Histological Study of the Effects of Empagliflozin Therapy on the Myocardium of Hypertensive Wister Adult Male Albino Rats

Original Article

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ABSTRACT

Introduction: Hypertension (HTN) is a common disease. A close relationship between HTN and high salt diet (HS) is recorded. Reduction of dietary HS in hypertensive patients causes improvements in blood pressure (BP) and cardiac architecture. Sodium–glucose cotransporter-2 inhibitors (SGLT2i) are oral anti diabetic drugs. Empagliflozin (EMPA) is one of these inhibitors. EMPA is an anti-hyperglycemic agent and reduces BP in patients with and without diabetes mellitus.

Aim of the Work: The aim was to assess the EMPA therapy effects on the hypertensive Wister adult male albino rat's myocardium and the regenerative role of telocytes.

Materials and Methods: Fifty adult male albino rats were utilized and divided into 2 main groups: group I (Control): included 20 rats and subdivided into subgroups IA (Standard diet & placebo) and IB (Standard diet& EMPA) and group II (HTN induced): involved 30 rats which took HS diet for eight weeks then subdivided into subgroup IIA (HS diet), IIB (HS diet & EMPA) and IIC (Low salt diet) for another 8 weeks. Systolic BP was measured at the start, 8 and 16 weeks following the experiment's start. Specimens from the myocardium left ventricle were prepared for Picrosirius Red, CD34 immunostaining and electron microscopic examination. SBP, telocytes number and percentage of collagen fibers were analyzed statistically.

Results: Subgroup IIA showed disrupted histological architecture of the myocardium but it was preserved in subgroups IIB and IIC. Systolic BP and collagen fibers deposition were high in IIA subgroup but showed levels near those of the control group in IIB and IIC subgroups. Area percentage of telocytes was high in IIB and IIC with marked decrease in IIA subgroups.

Conclusion: Relation between hypertension and HS diet intake is proved. HS diet has deleterious effects on the myocardium architecture. This study revealed that EMPA and dietary salt restriction preserve rat's myocardium architecture.

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Key Words: EMPA, heart, hypertension, salt, SGLT2i.

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INTRODUCTION

Hypertension (HTN) is a common disease. In the US, it affects about 116 million adults and worldwide, it affects more than 1 billion adults[1,2]. Additionally, it raises the risk of cardiovascular disease (CVD) such as coronary heart disease, stroke, heart failure (HF) and death[3]. However, it is considered as one of the most common preventable causes of death[4]. Sodium affects CVD and blood pressure (BP). High salt diet (HS) intake raises BP and CVD. However, low sodium intake lowers BP and the occurrence of hypertension. Furthermore, it lowers CVD-related morbidity and mortality[5,6]. Sodium–glucose cotransporter-2 inhibitors (SGLT2i) are new class of oral anti-hyperglycemic drugs that have been approved to treat type 2 diabetes mellitus (T2DM)[7]. In addition, SGLT2i cause significant decrease in diastolic blood pressure (DBP) and systolic blood pressure (SBP)[8]. Food and Drug Agency (FDA) approved that empagliflozin has a great selectivity for SGLT2 receptors. EMPA is an effective anti-hyperglycemic drug in addition to lowering elevated blood pressure in T2DM[9,10]. EMPA causes reduction in death from CVD, myocardial infarction and heart failure-related hospitalizations[11]. It improves left ventricle (LV) mass, decreases myocardial fibrosis markers which improve cardiac function. Initial decrease in BP is due to the effects of diuretic and volume depletion. long-term impacts may be attributable to suppression of the renin-angiotensin system and weight reduction[12].

Telocytes (TCs) are distinctive type of the interstitial cells. Its distribution within heart varies with normal or pathological states. They promote cardiac growth and regeneration[13]. Transmission electron microscope (TEM)

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is necessary in identification of the TCs. TCs have cell body containing nucleus and labyrinthine system made of telopodes (Tps) (prolongations). Cell body has variable shapes which may be piriform, spindle, triangular or stellate depending on the number of Tps which ranges from 1–5. Telopodes are constituted by an alternation of dilated segments (podoms) and thin segments (podomers). Their bodies with telopodes (Tps) form the network which guides regeneration of cardiac architecture[14,15]. TCs are sometimes referred to as CD34+ stromal cells due to the cell surface glycoprotein CD34 which is one of the most useful markers for the immuno-histochemical identification of TCs[16].

AIM OF THE WORK

The aim was to assess the EMPA therapy effects on the hypertensive Wister adult male albino rat's myocardium and regenerative role of telocytes.

MATERIALS AND METHODS

Drug and Chemicals

Empagliflozin

Commercially available as Jardiance tablets, a product of Boehringer Ingelheim company (Germany). It was approved in 2014 by the American Diabetes Association (ADA) and FDA (United States Food and Drug Administration). Each rat received empagliflozin (30mg/kg/day) orally by oral gavage Park *et al*.[17] in the form of a half of 10 mg tablet dissolved in 5 ml tape water per day in addition to the standard diet for 16 weeks.

Sodium chloride (NaCl) was obtained from Al Gomhoria Chemical Company, Tanta, Egypt.

Animals and Experimental Design

Fifty Wister adult male albino rats with average weight (200-250 gm) were included in this study. In the animal house of faculty of medicine, the animals were kept in a specific clean, pathogen-free environment. The rats were maintained in clean properly ventilated cages with steel wire tops at room temperature with a 12:12 light –dark cycle and free access to water and food ad libitum. All the experiment steps were done according to the rules and regulations laid down by the research ethical committee on animal's experimentation of Tanta Faculty of Medicine (approval code 33939/7/20).

The average baseline SBP of the rats was recorded using the non-invasive rat tail cuff method. Then rats were classified as follows:

Group I: (Control group) (20 rats): Each rat received an average 20 gm standard diet per day. Moreover, adult rats usually drink 20-50 ml/day (10 ml/100 g body weight/day)[18]. Rats of this group were subdivided into:

- Subgroup IA: (Standard diet & placebo) (10 rats): Rats received standard diet with normal salt (0.3% NaCl)[19]. It was added to their chow in addition to 77.15mg lactose anhydrous dissolved in 5 ml tape water[17] for 16 weeks.
- Subgroup IB: (Standard diet & EMPA) (10 rats): Each rat received empagliflozin (30mg/kg/day) by oral gavage) according to Park *et al*. (17) in the form of half a 10 mg tablet dissolved in 5 ml tape water per day in addition to the standard diet for 16 weeks.

Group II: (Hypertension induced group) (30 rats): Rats received high salt diet (8% NaCl)[20]. It was added to their chow for 8 weeks for induction of hypertension. Then SBP measurements using the non-invasive rat tail cuff method were done for all rats to confirm diagnosis of hypertension. After that rats were subdivided into:

- Subgroup IIA (High salt diet) (10 rats): Rats continued to receive high salt diet (8% NaCl)[20]. It was added to their chow for another 8 weeks.
- Subgroup IIB (High salt diet &EMPA) (10 rats): Each rat received empagliflozin (30mg/kg/day) according to Park *et al*.[17] by oral gavage in the form of half a 10 mg tablet dissolved in 5 ml tape water per day in addition to high salt diet (8% NaCl) which was added to their chow for another 8 weeks[20].
- Subgroup IIC (Low salt diet) (10 rats): Each rat received low salt diet (0.25% NaCl) for another eight weeks which was added to their chow[21].

After 16 weeks from the start of the experiment, final measurements of SBP were done for all rats of different groups.

Finally, rats were sacrificed under anesthesia using thiopental sodium followed by a midline thoraco-abdominal incision and chest bones were removed to get the heart away from the body. Samples from the caudal part of the left border down to the apex of the left ventricle of the heart were taken and fixed in an appropriate fixator (10% formol saline and 2.5% glutraldehyde) for light and electron microscopic histological examination respectively.

At last, according to safety and health precaution measures, sacrificed rats were carefully collected in a special package to be burnt later.

Blood pressure measurements[22,23]

For each rat, the systolic arterial blood pressure was measured 3 times; Baseline reading at day 0, at the end of 8th week of the experiment and at the end of 16th week of the experiment before scarification.

The non-invasive rat tail cuff method was the technique used for SBP measurements [Rat-tail sphygmomanometer (Harvard apparatus Ltd, Aden Berge, England) and pneumatic transducer (Harvard U.K.) connected to Harvard Universal oscillograph]. All measurements were done at the physiology department, faculty of medicine (Menuofia University). To avoid changes in blood pressure as a result

of day cycle, all measurements were done between 9.00am and 11.00am.

Rats of SBP (75-129 mmHg) were considered normal while those of 140 mmHg or more were considered hypertensive .

Histological study

Light microscopic study

1. Picrosirius Red (PR) Stain: to detect the collagen fibers of connective tissue in the left ventricle of rats in all groups[19].
2. CD34 Immunohistochemistry Stain: to detect telocytes in the left ventricle of heart of rats in all groups[24].

Electron microscopy (E.M) study

It was done to visualize the ultrastructure of the left ventricle of the rat's heart and telocytes in all experimental groups[25].

Morphometric study (quantitive assessment)[26]

i. Area percentage of CD34-positive immuno-stained TCs: It was counted in the left ventricles of the heart in 10 non-overlapping different fields at magnification 400/slide in every group by utilizing image J software program (National Institute of health, Bethesda, Maryland, USA). All data were analyzed statistically.

ii. Area percentage of interstitial collagen fibers: It was measured from Picrosirius Red stained slides to quantify the collagen fibers levels in 10 non-overlapping different fields by magnification 400/ slide in every group utilizing image J software program (National Institute of health, Bethesda, Maryland, USA). All data were analyzed statistically.

Statistical study[14]

The collected data including systolic blood pressure, telocytes quantity in CD34 immune-stained slides and collagen fibers in Picrosirius Red -stained slides were analyzed statistically. In our work, mean, standard deviation (S.D) and the (*P*) values were calculated by Statistical Package for the Social Sciences (SPSS) 16.0 software (IBM SPSS, Armonk, NY, USA). All data were expressed in histograms

Non-significant *P value* > 0.05 * Significant *p value* ≤ 0.05 ** Highly significant *p value* ≤ 0.001.

RESULTS

Histological and immunohistochemical results

Examination of the left ventricle of the heart by Light microscope

Picrosirius Red (PR) stained sections

Picrosirius Red stained sections of the rats' left ventricle revealed: In subgroups IA, restriction of reddish coloration of connective tissue collagen around the blood vessel and between the yellowish cardiac muscle fibers (Figure 1A). Subgroup IB had similar histological findings as subgroup IA. In subgroup IIA, extensive reddish coloration of connective tissue collagen infiltration between the yellowish cardiac muscle cells and increase of the reddish coloration of the connective tissue around thickened wall of the blood vessel was seen (Figure 1B). However, restriction of reddish coloration of connective tissue collagen around the blood vessel and between yellowish cardiac muscle cells in subgroup IIB was present (Figure 1C). In addition, slight increase of reddish coloration of connective tissue collagen fibers around the blood vessel and between yellowish cardiac muscle cells (Figure 1D) in subgroup IIC was seen.

CD34 immune-stained sections

Left ventricle sections of subgroup IA with CD34 immune-stain showed multiple, brown CD34-positive telocytes between cardiac muscle cells (Figure 2A). Subgroup IB had similar histological findings as subgroup IA. Sections of subgroup IIA revealed in some area's negative reaction of CD34 immunostaining between cardiac muscle cells. However, in other areas severe decrease in brown CD34-positive telocytes compared to control group was seen (Figure 2B). Sections of subgroup IIB showed multiple, brown CD34-positive telocytes between cardiac muscle cells apparently close to the control group (Figure 2C). CD34 immune-stained sections of subgroup IIC revealed multiple, brown CD34-positive telocytes between cardiac muscle cells apparently less than the control group (Figure 2D).

Electron microscopy (E.M) results

In subgroup IA, an ultra-thin section from left ventricle of adult male albino rat showed regular arrangement of cardiac muscle fibers with dark (A) band containing (M) line and light (I) band containing (Z) line. Regular nuclear membrane was obvious. Moreover, Abundant mitochondria with clear cristae in juxtanuclear regions were seen. Furthermore, regular scalloped sarcolemma with narrow subsarcolemmal space filled with mitochondria were clear (Figure 3A). Also, telocyte with triangular cell body and long telopode extending from cell body was obvious. Long, beaded string appearance of telopode due to alterations of podomere and podom segments were seen (Figure 4A). In addition, ladder step intercalated disk with transverse portions where desmosomes were abundant and longitudinal parts where a lot of gap junctions were clear (Figure 5A).Subgroup IB had similar histological findings as subgroup IA.

In subgroup IIA ,an ultra-thin section from left ventricle of adult male albino rat showed fragmented myofibrils, irregular and wavy sarcolemma with wide subsarcolemmal space filled with closely packed, variable sizes and shapes of distorted mitochondria with dense matrix.

However, rarefaction areas in subsarcolemmal space and in juxtanuclear region were seen (Figure 3B). Moreover, nucleus with indented and irregular nuclear membrane was seen. The central effacement of mitochondria was clear (Figure 3C). In addition, spindle shaped cell body of telocyte with large nucleus was present. Short and thick telopode were clear (Figure 4B). Furthermore, convoluted, distorted intercalated disk with ill-defined transverse and longitudinal portions were obvious (Figure 5B).

In subgroup IIB, an ultra-thin section from left ventricle of adult male albino rat showed regular and parallel myofibrils with rows of mitochondria in-between. Moreover, the Z and M lines were clear. The lace-like appearance of sarcoplasmic reticulum and corrugated nuclear membrane were present (Figure 3D). In addition, telocyte with spindle shaped cell body and large nucleus encircled by scanty cytoplasm ,podom , podomere and telopode were seen (Figure 4C). Also, the ladder step intercalated disks were clear. They had transverse portions where desmosomes were abundant as well as longitudinal portions with a lot of gap junctions (Figure 5C).

In subgroup IIC , an ultra-thin section from left ventricle of adult male albino rat showed regular arrangement of myofibrils with numerous mitochondria which had clear cristae in juxtanuclear zone. Regular nuclear membrane apparently close to the control group was seen (Figure 3E). Furthermore, regular and scalloped sarcolemma with narrow subsarcolemmal space were obvious. Irregular nuclear membrane was present (Figure 3F). Moreover, piriform cell body of telocyte containing large nucleus surrounded by scanty cytoplasm with short and thick telopode was seen. In addition, the large vacuole was clear (Figure 4D). Furthermore, ladder step intercalated disks with desmosomes and gap junction were clear (Figure 5D).

Statistical results

Systolic blood pressure measurements

The calculated mean baseline systolic blood pressure ± SD was 81.97± 2.23. Then, the rats were divided randomly into control group (20 rats) and experimental (Hypertension induced) group (30 rats). After 8 weeks, in subgroup IA (Control) the mean ± SD of SBP was 95.80 ± 5.34 while in the experimental (Hypertension induced) group which received high salt diet was 203.58 ± 15.23. The difference of SBP between experimental (Hypertension induced) and control group was highly significant.

Finally, after 16 weeks, in subgroup IA, mean ± SD of SBP was 116.76 ±2.45. IB subgroup had similar results to IA subgroup. In the experimental group, mean ± SD of SBP differed according to the different subgroups. Thus, in high salt diet (subgroup IIA), mean ± SD of SBP was 253.49 ± 7.81 with highly significant P value compared to the control, in EMPA treated (subgroup IIB) mean ± SD of SBP was 118.35 ± 2.20 with non-significant P value compared with the control group while in low salt diet (subgroup IIC) mean ± SD of SBP was 121.80 ± 2.69 with significant P value compared to the control group (Histogram 1).

The mean percentage area of collagen fibers deposition in all groups

After 16 weeks, the mean percentage of collagen area (mm) was calculated in different experimental groups. Subgroup IB had similar results to subgroup IA whose mean ± SD was 0.645 ± 0.19 which was high significant to subgroup IIA whose mean ± SD was 7.57± 0.75, non-significant to subgroup IIB whose mean ±SD was 0.738± 0.16 and significant to subgroup IIC whose mean ± SD was1.06± 0.19 (Histogram 2).

The mean percentage of CD34 expression in all groups

After 16 weeks, the mean percentage of CD34 expression was calculated in different experimental groups. Subgroup IB had similar results to subgroup IA whose mean ± SD was 6.82 ± 0.79 which was high significant to subgroup IIA whose mean ± SD was 1.14± 0.13, non-significant to subgroup IIB whose mean ± SD was 6.69± 0.83 and significant to subgroup IIC whose mean ± SD was 6.10 ± 0.32 (Histogram 3).

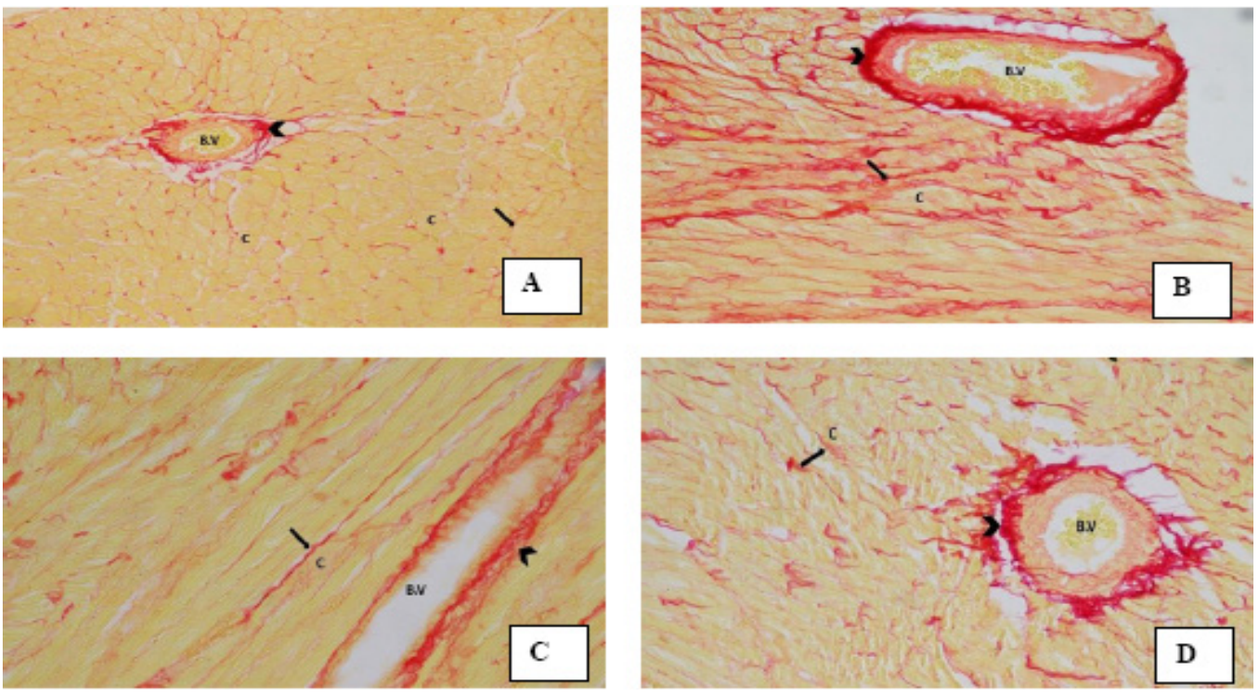


Fig. 1 : Left ventricle P.R × 400 A section in an adult male albino rat left ventricle showing: In subgroup IA , restriction of reddish coloration of connective tissue collagen fibers (arrowhead) around the blood vessel (B.V) and (arrow) between the yellowish cardiac muscle fibers (C) (Fig. 1A). Subgroup IB presents similar histological findings as IA subgroup. In subgroup IIA, extensive reddish coloration of connective tissue collagen fibers (arrow) infiltration between the yellowish cardiac muscle cells (C) and increase of the reddish coloration of the connective tissue (arrowhead) around thickened wall of the blood vessel (B.V) is seen (Fig.1B). However, in subgroup IIB (EMPA) restriction of reddish coloration of connective tissue collagen (arrowhead) around the blood vessel (B.V) and (arrow) between yellowish cardiac muscle cells (C) is present (Fig.1C). In addition, in subgroup IIC (low salt diet) slight increase of reddish coloration of connective tissue collagen (arrowhead) around the blood vessel (B.V) and (arrow) between yellowish cardiac muscle cells (C) (Fig.1D) is seen.

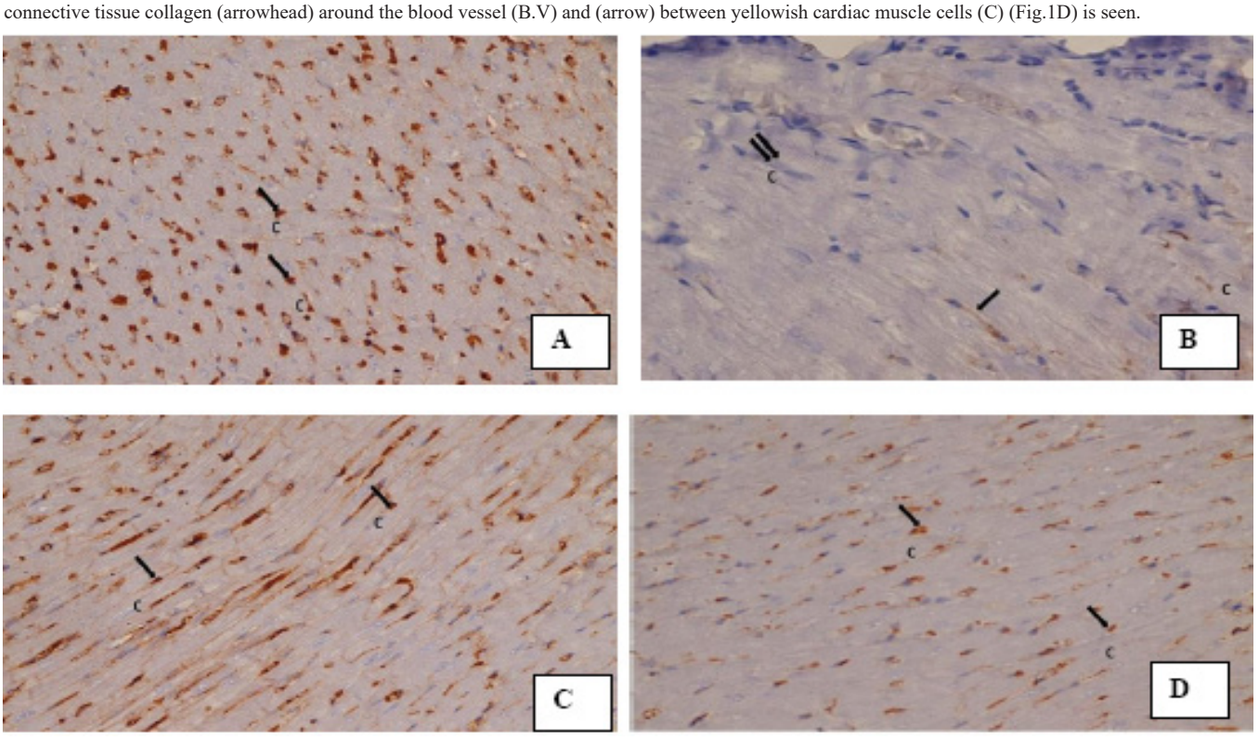


Fig. 2: CD34 immune-stain X 400 A section in an adult male albino rat left ventricle showing: In subgroup IA, positive reaction of CD34 immunostaining of telocytes which appear dark brownish in color (arrows) between cardiac muscle cells (C) (Fig. 2A). Subgroup IB has similar histological findings as IA subgroup. In subgroup IIA , different reaction of to CD34 immunostain , some sections show severe decrease in positive reaction of telocytes which appear dark brownish in color (arrow) between cardiac muscle cells (C) while others show negative reaction (double arrows) (Fig. 2B) In subgroup IIB, multiple, brown CD34-positive telocytes (arrows) between cardiac muscle cells (C) apparently close to the control group (Fig. 2C). In subgroup IIC, multiple, brown CD34-positive telocytes between cardiac muscle cells apparently less than the control group (Fig. 2D).

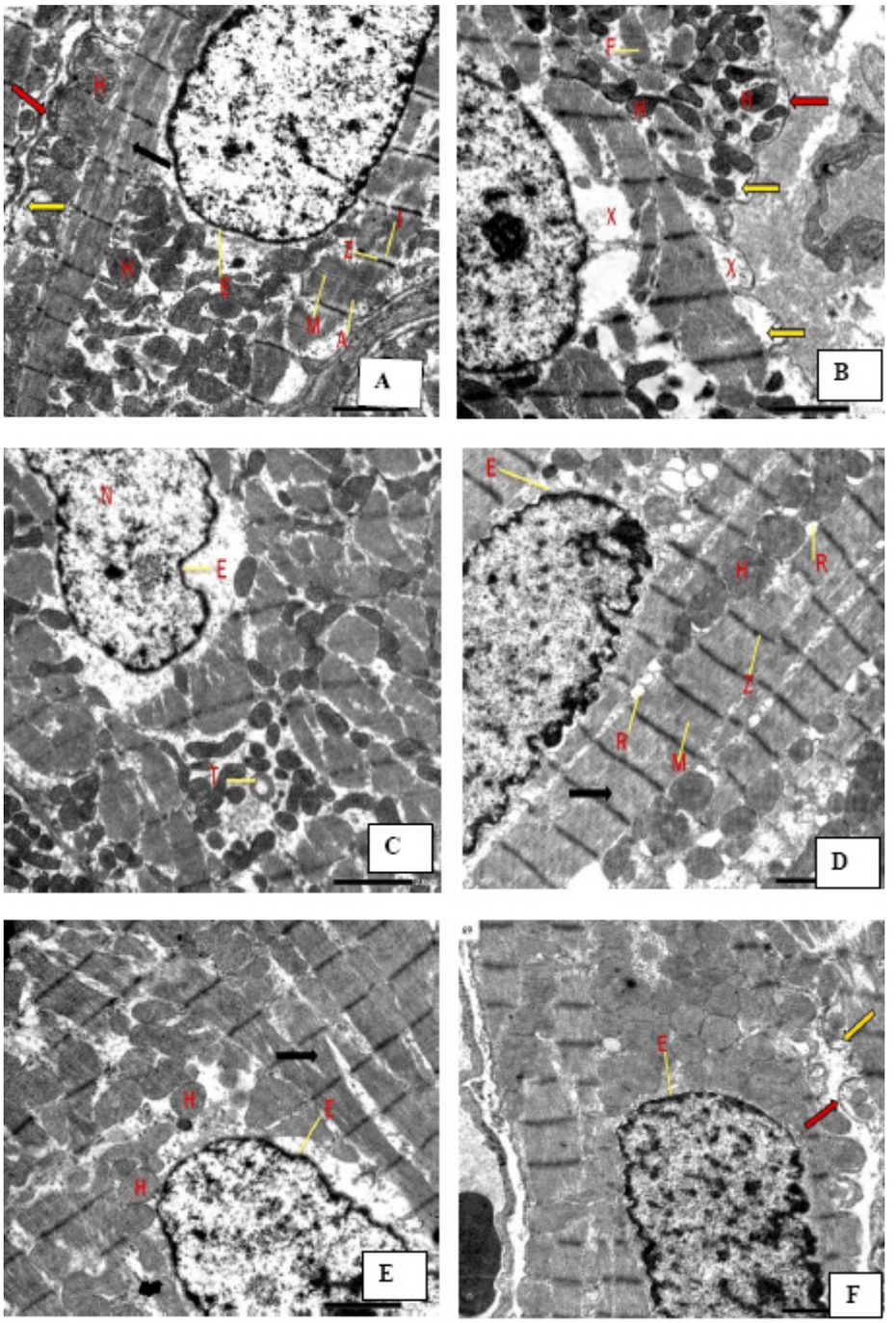


Fig. 3: Cardiac muscle (TEM) X 3000 An ultra-thin section from left ventricle of adult male albino rat showing: In subgroup IA, regular nuclear membrane (E). Abundant mitochondria with clear cristae (H) in juxtanuclear region are seen. Moreover, regular arrangement of cardiac muscle fibers (black arrow) with dark (A) band containing (M) line and light (I) band containing (Z) line are clear. Furthermore, regular scalloped sarcolemma (red arrow) with narrow subsarcolemmal space (yellow arrow) filled with mitochondria (H) are obvious (Fig. 3A). Subgroup IB presents similar histological findings as IA subgroup. In subgroup IIA, irregular and wavy sarcolemma (red arrow) with wide subsarcolemmal space (yellow arrow) filled with closely packed, variable sizes and shapes of distorted mitochondria (H) with dense matrix. However, rarefaction areas (X) in subsarcolemmal space and in juxtanuclear region are seen indicating depletion of mitochondria. Notice the fragmented (F) myofibrils (Fig. 3B). Moreover, nucleus (N) with indented and irregular nuclear membrane (E) is obvious . Central effacement of mitochondria (T) is clear (Fig. 3C). In subgroup IIB, regular and parallel myofibrils (black arrow) with rows of mitochondria (H) in-between. Moreover, Z and M lines are clear. Lace-like appearance of sarcoplasmic reticulum (R) and corrugated nuclear membrane (E) are seen (Fig. 3D). In subgroup IIC, regular arrangement of myofibrils (black arrow). Numerous mitochondria (H) with clear cristae in juxtanuclear zone and regular nuclear membrane (E) (Fig. 3E). Furthermore, regular and scalloped sarcolemma (red arrow) with narrow subsarcolemmal space (yellow arrow) are obvious. Irregular nuclear membrane (E) is seen (Fig.3F).

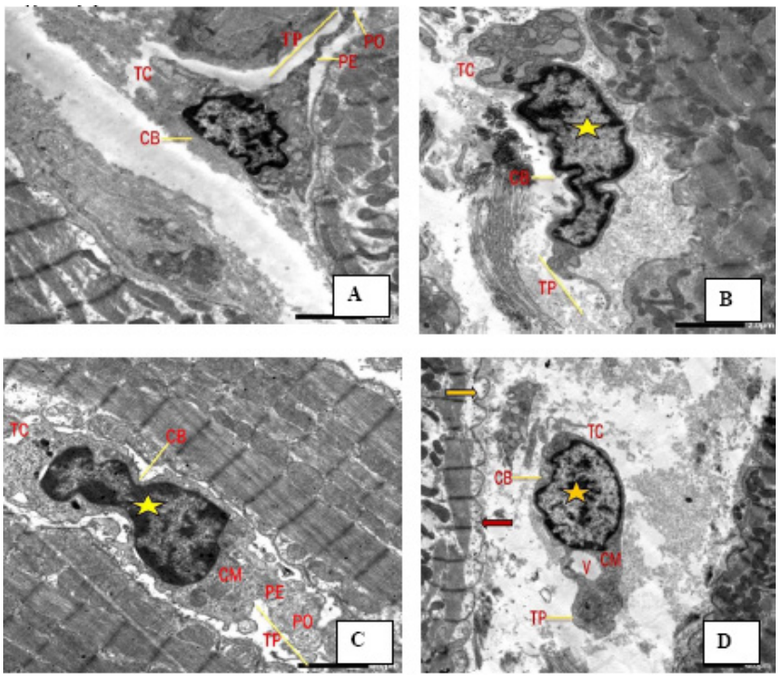


Fig. 4: Telocytes (TEM) X 3000 An ultra-thin section from left ventricle of adult male albino rat showing: In subgroup IA, telocyte (TC) with triangular cell body (CB) and long telopode (TP) extending from cell body. Long, beaded string appearance of telopode (TP) due to alterations of podomere (PE) and podom (PO) segments (Fig. 4A). Subgroup IB has similar histological results as IA subgroup. Subgroup IIA showing spindle shaped cell body (CB) of telocyte (TC) with large nucleus (star). Short and thick telopode (TP) is clear (Fig. 4B). Subgroup IIB showing telocyte (TC) with spindle shaped cell body (CB) and large nucleus (star) encircled by scanty cytoplasm (CM). Notice podom (PO), podomere (PE) and telopode (TP) (Fig. 4C). Subgroup IIC showing the piriform cell body (CB) of telocyte (TC) containing large nucleus (star) surrounded by scanty cytoplasm (CM). Short and thick telopode (TP) is seen. In addition, large vacuole (V) is clear. Notice the sarcolemma (red arrow) and the subsarcolemmal space (yellow arrow) (Fig. 4D).

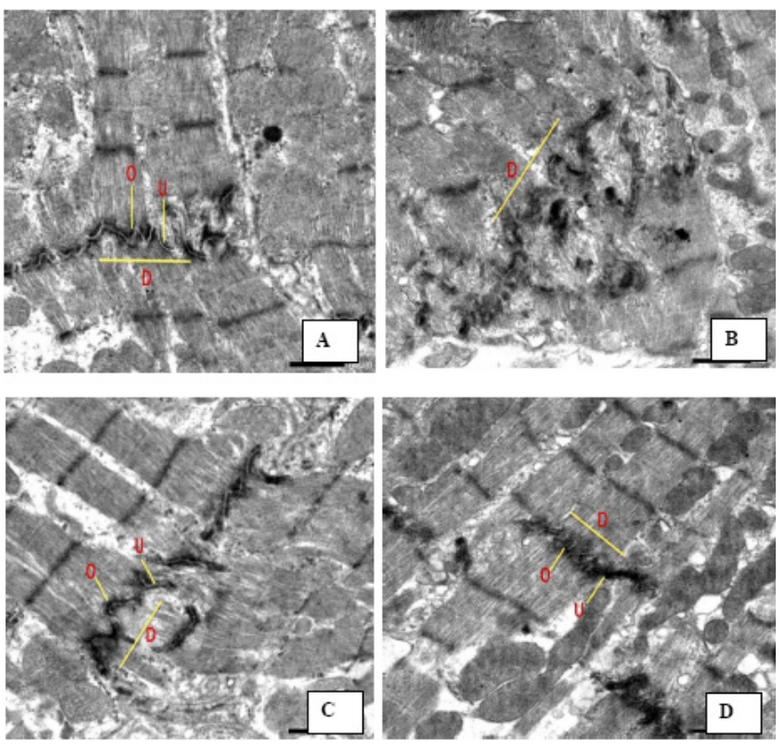
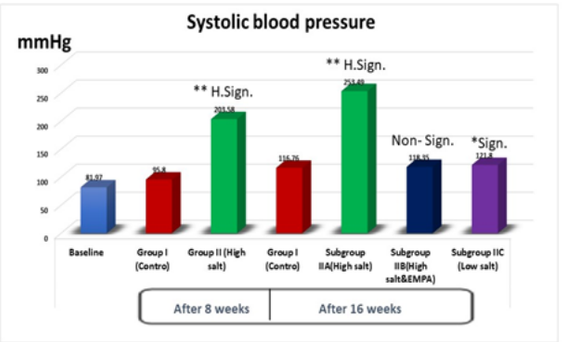
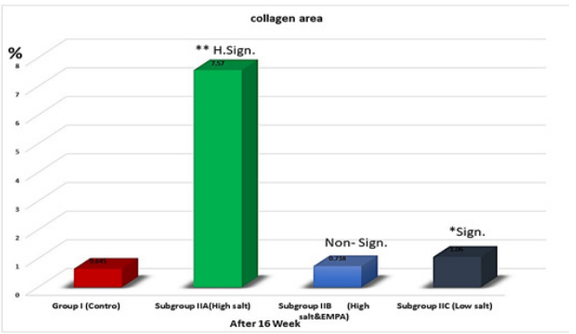


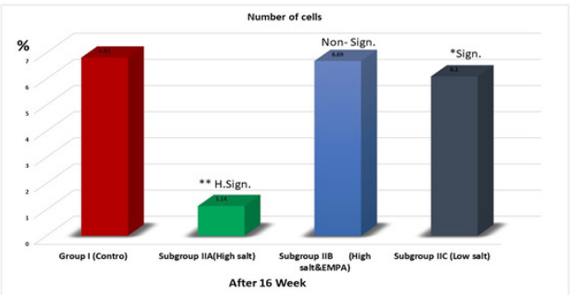
Fig. 5: Intercalated disk (TEM) X 5000 An ultra-thin section from left ventricle of adult male albino rat showing: A ladder step intercalated disc (D) in subgroup IA. It has transverse portions where desmosomes (O) are abundant as well as longitudinal portions rich in gap junctions (U) (Fig. 5A). Subgroup IB has similar histological results as IA subgroup. In subgroup IIA, convoluted and distorted intercalated disk (D) with ill-defined transverse and longitudinal portions (Fig. 5B).In subgroup IIB, ladder step intercalated disk (D) . It has transverse portions where desmosomes (O) are abundant as well as longitudinal portions rich in gap junctions (U) (Fig. 5C).In subgroup IIC, ladder step intercalated disk (D) with desmosomes (O) and gap junction (U) (Fig. 5D).



Histogram 1: Rat tail systolic blood pressure (SBP, mmHg) throughout the experiment in different groups



Histogram 2: Mean percentage (%) area of collagen fibers deposition after 16 weeks in different groups



Histogram 3: Mean percentage (%) of CD34 expression after 16 weeks in different groups.
P value were calculated and interrupted as follows:
Non-significant *P value* > 0.05
* Significant *P value* ≤ 0.05
** Highly significant *P value* ≤0.001

DISCUSSION

Hypertension (HTN) is a common disorder that affects ~25% of the adult population. HTN has negative effects on blood dynamics and target organs such as the heart, brain and kidney[27]. A high salt intake raises blood pressure and causes left ventricular hypertrophy and HF. Reduction in salt intake decreases blood pressure, cardiovascular morbidity and mortality[28].

SGLT2i are new oral anti-hyperglycemic drugs[12]. It was noticed that usage of SGLT2i was related to reduction in the elevated blood pressure and body weight in type 2 diabetic patients[29]. Moreover, they reduce cardiovascular complications and HF hospitalizations as SGLT2i can restore cardiac mitochondrial dysfunction[30,31]. EMPA is a highly selective SGLT2i which reduces hyperglycemia and has a potential cardiovascular protective effect as reported by Santos-Gallego *et al*.[31]. EMPA in hypertensive patients causes a decrease in both SBP and DBP[32]. In contrast, Kojima *et al*.[33] noticed that in type 2 diabetic rat model, chronic treatment with luseogliflozin which is an SGLT2i member normalized blood glucose levels and produced a sustained increase in glucose excretion. However, it hadn't affect blood pressure.

In the current work, after 8 weeks, statistical results of SBP of the rats of group (II) which received high salt diet showed a highly significant increase in comparison to rats of group (I) which received standard diet. In addition, after another 8 weeks, SBP in subgroup IIA (High salt) had a highly significant increase in comparison with the control group (I). These results previously revealed by Namai-Takahashi *et al*.[34] who demonstrated direct and progressive correlation between sodium intake and BP values. There was a consistent dose-response relation between salt intake and BP. Excessive sodium consumption produces an elevation in BP and has been related to onset of hypertension and cardiovascular complications. Hogas *et al*.[35] reported that high sodium intake for a long-term causes reduction the number of skin capillaries which would be the first step to develop hypertension.

Several mediators are involved in inducing hypertension with high sodium intake as angiotensin II which causes electrolyte balance regulation. Angiotensin II leads to vasoconstriction and blood pressure elevation through angiotensin type1 (AT1) receptors[36].

The current study showed statistical improvements of SBP measurements in subgroup IIB with no significant difference when compared to those of the control group (I). The current results are consistent with Chung *et al*.[37] and Fouqueh *et al*.[38] who demonstrated EMPA role in lowering blood pressure (SBP and DBP). The lowering effect of EMPA on BP even in diabetic and non-diabetic humans were similar between the 10 mg and 25 mg doses. The mechanisms of BP modulation observed with EMPA involve several pathways such as weight loss, natriuresis, urinary glucose excretion, osmotic diuresis and decreased arterial stiffness.

In this work, statistical improvement of SBP values in subgroup IIC with significant increase to those of the control group (I) were recorded. Current results are similar to Marketou *et al*.[36] who proved that the decrease in sodium intake for 4 or more weeks results in a significant reduction in BP, hypertension incidence and cardiovascular morbidity and mortality.

On the other hand, Patel and Joseph[39] reported that sodium-restricted diet has been related to activation of antidiuretic and anti-natriuretic systems in HF patients.

Moreover, there was higher level of plasma renin, aldosterone, adrenaline, noradrenaline, triglyceride and cholesterol in groups with low sodium intake. This increase in hormones cause activation of the pro-inflammatory and pro-oxidant genes within endothelial cells that further develop congestive symptoms and cardiorenal dysfunction.

In this study, sections stained with Picrosirius Red of the rats of subgroup IIA showed extensive reddish coloration of connective tissue collagen fibers infiltration between the yellowish cardiac muscle cells and the surrounding blood vessels. This was confirmed statistically by a significant increase in the percentage area of fibrosis (reddish color of collagen) in this subgroup in comparison with group I (control). Previous results were consistent with Hayakawa *et al*.[40] who demonstrated that diet with HS significantly increased the development of perivascular fibrosis and myocardial interstitial fibrosis.

The study of Khalil *et al*.[41] was agreed with current results which stated that pro-inflammatory cytokines like TGF-1 and its downstream effector Smad3 are involved in the combined and multistep mechanism of extracellular protein deposition, which is the cornerstone of fibrosis.

Picrosirius Red stained sections of subgroup IIB revealed restriction of reddish coloration of connective tissue collagen around the blood vessels and between cardiac muscle cells very close to the control group. These findings were proved statistically by a non-significant difference in the percentage portion of fibrosis in this subgroup when compared with group I (control). These results agree with Habibi *et al*.[42] who reported that significant reduction in myocardial interstitial fibrosis with EMPA treatment detected by picrosirius red staining which is an indication of collagen I and III deposition in the interstitium. Kang *et al*.[43] showed that EMPA improves ventricular remodelling by attenuating collagen deposition and fibrosis by lowering TGF-1 levels and the protein Smad3.

Sections stained with Picrosirius Red of subgroup IIC revealed a slight increase of the reddish coloration of the connective tissue collagen around the blood vessels and between the surrounding cardiac muscle cells compared to the control group. The current results were proved statistically by a significant difference in the percentage portion of fibrosis (reddish color of collagen) in this subgroup in comparison with control group. These results are in parallel with Berger *et al*.[44] who reported that low-sodium diets show a reduction in hypertension. The heart shows an increased contractility which may partially compensate for a larger exertion to sustain cardiac output. Rhee & Jeong.[45] proved that dietary salt restriction attenuates myocardial fibrosis. Hogas *et al*.[35] reported that restricting salt intake lowers BP, levels of B-type natriuretic peptide, aldosterone, plasma renin activity and oxidative stress.

In this work, CD34 immune-stained sections of subgroup IIA revealed negative reaction of CD34 immune-stain in some areas between cardiac muscle cells. However, in other areas decrease in brown CD34-positive telocytes were obvious. In addition, statistical results showed a highly significant difference in CD34 expression in this subgroup as compared to control one. The previous results are consistent with Galrinho *et al*.[14] who demonstrated that decreased number of TCs in the heart was noticed in different cardiac pathologies as HF due to dilated cardiomyopathy.

Bei *et al*.[46] and Ibba-Manneschi *et al*.[47] noted that fibrosis which is consequence of many pathological states in human heart is closely related with the number of TCs and their Tps . An increase in fibrillary collagen because of replacement fibrosis is correlated with the reduction of TCs and Tps quantity or even their absence. It is supposed that TCs may have an impact on fibrosis due to direct contacts and paracrine signalization between them and fibroblasts or myofibroblasts.

In the present study, ultrathin sections of telocytes, in subgroup IIA (High salt diet) showed spindle shaped cell bodies of telocyte containing large nucleus and surrounded by scanty cytoplasm. Short and thick telopodes were seen. These findings agree with Galrinho *et al*.[14] and Kucybala *et al*.[15] who proved that cardiac TCs of HF patients showed critical ultrastructural changes, as cytoplasmic vacuolization, absence of the labyrinthine components with inflammatory or ischaemic micro-environment rich in COX-2, lipid peroxide and shrinkage and shortening of telopodes.

In this study, multiple and brown CD34-positive telocytes between cardiac muscle cells apparently close to the control group were found in subgroup IIB. Moreover, statistically a non-significant difference in CD34 expression in this subgroup as compared to control group was recorded. In subgroup IIC, CD34 immunostained sections revealed multiple and brown CD34-positive telocytes between cardiac muscle cells apparently less than the control group. Furthermore, statistically, there was a significant difference in CD34 expression in subgroup IIC as compared to the control group. In addition, in subgroup IIB, ultrathin sections of telocytes revealed spindle shaped cell body of telocyte with podom and podomere segments that cause string beaded appearance of telopode. Moreover, in subgroup IIC, piriform shaped cell bodies of TC with large nucleus surrounded by scanty cytoplasm were clear. Short and thick telopodes and large vacuole were demonstrated. These findings are in parallel with Zhao *et al*.[13] and Galrinho *et al*.[14] who revealed that in the first days after induced myocardial infarction in rats, TCs were reduced in ischemic and infarcted zone, but after thirty days their number significant increase. After two weeks, the cell density increased in the non-ischemic areas. TCs play a role in neo-angiogenesis process following myocardial infarction either directly through physical nano-contacts with capillaries, or indirectly through microcrine production of pro-angiogenic microRNAs and by paracrine production of nitric oxide synthase 2 (NOS2) and vascular endothelial growth factor (VEGF).

In the present work, ultrathin sections of left ventricle of subgroup IIA showed irregular pattern and fragmented myofibril. Loss of uniform thickness of myofibrils was present. Nuclei with indented and irregular nuclear membrane, distorted and depleted mitochondria leaving rarefaction areas were noticed. Clusters of abnormal shape and size of mitochondria with dense matrix between myofibrils, central mitochondrial effacement indicating degeneration were found. Wavy and irregular sarcolemma with widened sub-sarcolemma space was found. Distorted intercalated disks with ill-defined parts were seen. Previous findings are consistent with Frisoli *et al*.[48] and Abas&Sabry[49] who reported altered organization of the myofibrils with abnormal and convoluted intercalated discs, hypertrophied cells with highly convoluted nuclear membranes due to increased transcription in parallel with the increased protein synthesis in hypertrophied cells, swollen mitochondria with fragmented cristae. In addition, salt intake causes hypertrophic affects the left ventricle as it sensitizes the heart to the hypertrophic stimulus of pressure load. Mechanisms for salt-dependent hypertension include volume expansion, renal disorders, impaired reaction of the renin-angiotensin-aldosterone-system, central excitation of the sympathetic nervous system and also inflammatory processes[50,51].

Ultrathin sections of the left ventricle muscle of subgroup IIB (High salt diet & EMPA) showed regular and parallel myofibrils with rows of mitochondria in between close to control group surrounding large and oval nuclei. Moreover, numerous mitochondria with clear cristae in juxtanuclear zone and between cardiac muscle fibers were seen. Lace-like appearance of sarcoplasmic reticulum scalloped and regular sarcolemma with abundant mitochondria in subsarcolemmal space, ladder step intercalated disk close to the control group were noticed. It had transverse parts where desmosomes were abundant and longitudinal parts with a lot of gap junctions. These observations agreed with Habibi *et al*.[42] who reported improvements in cardiac structure, appearance of sarcomeres, cardiomyocyte hypertrophy and ultrastructure of inter myofibrillar mitochondria by EMPA treatment. Yurista *et al*.[29] revealed that EMPA normalizes the mitochondria size and number, increases myocardial ATP in diabetic and non-diabetic failing hearts. Natriuresis gained by EMPA reduces sodium overload in cardiomyocytes through the suppression of the sarcolemmal sodium–hydrogen exchanger. Increased levels of intracellular sodium are believed to compromise mitochondrial calcium handling and contribute to mitochondrial dysfunction in HF thus partially reflect the decrease in intracellular sodium. Ultrathin sections of left ventricle muscle of subgroup IIC revealed regular arranged cardiac myofibril containing a central nucleus close to control group in some areas but thin and fragmented myofibril in other areas were seen. However, regular nuclear membranes and numerous mitochondria with clear cristae and ladder step intercalated disks were seen. These results are in parallel with Cashman *et al*.[52] and Xue *et al*.[53] who proved that one of the best strategies for lowering the burden of CVD has been shown to be salt reduction and improving the structure and functions of the heart. Many mechanisms involved in lowering BP, such as moderate salt restriction reduces volume load, inhibits the production of reactive oxygen species, reduces the activity of nitric oxide and improves peripheral vascular resistance which have cardiovascular benefits. Other possible mechanisms are that salt restriction inhibits inflammatory cell infiltration and decreases the production of inflammatory factors.

CONCLUSION

There is an important correlation between hypertension and high salt intake. In addition, high salt doses in diet alter the histological structure of the left ventricle of the heart. This study also revealed that EMPA treatment and salt restriction have beneficial effects on blood pressure and the histological architecture of the left ventricle of the heart with the highest improvement with EMPA drug. The important role of telocytes in regenerating tissues of the left ventricle of the heart is declared.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Mills, K. T.; Bundy, J. D.; Kelly, T. N.; Reed, J. E.; Kearney, P. M.; Reynolds, K. and He, J. (2016). Global disparities of hypertension prevalence and control: a systematic analysis of population-based studies from 90 countries. Circulation, 134(6): 441-450. https://doi.org/10.1161/CIRCULATIONAHA.115.018912
2. Whelton, P. K.; Carey, R. M.; Aronow, W. S.; Casey, D. E.; Collins, K. J.; Himmelfarb, C. D. and MacLaughlin, E. J. (2018).2017ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and management of high blood pressure in adults: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. Journal of the American College of Cardiology, 71(19): e127-e248. https://doi.org/10.1016/j.jacc.2017.11.006
3. Georgiopoulos, G.; Tsioufis, C.; Tsiachris, D.; Dimitriadis, K.; Liatakis, I.; Kalos, T. and Tousoulis, D. (2015): Metabolic syndrome predicts independently from its component's adverse events in essential hypertensive subjects. Journal of Hypertension, (33): e91. DOI: 10.1097/01.hjh.0000467597.19477.b9
4. Oh, G. C. and Cho, H. J. (2020). Blood pressure and heart failure. Clinical hypertension, 26 (1): 1-8. https://doi.org/10.1186/s40885-019-0132-x
5. Murtaugh, M. A.; Beasley, J. M.; Appel, L. J.; Guenther, P. M.; McFadden, M.; Greene, T. and Tooze, J. A. (2018). Relationship of sodium intake and blood pressure varies with energy intake: secondary analysis of the DASH (Dietary Approaches to Stop Hypertension)–sodium trial. Hypertension, 71 (5): 858-865. https://doi.org/10.1161/HYPERTENSIONAHA.117.10602

6. Schutte, A. E.; Srinivasapura Venkateshmurthy, N.; Mohan, S. and Prabhakaran, D. (2021). Hypertension in low-and middle-income countries. Circulation research, 128 (7): 808-826. https://doi.org/10.1161/CIRCRESAHA.120.318729

7. Cefalu, W. T.; Leiter, L. A.; de Bruin, T. W.; Gause-Nilsson, I.; Sugg, J. and Parikh, S. J. (2015): Dapagliflozin's effects on glycemia and cardiovascular risk factors in high-risk patients with type 2 diabetes: a 24-week, multicenter, randomized, double-blind, placebo-controlled study with a 28-week extension. Diabetes care, 38 (7): 1218-1227. https://doi.org/10.2337/dc14-0315

8. Desouza, C. V.; Gupta, N. and Patel, A. (2015). Cardiometabolic effects of a new class of antidiabetic agents. Clinical therapeutics, 37 (6): 1178-1194. https://doi.org/10.1016/j.clinthera.2015.02.016

9. Shubrook, J. H.; Bokaie, B. B. and Adkins, S. E. (2015). Empagliflozin in the treatment of type 2 diabetes: evidence to date. Drug design, development and therapy, (9): 5793. https://doi.org/10.2147/DDDT.S69926

10. Kim, S.; Jo, C. H. and Kim, G. H. (2019). Effects of empagliflozin on nondiabetic salt-sensitive hypertension in uninephrectomized rats. Hypertension research: official journal of the Japanese Society of Hypertension, 42 (12) :1905–1915. https://doi.org/10.1038/s41440-019-0326-3

11. Shao, Q.; Meng, L.; Lee, S.; Tse, G.; Gong, M.; Zhang, Z. and Liu, T. (2019). Empagliflozin, a sodium glucose co-transporter-2 inhibitor, alleviates atrial remodeling and improves mitochondrial function in high-fat diet/streptozotocin-induced diabetic rats. Cardiovascular diabetology, 18 (1): 1-14. https://doi.org/10.1186/s12933-019-0964-4

12. Tan, Y.; Yu, K.; Liang, L.; Liu, Y.; Song, F.; Ge, Q. and Wang, P. (2021). Sodium–Glucose Co-Transporter 2 Inhibition with Empagliflozin Improves Cardiac Function After Cardiac Arrest in Rats by Enhancing Mitochondrial Energy Metabolism. Frontiers in pharmacology, (12): 758080. https://doi.org/10.3389/fphar.2021.758080

13. Zhao, B.; Chen, S.; Liu, J.; Yuan, Z.; Qi, X.; Qin, J. and Cai, D. (2013). Cardiac telocytes were decreased during myocardial infarction and their therapeutic effects for ischaemic heart in rat. Journal of cellular and molecular medicine, 17(1): 123-133. https://doi.org/10.1111/j.1582-4934.2012.01655.x

14. Galrinho, R. D.; Manole, C. G. and Vinereanu, D. (2016). Telocytes - a Hope for Cardiac Repair after Myocardial Infarction. Maedica, 11(4): 325–329. PMID: 28828051; PMCID: PMC5543526

15. Kucybala, I.; Janas, P.; Ciuk, S.; Cholopiak, W.; Klimek-Piotrowska, W. and Holda, M. K. (2017). A comprehensive guide to telocytes and their great potential in cardiovascular system. Bratislavske lekarske listy, 118(5): 302-309. https://doi.org/10.4149/bll_2017_059

16. Klein, M.; Csöbönyeiová, M.; Žiaran, S.; Danišovič, Ľ. and Varga, I. (2021). Cardiac telocytes 16 years on—What have we learned so far, and how close are we to routine application of the knowledge in cardiovascular regenerative medicine?. International Journal of Molecular Sciences, 22(20): 10942. https://doi.org/10.3390/ijms222010942

17. Park, S. H.; Farooq, M. A.; Gaertner, S.; Bruckert, C.; Qureshi, A. W.; Lee, H. H. and Lessinger, J. M. (2020). Empagliflozin improved systolic blood pressure, endothelial dysfunction and heart remodeling in the metabolic syndrome ZSF1 rat. Cardiovascular Diabetology, 19(1): 1-14.https://doi.org/10.1186/s12933-020-00997-7

18. National academy press (1995): Nutrient requirements of laboratory animals.Ch.I (general consideration for feeding and diet formulation). 4th edition. Washington, D.C. PP:3-9

19. Wang, Y.; Mu, J. J.; Liu, F. Q.; Ren, K. Y.; Xiao, H. Y.; Yang, Z. and Yuan, Z. Y. (2014). Salt-induced epithelial-to-mesenchymal transition in Dahl salt-sensitive rats is dependent on elevated blood pressure. Brazilian Journal of Medical and Biological Research, 47(3): 223-230. https://doi.org/10.1590/1414-431X20133554

20. Ding, Y.; Wang, Y.; Jia, Q.; Wang, X.; Lu, Y.; Zhang, A. and Zhang, J. (2020). Morphological and functional characteristics of animal models of myocardial fibrosis induced by pressure overload. International Journal of Hypertension, 2020. https://doi.org/10.1155/2020/3014693

21. Walkowska, A.; Kuczeriszka, M.; Sadowski, J.; Olszynski, K. H.; Dobrowolski, L.; Červenka, L. and Kompanowska-Jezierska, E. (2015). High salt intake increases blood pressure in normal rats: putative role of 20-HETE and no evidence on changes in renal vascular reactivity. Kidney and Blood Pressure Research, 40(3): 323-334. https://doi.org/10.1159/000368508

22. Ullian, M. E.; Islam, M. M.; Robinson, C. J.; Fitzgibbon, W. R.;Tobin, E. T. and Paul, R. V. (1997). Resistance to mineralocorticoids in Wistar-Furth rats. American Journal of Physiology-Heart and Circulatory Physiology, 272(3): H1454-H1461. https://doi.org/10.1152/ajpheart.1997.272.3.H1454

23. Al Mwafy, M. E.; El-Sawaf, M. E. S.; Salem, M. F. and Naguib, Y. M. (2020). Effect of different diets on left ventricle and area postrema in adult male albino rats predisposed to prolonged intake of high salt. Tanta Medical Journal, 48(1): 28. DOI: 10.4103/tmj.tmj_1_19.

24. Suvarna, K. S.; Layton, C. and Bancroft, J. D. (Eds.). (2018). Bancroft's theory and practice of histological techniques E-Book. Elsevier Health Sciences 8th ed. Philadelphia: Churchil Living Stone; 2018: 78–80.

25. Kuo, J. (2007): Electron microscopy: Methods and protocols. Acta Biochimica Polonica, 56(1): 195-197.

26. Drozdz, D. and Kawecka-Jaszcz, K. (2014). Cardiovascular changes during chronic hypertensive states. Pediatric Nephrology, 29(9): 1507-1516. https://doi.org/10.1007/s00467-013-2614-5

27. Soliman, E. Z.; Ambrosius, W. T.; Cushman, W. C.; Zhang, Z. M.; Bates, J. T.; Neyra, J. A. and Lewis, C. E. (2017). Effect of intensive blood pressure lowering on left ventricular hypertrophy in patients with hypertension: SPRINT (Systolic Blood Pressure Intervention Trial). Circulation, 136(5): 440-450. https://doi.org/10.1161/CIRCULATIONAHA.117.028441

28. Loutradis, C.; Papadopoulou, E.; Theodorakopoulou, M.; Karagiannis, A. and Sarafidis, P. (2019). The effect of SGLT-2 inhibitors on blood pressure: a pleiotropic action favoring cardio-and nephroprotection. Future medicinal chemistry, 11(11): 1285-1303. https://doi.org/10.4155/fmc-2018-0514

29. Yurista, S. R.; Silljé, H. H.; Oberdorf-Maass, S. U.; Schouten, E. M.; Pavez Giani, M. G.; Hillebrands, J. L. and Westenbrink, B. D. (2019). Sodium–glucose co-transporter 2 inhibition with empagliflozin improves cardiac function in non-diabetic rats with left ventricular dysfunction after myocardial infarction. European journal of heart failure, 21 (7): 862-873. https://doi.org/10.1002/ejhf.1473

30. Williams, D. M.; Nawaz, A. and Evans, M. (2021). Sodium-Glucose Co-Transporter 2 (SGLT2) Inhibitors: Are They All the Same? A Narrative Review of Cardiovascular Outcome Trials. Diabetes Therapy, 12(1): 55-70. https://doi.org/10.1007/s13300-020-00951-6

31. Santos-Gallego, C. G.; Requena-Ibanez, J. A.; San Antonio, R.; Ishikawa, K.; Watanabe, S.; Picatoste, B. and Badimon, J. J. (2019). Empagliflozin ameliorates adverse left ventricular remodeling in nondiabetic heart failure by enhancing myocardial energetics. Journal of the American College of Cardiology, 73(15): 1931-1944. https://doi.org/10.1016/j.jacc.2019.01.056

32. Ferreira, J. P.; Fitchett, D.; Ofstad, A. P.; Kraus, B. J.; Wanner, C.; Zwiener, I. and Zannad, F. (2020). Empagliflozin for patients with presumed resistant hypertension: a post hoc analysis of the EMPA-REG OUTCOME trial. American journal of hypertension, 33(12): 1092-1101. https://doi.org/10.1093/ajh/hpaa073

33. Kojima, N.; Williams, J. M.; Takahashi, T.; Miyata, N. and Roman, R. J. (2013). Effects of a new SGLT2 inhibitor, luseogliflozin, on diabetic nephropathy in T2DN rats. Journal of Pharmacology and Experimental Therapeutics, 345(3): 464-472. DOI: https://doi.org/10.1124/jpet.113.203869

34. Namai-Takahashi, A.; Sakuyama, A.; Nakamura, T.; Miura, T.; Takahashi, J.; Kurosawa, R. and Ito, O. (2019). Xanthine Oxidase Inhibitor, Febuxostat Ameliorates the High Salt Intake–Induced Cardiac Hypertrophy and Fibrosis in Dahl Salt-Sensitive Rats. American journal of hypertension, 32(1): 26-33. https://doi.org/10.1093/ajh/hpy143

35. Hogas, M.; Statescu, C.; Padurariu, M.; Ciobica, A.; Bilha, S. C.; Haisan, A. and Hogas, S. (2022). Salt, Not Always a Cardiovascular Enemy? A Mini-Review and Modern Perspective. Medicina, 58(9): 1175. https://doi.org/10.3390/medicina58091175

36. Marketou, M. E.; Maragkoudakis, S.; Anastasiou, I.; Nakou, H.; Plataki, M.; Vardas, P. E. and Parthenakis, F. I. (2019). Salt-induced effects on microvascular function: A critical factor in hypertension mediated organ damage. The Journal of Clinical Hypertension, 21(6): 749-757. https://doi.org/10.1111/jch.13535

37. Chung, S.; Kim, S.; Son, M.; Kim, M.; Koh, E. S.; Shin, S. J. and Kim, H. S. (2019). Empagliflozin contributes to polyuria via regulation of sodium transporters and water channels in diabetic rat kidneys. Frontiers in physiology, (10): 271. https://doi.org/10.3389/fphys.2019.00271

38. Fouqueh, C.E.; A.Aly Labib, D.; Selim, M.S. and Awad, H.A. (2020). Potential Effects of Empagliflozin in L-NAME- Induced Hypertensive Nephropathy in Albino Rats. Biomedical and Pharmacology Journal, (13): 399-416. https://dx.doi.org/10.13005/bpj/1900

39. Patel, Y. and Joseph, J. (2020). Sodium Intake and Heart Failure. International journal of molecular sciences, 21(24): 9474. https://doi.org/10.3390/ijms21249474

40. Hayakawa, Y.; Aoyama, T.; Yokoyama, C.; Okamoto, C.; Komaki, H.; Minatoguchi, S. and Minatoguchi, S. (2015). High salt intake damages the heart through activation of cardiac (pro) renin receptors even at an early stage of hypertension. PloS one, 10(3): e0120453. https://doi.org/10.1371/journal.pone.0120453

41. Khalil, H.; Kanisicak, O.; Prasad, V.; Correll, R. N.; Fu, X.; Schips, T. and Molkentin, J. D. (2017). Fibroblast-specific TGF-β–Smad2/3 signaling underlies cardiac fibrosis. The Journal of clinical investigation, 127(10), 3770-3783. https://doi.org/10.1172/JCI94753

42. Habibi, J.; Aroor, A. R.; Sowers, J. R.; Jia, G.; Hayden, M. R.; Garro, M. and DeMarco, V. G. (2017). Sodium glucose transporter 2 (SGLT2) inhibition with empagliflozin improves cardiac diastolic function in a female rodent model of diabetes. Cardiovascular diabetology, 16(1) :1-15. https://doi.org/10.1186/s12933-016-0489-z

43. Kang, S.; Verma, S.; Hassanabad, A. F.; Teng, G.; Belke, D. D.; Dundas, J. A. and Fedak, P. W. (2020). Direct effects of empagliflozin on extracellular matrix remodelling in human cardiac myofibroblasts: novel translational clues to explain EMPA-REG OUTCOME results. Canadian Journal of Cardiology, 36(4), 543-553. https://doi.org/10.1016/j.cjca.2019.08.033

44. Berger, R. C. M.; Benetti, A.; Girardi, A. C. C.; Forechi, L.; de Oliveira, R. M.; Vassallo, P. F. and Mill, J. G. (2018). Influence of Long-Term Salt Diets on Cardiac Ca2+ Handling and Contractility Proteins in Hypertensive Rats. American journal of hypertension, 31(6):726-734. https://doi.org/10.1093/ajh/hpy023

45. Rhee, M. Y. and Jeong, Y. J. (2020). Sodium intake, blood pressure and cardiovascular disease. Korean circulation journal, 50(7): 555-571. https://doi.org/10.4070/kcj.2020.0042

46. Bei, Y.; Zhou, Q.; Sun, Q. and Xiao, J. (2016). Telocytes in cardiac regeneration and repair. In Seminars in cell & developmental biology, Academic Press, (55): 14-21. https://doi.org/10.1016/j.semcdb.2016.01.037

47. Ibba-Manneschi, L.; Rosa, I. andManetti, M. (2016). Telocyte implications in human pathology: An overview. In Seminars in Cell & Developmental Biology. Academic Press, (55): 62-69. https://doi.org/10.1016/j.semcdb.2016.01.022

48. Frisoli, T. M.; Schmieder, R. E.; Grodzicki, T. and Messerli, F. H. (2012). Salt and hypertension: is salt dietary reduction worth the effort?. The American journal of medicine, 125(5): 433-439. https://doi.org/10.1016/j.amjmed.2011.10.023

49. Abas, E., & Sabry, M. M. (2020). Intermittent fasting attenuates apoptosis, modulates autophagy and preserves telocytes in doxorubicin induced cardiotoxicity in albino rats: A Histological Study. Egyptian Journal of Histology, 43(3), 663-683. https://dx.doi.org/10.21608/ejh.2020.22181.1230

50. Soliman, M., Zakaria, M. M. A., Nadim, H. S., El-Fakharany, W. A. H., Shokry, M. M., & Sadek, A. S. (2024). Platelet-Rich Plasma Attenuates Isoproterenol-Induced Myocardial Injury in Adult Male Albino Rat: Histological and Immunohistochemical Study. Egyptian Journal of Histology, 47(2), 617-631. https://dx.doi.org/10.21608/ejh.2023.200537.1874

51. Rust, P. and Ekmekcioglu, C. (2016). Impact of salt intake on the pathogenesis and treatment of hypertension. Hypertension: from basic research to clinical practice, 61-84.

52. Cashman, K. D.; Kenny, S.; Kerry, J. P.; Leenhardt, F. and Arendt, E. K. (2019). 'Low-Salt'bread as an important component of a pragmatic reduced-salt diet for lowering blood pressure in adults with elevated blood pressure. Nutrients, 11 (8): 1725. https://doi.org/10.3390/nu1108172

53. Xue, Y.; Wen, Q.; Xu, C.; Zhang, X.; Zeng, J.; Sha, A. M. and Zeng, C. (2020). Elevated salt taste threshold is associated with increased risk of coronary heart disease. Journal of cardiovascular translational research, 13(6): 1016-1023. https://doi.org/10.1007/s12265-020-10017-4

الملخص العربى

دراسة هستولوجية لآثار علاج الامباجليفلوزين على عضله القلب في ذكور جرذان الويستر البيضاء البالغة المستحث لها ارتفاع ضغط الدم

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مقدمه البحث: ان ضغط الدم المرتفع مرض شائع. تم تسجيل العلاقة بين ارتفاع الملح الغذائي العالي وضغط الدم المرتفع. يؤدي انخفاض ملح الطعام لدى مرضى ضغط الدم المرتفع إلى بعض التحسينات في ضغط الدم وأنسجة القلب. ان مثبطات جلوكوز الصوديوم٢ هي أحدث الأدوية المضادة لمرض السكر. إمباجليفلوزين هو أحد هذه المثبطات وهو عامل فعال للغاية في تقليل السكر المرتفع في الدم ويساعد في خفض ضغط الدم المرتفع لدى مرضى السكري وايضا غير المصابين بالسكري.

الهدف من البحث: الهدف هو دراسه لآثار علاج الامباجليفلوزين على عضله القلب في ذكور جرذان ويستر البيضاء البالغة االمستحث لها ارتفاع ضغط الدم ودور التيلوسيت في تجديد الخلايا.

المواد والطرق: تم استخدام خمسون ذكر بالغ من الجرذان البيضاء في هذه الدراسة. تم تصنيفها على النحو التالي: المجموعة الأولى (الضابطة) تضمنت عشرون فأرًا ومقسمة إلى مجموعتين فرعيتين ١أ (نظام غذائي متوازن) و١ب (نظام غذائي متوازن و امباجليفلوزين) والمجموعة الثانية (مستحث لها ارتفاع في ضغط الدم) تضمنت ثلاثون فأرًا وتلقت نظامًا غذائيًا عالي الملح لمدة ٨ أسابيع ثم تم تصنيفها على أنها مجموعة فرعية ٢أ (غذاء عالى الملح)، ٢ب (غذاء عالي الملح والامباجليفلوزين) و ٢ج (غذاء قليل الملح) لمدة٨ أسابيع أخرى. تم قياس ضغط الدم الانقباضي عند خط الأساس، بعد ٨ أسابيع وبعد ١٦ أسبوعًا من بداية التجربة. تم إخضاع عينات من البطين الأيسر للقلب للفحص النسيجي والكيميائي المناعي. تم تحليل بيانات ضغط الدم الانقباضي وعدد الخلايا التيلوسيتية ونسبة مساحة الكولاجين إحصائياً.

النتائج: أظهرت المجموعة الفرعية ٢أ بنية نسيجية مضطربة لعضله القلب، ولكن في المجموعتين الفرعيتين ٢ب و٢ج لوحظ تحسن فى البنية النسيجية لعضلة القلب. كان ضغط الدم الانقباضي و واالنسبة المئوية للتليف مرتفعين في المجموعة الفرعية ٢أ لكنهما تحسنا في المجموعات الفرعية ٢ب و٢ج. كانت عدد الخلايا التيلوسيتية عالية في المجموعات الفرعية ٢ب و٢ج مع انخفاض ملحوظ في المجموعة الفرعية ٢أ.

خلاصة البحث: تم إثبات العلاقة بين تناول غذاء عالى الملح وضغط الدم المرتفع. الجرعات الغذائية العالية من الملح لها آثار ضارة على عضله القلب. كشفت هذه الدراسة أيضًا أن علاج الامباجليفلوزين وتقليل الملح يحسن هذه الآثار السيئة.