from TMZ 10 mg group) was significantly decreased compared with ISP-induced myocardial hypertrophic group (Figure 10-C, D, E). TEL treated group showed a significant decrease in LC3-II/LC3-I ratio as compared to the ISP group and TMZ 10 mg treated group. Rats treated with TMZ in 20 mg dose only demonstrated a statistically significant decrease in LC3-II/LC3-I ratio as compared to the ISP group. Combinations treated rats also demonstrated a significant decrease in LC3-II/LC3-I ratio as compared to the ISP group, Figure 10-D, E.

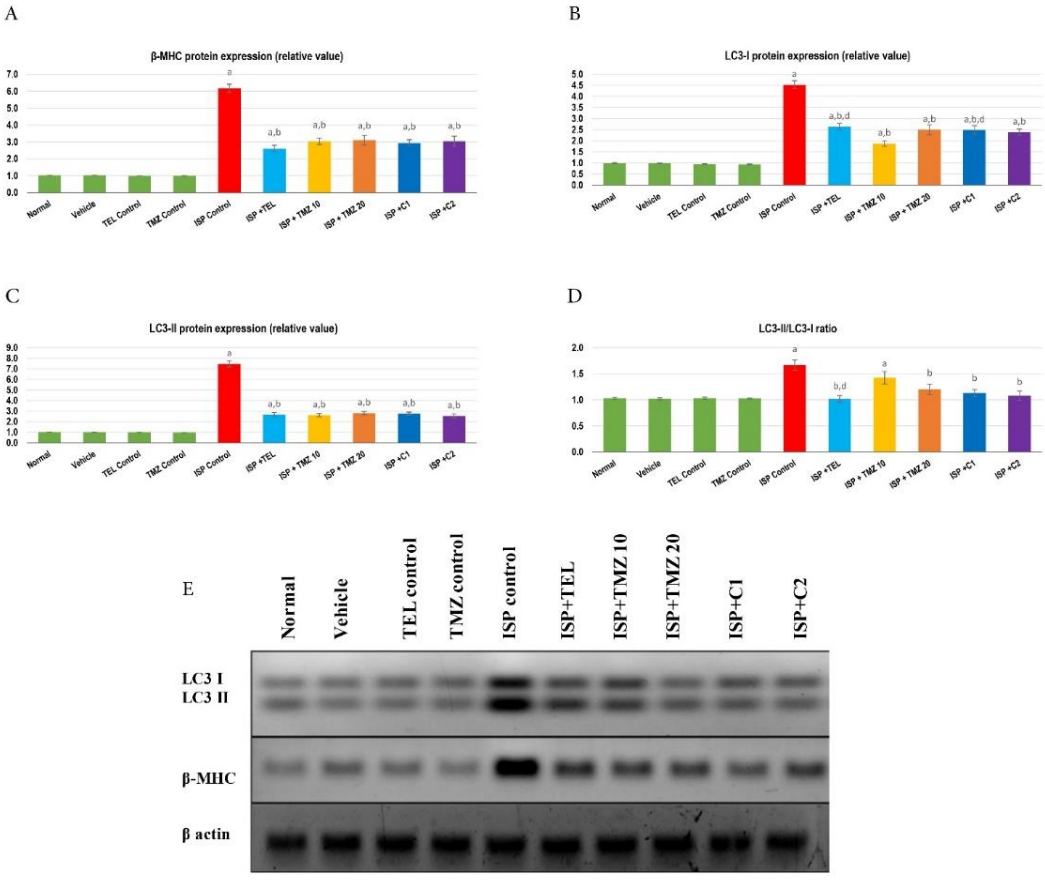

Figure 10. Effect of telmisartan and trimetazidine monotherapy and their combinations on the expression level of A. β-MHC, B. LC3I, C. LC3II, and D. LC3II/LC3I ratio in isoproterenol-induced cardiac hypertrophy. E. Western blot analysis of autophagy-related proteins (LC3 I and LC3 II) and β-MHC. ISP, Isoproterenol; TEL, Telmisartan; TMZ 10, Trimetazidine 10 mg; TMZ 20, Trimetazidine 20 mg; C1, combination 1 TEL+TMZ (10 mg); C2, combination 2 TEL+TMZ (20 mg). Data are expressed as mean ± SEM, n = 8 for all groups, except for ISP control and ISP +TMZ 10; n=7. [a] $p<0.05$ in comparison to the normal-control group, [b] $p<0.05$ in comparison to ISP-control group using one-way ANOVA followed by Bonferroni Post hoc multiple comparison test.

Discussion

The present study assessed the potential preventive role of TEL and TMZ separately versus their combination in ISP-induced myocardial hypertrophy in rats and examined if autophagy is one of the possible mechanisms for these effects. The findings of the study demonstrated the ability of TMZ, TEL, or their combination to prevent myocardial hypertrophy and that modulation of autophagy is implicated in the process. However, the two drug combinations tried were not superior to either of the drugs alone. In this work, we employed ISP to induce myocardial hypertrophy which is a widely accepted model having well-described hypertrophic pathways[26]. In our study, one week of ISP (4 mg/Kg/day) was successful in inducing myocardial hypertrophy and fibrosis in rats as evidenced by higher HW/BW ratio increased β-MHC & TGF-β expression, elevated CK-MB, ECG changes, weaker cardiac contractility and by histopathological changes in cardiac tissues. Those findings are consistent with prior literatures[27,28]. The mechanisms behind ISP-hypertrophy and fibrosis are multiple encompassing longstanding stimulation of β adrenergic receptors, enhanced protein synthesis, oxidative stress, inflammation, upregulation of TGF-β1, and modulation of autophagy [12,29]. We reported 30% overall mortality in the ISP-model group which is comparable to percentages reported previously[30]. This could be related to arrhythmia caused by ISP-induced ischemic changes, extensive infarction, or acute heart failure. Our data showed that ISP administration caused elevation in SBP and DBP, and ECG changes which came in agreement with previous studies that used identical model[27,29]. In myocardial hypertrophy, repolarization may be affected producing a left ventricular strain pattern in the form of abnormal ST segments or T wave inversion. This pattern was similar to one found and reported previously[20]. Myocardial fibrosis was found to correlate with QRS prolongation, fQRS complex, ECG strain and T-wave inversion[31]. In this work, about one-third of rats in the ISP group showed fQRS which indicates the effect of ischemia and fibrosis of the ventricles resulting in heterogeneous depolarization of the myocardium. In this experiment, TEL and TMZ ameliorated ISP-induced myocardial hypertrophy and fibrosis. Our results are in accordance with earlier findings stating that TEL prevented detrimental cardiac remodeling by reducing cardiac hypertrophy and fibrosis[32,33]. Also, TMZ, particularly in the high dose (20 mg/kg), had a comparable effect to TEL in all the examined parameters. In line with the cardioprotective effects manifested in our experiment, TMZ prevented metabolic and structural cardiac remodeling in the ISP-heart failure model and ischemia-reperfusion injury[10]. The protective influence of TMZ on the heart was explained in other studies by the drug ability to optimize cardiac metabolism, regulate adenosine monophosphate-activated protein kinase, and increased the expression of peroxisome proliferator-activated receptor α[34]. The impact of both drugs was also manifested by the reduction of BP and ECG changes induced by ISP. The antihypertensive effect of TEL is well-documented in experimental and clinical settings with a clear mechanism of action. Telmisartan blocks AT1 receptor-mediated actions of Ang II with less vasoconstriction and reduced aldosterone secretion. It also activates PPAR-γ and PPAR-α receptors with indirect percussions on the proliferation of vascular smooth muscle cells. Moreover, it improves endothelial function and has anti-oxidant effect[4]. In contrast, the mechanisms mediating TMZ antihypertensive effect are less clear. It might involve attenuating

endothelial dysfunction via increasing nitric oxide and antioxidant effect[35,36] with less vasoconstriction. A much older clinical work, in patients with hypertension comorbid with ischemic heart disease, declared that TMZ reduces peripheral resistance both in resting and post-exercise states[37]. ECG of rats treated with TEL, TMZ, or their combination showed better ECG patterns compared with those from ISP-treated rats. This reflects the mitigation of cardiac hypertrophy and fibrosis. The improvement in ECG parameters by TEL and TMZ is matching that of earlier studies which showed that TEL was effective in reducing the left ventricular strain pattern evident in ECG[38] and that TMZ treatment reduced HR and QT and QTc intervals as compared with ISP model rats[39]. The Hemodynamic and biochemical studies were further strengthened by histopathological examination of heart specimens from the animal model. ISP model rats had a marked increase in left ventricular wall thickness and fibrosis compared to normal rats. This was reflected in evident weaker cardiac contractility in the ISP-treated group. Not only our results but also earlier work supported the deteriorating effects of myocardial fibrosis and pressure overload on cardiac contractility[40]. Rats pretreated with TEL or TMZ 20 mg/kg had less ISP-induced pathological changes with a marked reduction in ventricular hypertrophy, fibrotic area, and better myocardial contractility than in ISP rat group. This result is in agreement with many other studies as in which TEL improved left ventricular contraction and alleviated remodeling[41,42]. The antifibrotic effect of TEL is crucial to its anti-remodeling effect following myocardial infarction[33], hypertension[43], ISP-induced heart failure[44], and diabetic cardiomyopathy[45]. Then again, TMZ dampened fibrosis induced by ISP[34], radiation[46], streptozotocin-diabetes[47], and pressure overload[48]. Cardiac fibrosis induced by ISP is coupled with high TGF-β1 levels and conversion of cardiac fibroblast to myofibroblast phenotype and Smad signaling[49]. Myofibroblast proliferation leads to excessive deposition of collagens impairing cardiac function with deterioration to heart failure[50]. In this report, myocardial fibrosis induced by ISP was accompanied by a higher cardiac expression of β-MHC and TGF-β1. The former represents the re-expression of fetal genes and is viewed as an important molecular indicator of pathological hypertrophy while the latter is known to be overexpressed in hypertrophic myocardium, especially during the transition to decompensation and heart failure. It is worth noting that both are implicated in cardiac fibrosis[51,52]. TGF-β is a critical player in cardiac remodeling acting via direct and indirect pathways to induce hypertrophy of cardiomyocytes, the proliferation of fibroblasts, and changes in the extracellular matrix[53]. In our study, pretreatment with TEL and/or TMZ ameliorated myocardial fibrosis and caused lower levels of TGF-β1 protein expression as compared to the ISP group. Other researchers reported the effect of TEL in the downregulation of TGF-β1 in myocardial infarction[54], this effect could be mediated by modulation of PPAR-γ receptors independent of AT1 receptor blockade[55]. TMZ results corroborated with earlier studies which explained its antifibrotic effect by reducing TGF-β levels and alleviating oxidative stress[46,47]. In our study, cardiac β-MHC protein expression in ISP-induced myocardial hypertrophic rats was significantly increased compared with the normal control group. Other studies supported our result[12,52]. In our study, TEL and TMZ treatment separately or in combination significantly prevented the rise in β-MHC protein expression in cardiac tissue induced by ISP. A previous study showed that TEL treatment normalized the expression of β-MHC gene in hypertrophic hearts in mice[56]. Pretreatment with TMZ in double the higher

dose we used inhibited the upregulation of β-MHC gene in cardiac hypertrophy caused by constriction of abdominal aorta [57]. Accumulating data has revealed a close connection between autophagy processes in cardiomyocytes and myocardial hypertrophy. However, there is a fine line that determine beneficial or exacerbating role of cell autophagy in myocardial hypertrophy[7,12]. Optimizing autophagy should be the target of any pharmacological intervention aiming at limiting myocardial damage and remodeling related to ischemia or pressure overload[58]. In this work, autophagy was assessed using protein expression of LC3I, LC3II and the ratio between them which is considered as an indication of autophagy activation[59]. Our study indicated that LC3-II protein expression in cardiac tissue and the LC3-II/LC3-I ratio in myocardial hypertrophic model rats was markedly increased compared to normal control groups. Our result is consistent with another study in which ISP treatment in a dose of 5 mg/kg/day for 7 days caused marked increase in autophagy in the hypertrophied hearts of rats with high LC3-II/LC3-I ratio[12]. A study explained the effect of ISP in different doses concluded that ISP stimulated autophagy in a dose-dependent manner, and that at high doses of 5 and 50 mg the cross talk between autophagy and cell death pathways was impaired leading to myocardial damage by apoptosis and necrosis[16]. In our study, TEL, TMZ and their combination attenuated the effect of ISP. Up to our knowledge, this the first work to reveal the link between the two studied drugs and autophagy in ISP cardiac hypertrophic models. However, TEL ameliorated excessive autophagy induced by intermittent hypoxia in mice kidneys[60]. Moreover, losartan, the prototype AT1 receptor antagonist prevented cardiomyocyte autophagy and hypertrophy caused by aortic constriction in mice[7]. Our data indicated that only TMZ in the large dose (20mg) attenuated ISP induced autophagy. The same dose was effective in halting excessive autophagy in ischemia-reperfusion model in rats[10]. TMZ (30 mg/kg/day for 8 weeks) enhanced cardiac function by modulating cardiomyocyte autophagy in diabetic cardiomyopathy [61]. Nevertheless, there are studies documenting induction of autophagy by TMZ emphasizing again the critical effect of dose and the surrounding milieu caused by specific pathological condition[62]. And this might explain why the combination of TEL and TMZ was effective but not better than either of the drugs alone. In our study, combination groups showed attenuated ISP induced LC3-II protein expression and LC3-II/LC3-I ratio but was not different from TEL or TMZ treated groups. Also, combination groups exhibited marked decrease in LV wall thickness as opposed to ISP group. Coadministration of TEL and TMZ led to a significant improvement in myocardial contractility compared to the ISP group, but it was not better than monotherapy. Our study is the first to demonstrate that coadministration of TEL and TMZ prevented myocardial hypertrophy induced by ISP but unfortunately the combination was not statistically different from pretreatment with either of the drugs. This might be related to the used doses of the drugs, the augmented effect on modulation of autophagy. It is hard to exclude the possibility of other pathways where each drug might had affected differently. Explanation of this result might be offered in future experiments.

Conclusion

This study for the first time clearly demonstrated the preventive effect of TEL and TMZ separately versus their combination in experimental model of myocardial hypertrophy. Interestingly, this study showed that the combined therapy with TEL and TMZ significantly prevented the myocardial

hypertrophy induced by ISP in this animal model. The study clearly demonstrates, for the first time, the role of autophagy as a mechanism of the preventive effects produced by TEL and TMZ in ISP-induced myocardial hypertrophy in rats. Finally, co-administration of TEL and TMZ was not superior to their individual use on protecting against myocardial hypertrophy induced by ISP. Therefore, we could conclude that TEL and TMZ could be used separately or in combination in patients with a higher risk for developing myocardial hypertrophy like hypertensive patients to protect against myocardial hypertrophy and its progression to HF.

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