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Abstract

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Keywords

dapagliflozin, eplerenone, heart failure, aldosterone, neurohormonal change.

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RESEARCH ARTICLE

The Impact of Dapagliflozin on Aldosterone Hormone in Rats with Heart Failure

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ABSTRACT

Background: The latest anti-diabetic medication sodium-glucose co-transporter inhibitor along with the lowering of blood glucose has a cardioprotective and reno-protective effect, it has the ability to reduce the development of heart failure and decrease hospitalization in heart failure subjects with and without diabetes. This study was conducted to evaluate the effect of sodium-glucose co-transporter inhibitor dapagliflozin alone and in combination with eplerenone on the aldosterone hormone in the treatment of rats with experimentally induced heart failure.

Method: Thirty Wister rats were randomly divided into five groups each of six rats, the first group served as a control group. The heart failure model was induced experimentally by intraperitoneal injection of isoprenaline 5mg/kg/day for one week for the rest of the experimental rat groups. The second group was a positive control. The third, fourth, and fifth groups received oral daily doses of dapagliflozin 10 mg/kg/day, eplerenone 100 mg/kg/day, and dapagliflozin-eplerenone 10,100 mg/kg/day respectively for four weeks.

Results: Induction of heart failure in rats has significantly raised circulating BNP, NT-Pro BNP, aldosterone, troponin I, serum urea, and creatinine. Rats treated with dapagliflozin showed statistically significant decreases in BNP, NT-Pro BNP, aldosterone, troponin I, blood urea nitrogen, and serum creatinine. Non-significant changes are seen in decreasing mean blood pressure. Dapagliflozin-eplerenone administration produced a significant reduction in plasma aldosterone level, heart rate, and diastolic blood pressure.

Conclusion: The study demonstrates the cardiovascular benefit of sodium-glucose co-transporter inhibitor dapagliflozin in rats with experimentally induced heart failure, reducing the myocardial stretch indicates the prominent role of dapagliflozin in reducing the development of heart failure and decreasing cardiovascular complications in subjects with and without diabetes. Moreover, the impact of dapagliflozin on renal function further contributes to cardiovascular benefits by reducing volume overload and neurohormonal activation which are features of cardiorenal syndrome.

Keywords: dapagliflozin, eplerenone, heart failure, aldosterone, neurohormonal change.

INTRODUCTION

Heart failure (HF) is a clinical syndrome characterized by a reduction in the ability of the cardiac muscle to provide efficient blood for metabolic needs, which are leading to elevation of pulmonary and systematic venous pressure due to cardiac-related, resulting in organ congestion, and this will be associated with typical symptoms such as shortness of breath and ankle swelling (Lilly, 2012).

Identification of HF pathophysiology is essential for finding a good treatment option, including the activated sympathetic nervous system, neurohormonal changes, renin-angiotensin-aldosterone system, natriuretic peptide, and antidiuretic hormone all are the leading causes of vasoconstriction, elevating blood volume for inducing ventricular filling. in the early stage, these compensatory mechanisms increase cardiac output, while in the later phase result in a structural and functional defect in the heart (Hartupee and Mann, 2017). Triggering the renin-angiotensin-aldosterone system (RAAS) will result in increasing the level of renin, angiotensin II and aldosterone. Angiotensin II (Ag II) is a potent blood vessel constrictor mainly on the renal efferent arteriole, it stimulates the sympathetic efferent arteriole for releasing noradrenaline, inhibiting vagal tone, in addition to encouraging aldosterone release. Aldosterone hormone increases water-sodium reabsorption and enhances potassium excretion. These are resulting in sodium water retention, and induce potassium excretion (Choi *et al*., 2019).

An increasing stretch of the cardiac wall leads to the release of an endogenous hormone, a natriuretic peptide which is released in response to volume or pressure overload. There are three main types of natriuretic peptides; first atrial natriuretic peptide (ANP) which is released from the atrium as a result of increased volume

overload, second brain natriuretic peptide (BNP), which is mainly released from the ventricle due to increased preload (filling pressure) while the third type, C-natriuretic peptide (CNP) is predominately found in the central nervous system (Das and Solinger, 2009).

Aldosterone hormone has a critical role in the pathophysiology of cardiovascular disease. in advanced-stage HF, the Aldosterone antagonist shows a beneficial effect, since increased aldosterone levels in HF cause sodium retention, electrolyte imbalance, endothelial dysfunction, and fibrosis of cardiac muscle. Accordingly, selective mineralocorticoid receptor antagonist eplerenone and non-selective spironolactone show a reduction in mortality and hospitalization with a significantly lowering sudden cardiac death (SCD) (Ramani *et al*., 2010).

The antihyperglycemic agent, sodium-glucose co-transporter inhibitor (SGCT2) despite lowering the blood glucose, has a beneficial cardiovascular outcome. It can reduce the development of HF and decrease hospitalization in HF subjects with and without diabetes. In addition to the favorable effect on body weight, lipid profile, blood pressure, arterial stiffness, and endothelial function. (Tentolouris *et al*., 2019).

The cardioprotective effects of SGLT2 inhibitors may be related to several mechanisms including improvement in the metabolism of the cardiac cell, inhibition of myocardial cell Na/ H exchange, increasing sodium-water excretion, osmotic diuresis, they reduce the plasma volume and preload, lowering blood pressure, arterial stiffness, and afterload which is believed to improve the blood flow to the subendocardial area in subject with HF. In addition to changing adipokine and cytokine production and decreasing cardiac cell necrosis and fibrosis (Lytvyn *et al.*, 2017).This study aimed to evaluate the effect of sodium-glucose co-transporter inhibitor dapagliflozin alone and in combination with eplerenone on the aldosterone hormone in treating rats with experimentally induced HF.

2. Material and Method

2.1 Animals

Thirty female Wistar albino rats weighing (180-200) grams with an age range of (8-12) weeks old were involved in the study. The animals were purchased from an animal laboratory house for experimental animals (Iraq, Erbil). Rats were kept in special cages in the animal house of the college of pharmacy, Hawler Medical University (Iraq, Erbil). The animal room with a controlled ambient temperature of 25 ± 2 ℃ was programmed on 12/12 hours of light-dark cycles. They were having free access to water and a standard rat food pallet.

2.2 Materials

Serum NT-proBNP, Brain natriuretic peptide (BNP), and aldosterone enzyme-linked immunosorbent assay (ELISA) rat kits were purchased from Sun Red biotechnology company (China). Isoprenaline HCl pure powder was purchased from Glentham Life Sciences (UK), Dapagliflozin 10 mg tablet, and Eplerenone 25 mg tablet were purchased in a verified and licensed pharmacy manufactured by AstraZeneca (British Swedish) and Pfizer (USA) respectively.

2.3 Induction of myocardial injury

Isoprenaline was dissolved in distilled water and injected intraperitoneally into rats (5mg/kg) once daily for seven consecutive days to induce an experimental heart failure (Dizaye and Ali, 2019).

2.4 Experimental protocol

Thirty female Wister Albino rats were randomly divided into five groups each of six rats.

Group I: was served as a normal control group and they were injected with physiological saline for seven days and then received a placebo for four weeks.

Group II: served as a positive control in which heart failure was induced experimentally by intraperitoneal injection of 5 mg/kg/day isoprenaline for seven days, then rats were administered placebo through the oral gavage for four weeks.

Group III, IV, and V have induced heart failure through isoprenaline injection using the above-mentioned method. Then each group received medication treatment as follows:

Group III: received an oral daily dose of Dapagliflozin 10 mg/kg for four weeks (Bahriz *et al.*, 2021).

Group IV: received an oral daily dose of Eplerenone 100mg/kg for four weeks ((Dizaye and Mustafa, 2019).

Group V: received the oral combination of Dapagliflozin 10 mg tablet with Eplerenone 25 mg tablet for four weeks.Medication administration was performed through oral gavage by dissolving the drug in distill water and calculating the exact required dose. Measuring Blood pressure and heart rate was obtained from the tail of the rat by using a noninvasive coda monitoring system. Blood pressure, heart rate, and body weight were recorded on the 1st day of the study in all rat groups, on the 8th day of the experiment after HF induction, and on

the last day three hours after the final dose of the drug, for the whole group. During oral drug administration, the body weight is recorded weekly.

On the 30th day of drug administration, all rats were anesthetized by injecting xylazine 10 mg/kg and ketamine 125 mg/kg intraperitoneally (Konecny, 2021). The blood sample was obtained through the cardiac puncture technique. The blood specimen was left to be coagulated and then centrifuged, and the separated serum was kept in tubes for various tests. Serum samples were coded with random numbers and after all samples have been assessed, identification for each sample was made.

Since the rats were anesthetized at the end of the experiment, they were euthanized through cervical dislocation. For the confirmation of death, heartbeat and pupillary response to light was assessed.

2.5 Biochemical assay : Biochemical assays were obtained by measuring plasma NT-proBNP, BNP, and aldosterone using (ELISA) rat kits specific for each parameter. In addition to measuring plasma troponin I. Serum creatinine and blood urea nitrogen were measured using specific reagents and channels for each mentioned parameter by using Cobas-6000 (Roche).

2.6 statistical analysis Graph pad prism version 9.0.0 was applied for performing data analysis. The difference in all parameters of the control and medication-treated rats group were analyzed by one-way analysis of variance ANOVA test. Tukey's multiple comparison test was applied to compare the groups individually. All data were expressed as mean and standard error, the graphs were presented as bar charts. The standard curve of Elisa tests was prepared through interpolation, linear and non-linear curve fit according to the nature of the ELISA test. P< 0.05 was set as significant.

3. Results

3.1 Effect of dapagliflozin, eplerenone, and dapagliflozin- eplerenone combination on a biochemical assay

The observed data showed the plasma concentration of aldosterone in the isoprenaline received group was significantly increased (2930 ± 498.9 pg/ml) compared to the normal control group (1873 ± 64.59 pg/ml) (figure 1C). Compared to the HF-induced group, the combination of dapagliflozin- eplerenone showed a vast reduction in the level of aldosterone (885.1 ± 47.08 pg/ml), whereas rats treated with dapagliflozin and eplerenone decreased aldosterone hormone by (1347 ± 131.8 pg/ml), (1016 ± 106.18 pg/ml) respectively. The decrement was considered significant in all treatment groups ($P < 0.05$).

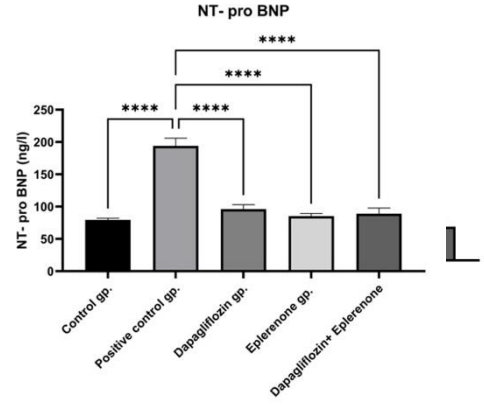
Results addressed in (figure 1D) showed that the plasma level of BNP in the heart failure-induced group (group II) was statistically higher (100 ± 3.31ng/l) than in the control group (31.23 ± 3.81ng/l). The BNP level was reduced by all treatment groups and the reduction was statistically significant ($P < 0.05$) in comparison to positive heart failure group II, the group that received dapagliflozin treatment significantly lowered the BNP to (46.86 ± 9.24 ng/l), whereas rats treated with eplerenone decreased the raised number of BNP into (48.61 ± 5.20 ng/l), in addition to (57.28 ± 0.87 ng/l) reduction in BNP level by a combination of dapagliflozin and eplerenone. However, none of the treatment groups were able to return the BNP level back to normal.

Similarly, the positive HF group showed NT- pro-BNP increased significantly in comparison to the control group, each treatment group including dapagliflozin, eplerenone, and dapagliflozin- eplerenone combination significantly decreased the circulating NT- pro-BNP and the highest reduction observed with eplerenone received group as appear in (figure 1B).

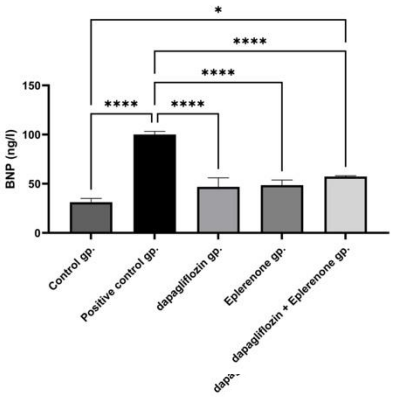
The intraperitoneal injection of isoprenaline in group II rats raised troponin levels significantly (906 ± 198.4 pg/ml) when compared to group I (353.5 ± 60.61ng/ml) ($P < 0.05$). As shown in (figure 1 A), the raised troponin level was extremely decreased by dapagliflozin (233.8 ± 30.71 ng/ml) which was significant statistically ($P < 0.05$). Moreover, the eplerenone group and combination of dapagliflozin-eplerenone lowered plasma troponin significantly to (394 ± 58.92 ng/ml), and (315.8 ± 73.29 ng/ml) respectively.

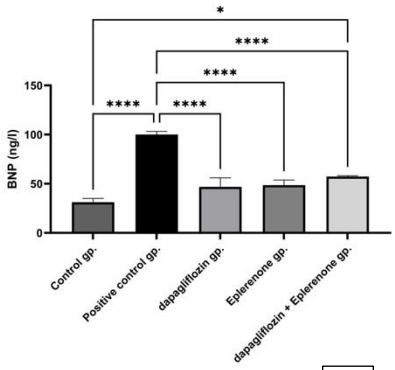
A

B



C





D

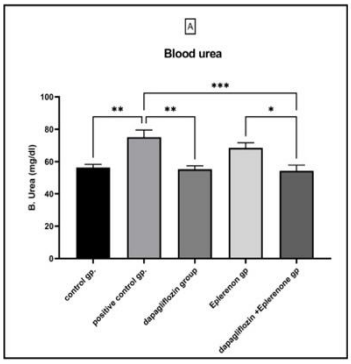
Fig. 1. Effect of dapagliflozin, eplerenone, dapagliflozin-eplerenone combination on BNP, NT- pro BNP, aldosterone, troponin.

* p-value < 0.05, ** p value < 0.01, *** p value < 0.001, **** p-value < 0.0001

3.2 Effect of dapagliflozin, eplerenone, and dapagliflozin-eplerenone combination on renal function

Effects of different treatment groups on renal function particularly blood urea nitrogen (BUN) and serum creatinine were obtained in this study, isoprenaline pretreated rats have raised the level of BUN over the control group, this increment statistically was significant ($P < 0.05$). The result (figure 2. A) explained that BUN was significantly reduced in all treatment groups, among the treatment groups dapagliflozin-eplerenone combination presented the highest reduction (54.25 ± 3.56 mg/dL) compared to group II (75.12 ± 4.41 mg/dL).

Serum creatinine level was significantly increased in rats injected with a high dose of isoprenaline (0.68 ± 0.009 mg/dL) when compared to the control (0.41± 0.01 mg/dL) as shown in (figure 2 B). All treatment groups presented a decrease in the level of serum creatinine, the greater reduction was achieved through dapagliflozin (0.38 ± 0.01 mg/dL), and the reduction was significant statistically when compared to group II ($P < 0.05$).



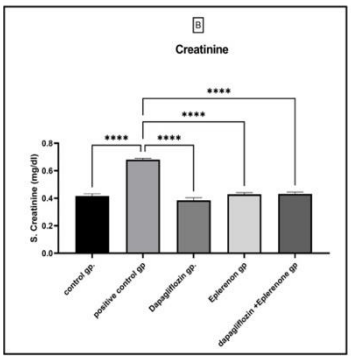


Fig. 2. **Effect of study medication on (A) blood urea nitrogen and (B) serum creatinine.**

3.3 Effect of dapagliflozin, eplerenone, dapagliflozin-eplerenone combination on blood pressure and heart rate

The effect of study medication on mean blood pressure and systolic blood pressure shows no appreciable change over control groups, however group IV, and V shows a significant reduction in diastolic blood pressure when compared to control groups.

Measurement of heart rate during the study assessed that rat's heart rate was significantly increased in isoprenaline induced HF group, in comparison to group I. Whenever, lowering heart rate was statistically significant with each of the treatment groups, heart rate decreased to (340 ± 14 beat/minute) by eplerenone and (355 ± 11 beat/minute) by dapagliflozin when compared to group II (427 ± 13 beat/minute). The combination of dapagliflozin and eplerenone was able to return the elevated heart rate back to normal (316 ± 5 beats/minute).

Table 1. Effect of Dapagliflozin, Eplerenone, Dapagliflozin-Eplerenone on arterial blood pressure and heart rate.

Parameter	Control	Heart failure	Dapagliflozin	Eplerenone	Dapagliflozin-Eplerenone
Systolic Blood pressure mmHg	113.3 ± 2.61	107 ± 2.12	101.7 ± 6.76	108 ± 3.45	105.2 ± 1.16
Diastolic blood pressure mmHg	80.33 ± 1.85	72.17 ± 1.13	70.67 ± 4.08	67 ± 2.98 [a]	67.17 ± 2 [a]
Mean blood pressure mmHg	91.17 ± 2.04	83.67 ± 1.25	80.83 ± 4.92	80.67 ± 2.9	79.83 ± 1.32
Heart rate beat/minute	335 ± 8 [d]	427 ± 13	355 ± 11 [b]	340 ± 14 [d]	316 ± 5 [d]

value are expressed as mean mean ± SEM, [a] p-value < 0.05, [b] p value < 0.01, [c] p value < 0.001, [d] p-value < 0.0001

4. Discussion

The result shown in table 1 clarifies that the intraperitoneal injection of isoprenaline 5mg/kg for 7 days result in cardiac hypertrophy, necrosis of cardiac muscle cells, and inflammatory cell infiltration, this demonstrated physiological and pathological changes which resembles HF in the rats. Stimulating the RAAS system plays an important role in the buildout of hemodynamic alteration and cardiac remodeling. This model has been confirmed to successfully introduce HF in the rats (Nichtova *et al.*, 2012).

Mechanism belongs to the inductive effect of isoprenaline in HF possibly including an impaired balance between oxygen demand and supply by cardiac myocyte, due to a high increase in heart rate and contraction force of cardiac muscle, this is because of non-selective beta-adrenergic agonist (Lobo Filho *et al.*, 2011).
NT pro-BNP and BNP both are metabolic products of pro-BNP, during myocardial stress they are secreted into the blood which renders them to be an important monitoring tool in various cardiomyopathy conditions. The result of the present study shows that both BNP and NT-proBNP significantly increased in rats with heart failure. As shown in (figure 1B, D) dapagliflozin significantly reduced the workload on the ventricular wall to a higher degree than a combination of dapagliflozin-eplerenone in rats with heart failure. To note, both BNP and NT-proBNP were used in this study owing to the longer half-life duration of NT-proBNP (72hr) compared to 4 hr for BNP and the fact that NT-proBNP is not affected by neprilysin inhibitor treatment and or less impacted by other condition like obesity (Inamdar and Inamdar, 2016).

An increase in the level of the aldosterone hormone is expected in heart failure. The plasma concentration of aldosterone hormone is increased in patients with HF as a consequence of neurohormonal changes that are activated in heart failure, which has a negative effect on overall health conditions.
The present study demonstrated that dapagliflozin significantly lowered the increased plasma aldosterone in rats with heart failure. This is probably due to a reduction in the activity of the RAAS system, owing to a decrease in plasma concentration of renin and vasoconstrictor angiotensin II, which in turn decreases the aldosterone level (Ghanim *et al.*, 2021). furthermore, decreasing the sympathetic activity in the kidney further reduces the secretion of aldosterone as the consequent natriuresis effect of dapagliflozin reduces the fluid overload (Sawamura *et al.*, 2020).

The combination of dapagliflozin-eplerenone presented a significant reduction in aldosterone levels. Since eplerenone is selectively blocking mineralocorticoid receptors, hence reducing the plasma activity of aldosterone which in turn inhibits renal epithelial sodium channels' ability to be expressed and moved around by the aldosterone. Consequently, this could lead to a decrement in the neurohormonal activity through the tubuloglomerular feedback (Narasimhan *et al.*, 2021).

circulating cardiac troponin I is the most sensitive indicator among all troponin subtypes, in detecting myocardial infarction. In subjects with the normal physiological action of the heart, the plasma troponin I is very low or near zero. In the present study, troponin was significantly increased after induction of heart failure in groups; II, III, IV, and V. this increment in plasma troponin level is due to myocardial injury, and troponin was released as a consequence of this damage. This finding was in agreement with other studies (Singh *et al.*, 2010). Among significant reductions in plasma troponin by all treatment groups, dapagliflozin demonstrated the greatest reduction, this may be due to decreasing the load on the cardiac wall (Berg *et al.*, 2021).

Despite the cardioprotection effect of SGCT2 inhibitor, dapagliflozin provides a favorable effect in preventing the progression of kidney dysfunction. Inducing HF in rats showed significant elevation of BUN and serum creatinine, this is probably owing to physiological adaptation are not efficient to compensate for a failing heart which leads to a subsequent reduction in overall kidney function. Additionally, the raised BUN and serum creatinine in rats with heart failure is possibly due to decreased renal blood flow, which was significantly decreased by dapagliflozin and eplerenone, this may be due to their diuresis and natriuresis effects. This finding was in agreement with other studies (Rajasekeran *et al.*, 2016).

Furthermore, dapagliflozin reduces the activity of the neprilysin enzyme which is responsible for the degradation of natriuretic peptide, since natriuretic peptide increases sodium excretion excessively and participates in increasing urea and creatinine clearance, this activity may responsible for the attenuation of the increased blood level of urea and creatinine (Sano, 2018).

Adding dapagliflozin to eplerenone will further improve renal function by interfering with the RAAS system, reducing the plasma renin concentration, inhibiting the production of Ag II, and subsequently reducing aldosterone hormone secretion (Isshiki *et al.*, 2020).

Heart rate is significantly increased in rats with heart failure. Increased heart rate and the inability of the heart to pump blood efficiently, are a reflection of increased sympathetic activity in compensatory heart failure. In this study, Heart rate was significantly increased in rats with isoprenaline-induced heart failure, all the treatment groups were able to significantly reduce heart rate, reduction in heart rate by eplerenone may refer to decreasing the catecholamine effect on the cardiac tissue (Chin *et al.*, 2019).

Additionally, the combination of dapagliflozin-eplerenone was able to turn the heart rate back to normal, this may be owing to the reduction of preload on the heart, and reducing sympathetic activity by dapagliflozin will further decrease heart rate, this is assured the cardioprotective effect of SGCT2 inhibitor (Tentolouris *et al.*, 2019).

Along with a reduction in the ability of myocardium in compromised heart function induced by isoprenaline, neprilysin reduced activity by dapagliflozin will stimulate natriuresis, as a result, drop the arterial blood pressure. In this study, the physiological changes triggered by the treatment groups do not show an obvious change in mean blood pressure and systolic blood pressure, which failed to obtain a significant decrement in blood pressure compared to group II. However, the diastolic blood pressure is significantly reduced by eplerenone and a combination of dapagliflozin-eplerenone, which may refer to the diuretic effect of eplerenone (Rossignol *et al.*, 2011).

All the aforementioned parameters indicate the reduction in workload on the heart is achieved through the effect of study treatment. This decrement appears in the form of reduced BNP, NT pro BNP, aldosterone, and troponin I which are released upon myocardial injury, cardiac remodeling, and ventricular wall stress.

5. Conclusion

1. Dapagliflozin has significantly reduced the plasma level of cardiac biomarkers: NT pro-BNP, BNP, aldosterone, and troponin in rats with heart failure, and the result shows the combination of dapagliflozin with eplerenone shows a preference in reducing the aldosterone hormone.

2. impact of dapagliflozin on renal function further contributes to cardiovascular benefit by reducing volume overload, and decreasing neurohormonal activation which are features of cardiorenal syndrome.

3. Medications used in different groups have ameliorated compromised renal function in heart failure, imparted by increasing dropped down the level of serum creatinine and blood urea nitrogen which is clearly seen with dapagliflozin alone and in combination with eplerenone.

4. Eplerenone alone and in combination with dapagliflozin significantly reduced diastolic blood pressure and heart rate.

5. Conclusion

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2. impact of dapagliflozin on renal function further contributes to cardiovascular benefit by reducing volume overload, and decreasing neurohormonal activation which are features of cardiorenal syndrome.

3. Medications used in different groups have ameliorated compromised renal function in heart failure, imparted by increasing dropped down the level of serum creatinine and blood urea nitrogen which is clearly seen with dapagliflozin alone and in combination with eplerenone.

4. Eplerenone alone and in combination with dapagliflozin significantly reduced diastolic blood pressure and heart rate.

5. dapagliflozin alone failed to show a significant reduction in arterial blood pressure. Dapagliflozin produced a non-significant weight reduction, however, a non-increase in rats' weight was observed.

6. Ethical approval

Ethical approval for this work was obtained from the ethics committee of Hawler Medical University, College of pharmacy with approval number 17102021-ECPH HMU-210.
This research work was performed by the Guide for the care and use of laboratory animals provided by the National Academy of Science and published by the National Institute of Health.

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