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Canagliflozin Reduces the Contractile Response of Isolated Heart of Normal Adult Rats

Canagliflozin Reduce la Respuesta Contráctil del Corazón Aislado de Ratas Adultas Normales

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SUMMARY: Sodium-glucose cotransporter 2 inhibitors (SGLT2i) represent a unique class of glucose-declining renal-targeted drugs. The SGLT2i Canagliflozin (CANA) is an anti-hyperglycemic drug that reduces various cardiovascular and renal outcomes in patients with type 2 diabetes mellitus. This study aimed to explore the potential effects of CANA on the isolated healthy adult rat hearts to show if CANA has positive inotropic or cardiac depressant effects via analyzing the amplitude and frequency of cardiac contractions. In isolated normal adult rat hearts, the effects of CANA on cardiac contractility were examined. In a dose-response curve, CANA led to a significant cardiac depressant effect in a dose-dependent manner. This cardiac depressant effect of CANA (10^{-6} M) was not prevented by atropine. However, this cardiac depressant effect was partially antagonized by both Isoproterenol (10^{-5} M) and Calcium chloride (10^{-6} M), suggesting beta-adrenoceptor and calcium channel blocking actions. In addition, the cardiac depressant effect of CANA (10^{-6} M) was mitigated in part by Nitric oxide synthase inhibitor, L-NAME, suggesting that its action probably depends to some extent on the accumulation of nitric oxide, which decreases the rise of intracellular Calcium. Data from this study demonstrate that CANA has a significant cardiac relaxant effect in isolated hearts of healthy adult rats by different possible mechanisms. This inhibitory effect on cardiac contractility may help improve the diastolic ventricular filling providing a therapeutic potential to help the other cardioprotective mechanisms of CANA in the prevention and treatment of heart failure.

KEY WORDS: Canagliflozin; Isoproterenol; L-NAME; Heart contractility; Rats.

INTRODUCTION

The risk of cardiovascular diseases is common among patients with type 2 diabetes mellitus (Whiting *et al*., 2011). The inhibitors of sodium-glucose cotransporter 2 (SGLT2) have safety outcomes on the regulation of blood glucose, blood pressure, body weight, and renal hemodynamics (Vasilakou *et al*., 2013; Cherney *et al*.,2014).They lower the hazard of severe cardiovascular and renal complications and eventually mortality (Wu *et al*., 2016). CANA declines the hospitalization for heart failure and serious renal events in type 2 diabetic patients by 33 % and 40 %, respectively (Neal *et al*., 2017; Sayour *et al*., 2019). Interestingly, in non-diabetic male rats, acute CANA administration may protect against *in vivo* myocardial ischemia-reperfusion damage supporting the idea that CANA may have a direct cardiovascular protective role independent of its anti-hyperglycemic impact (Sayour *et al*., 2019). The anti-diabetic

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action of CANA by blockade of glucose reabsorption in the renal proximal tubules does not completely explain its cardiovascular protective role (Reddy & Inzucchi, 2016; Zelniker & Braunwald, 2018). Despite all suggested mechanisms of the cardio-protective actions of CANA against heart failure in T2DM patients, no studies have been done to elucidate the direct impacts of CANA on the cardiac contractility of isolated normal rat hearts independent of its anti-hyperglycemic action. Here, we examine the effects of CANA on the contractility of isolated hearts of healthy adult rats and to what extent.

MATERIAL AND METHOD

Experimental animals and protocol. A total of 36 adult male Wistar rats, aged 6-8 weeks and weighing 250-300 gm, were used in this experiment. They were collected from Zagazig University's veterinary medical faculty's animal house. The rats were kept in animal housing circumstances for two weeks prior to the trials. The animals were housed in sanitary conditions in steel wire cages at the animal house at Zagazig University's faculty of medicine. The animals were provided a chow feed and had unlimited access to water. They were kept at room temperature with a 12-hour light/dark cycle.The hearts of rats were removed and assigned into 6 groups (6 isolated heart/group). CANA group (for the study of the effect of CANA alone), isoproterenol group (for the study of the effect of isoproterenol alone), CANA+ isoproterenol group (to investigate the concomitant effect of CANA and b-agonist isoproterenol) , calcium chloride ($CaCl_2$) group (for the study of the effect of CaCl2 alone), CANA+$CaCl_2$ group (to study the impact of CANA application on the $CaCl_2$ induced contractions of isolated hearts), and CANA+L-NAME group (L-NAME, a nitric oxide synthase (NOS) inhibitor, was used to investigate the influence of CANA on the relaxing response). In the assigned groups, the amplitude and frequency of cardiac contractility were evaluated.

Isolated heart preparations. Before sacrifice, animals were anesthetized with ethyl ether. Then, rapid thoracotomy was performed to remove the hearts after dissecting the great vessels, which placed in Krebs Ringer bicarbonate solution with the following composition (NaCl 6.895 mg/L, KCl 0.350 mg/L, $CaCl_2$ 0.280 mg/L, NaH_2PO_4 0.160 mg/L, $MgSO_4$ 0.290 mg/L, $NaHCO_3$ 2.1 mg/L, Glucose 2.1 mg/L). The solution was equilibrated with a mixture of 95 % O_2 and 5 % CO_2 to a pH of 7.4. In the laboratory, the hearts were rapidly cleaned from lung tissue and pericardium with dissection of muscles, which placed into organ baths (Williams *et al*., 1981). The preparation was squeezed several times to remove as much blood as possible. The heart was allowed to stabilize for 5 minutes before any manipulation (Li *et al*., 2000). An isotonic lever was attached to the ventricular apex and used to record the developed tension (amplitude of contraction) on the power lap connected to a computer. Cardiac performance was assessed by recording Amplitude of contraction (mm) and Frequency (beat/minute) before and after any drug given.

Chemicals and Reagents. Canagliflozin, isoproterenol, L-NAME were provided by sigma-Aldrich (St.louis, MO, USA). El-Gomhouria Co. for drugs and medical supplies (Egypt) provided the physiologic buffer solution chemicals. All stock solutions were prepared and kept in accordance with the manufacturer's guidelines.

Functional study on the cardiac contractile response. The efficacy of CANA on cardiac contractile response was assesed through a series of established *in vitro* tests. The impact of CANA alone on spontaneous basal contractions of isolated normal adult rat hearts was explored in a dose-dependent manner. Various concentrations of CANA (10^{-12}, 10^{-9}, 10^{-6}, 10^{-2}, and 10^{-3} M) were applied. The strips were flushed with the buffer solution after each dosage was administered for 10 min, and the recovery of contractions was recorded. CANA concentration of 10^{-6} M was considered the IC50 used throughout the study.

The influence of CANA (10^{-6} M) on the isoproterenol-induced cardiac contractions was studied. A curve for dose-response to isoproterenol (10^{-9} to 10^{-3} M) was accomplished. The amplitude and the frequency of spontaneous cardiac contractions were enhanced by adding isoproterenol to the isolated rat heart in the bath. The isoproterenol (10^{-5} M) was used as a control dose at the beginning of each experiment. The cardiac preparation was incubated first with CANA (10^{-6} M) followed by isoproterenol (10^{-5} M) to assess this effect cardiac contractions. Moreover, the CANA application on top of isoproterenol-evoked cardiac contraction was also investigated.

In addition, the consequence of CANA application on the $CaCl_2$-induced contractions of isolated rat hearts was evaluated. We assessed the contractile responses of heart preparations to different doses of calcium (from 10^{-9} M to 10^{-5} M) alone. The impact of calcium (10^{-6} M) on CANA (10^{-6} M) pre-treated heart preparations was also examined.

The influence of CANA (10^{-6} M) on the amplitude and frequency of cardiac contractility of isolated hearts pre-treated with different doses of L-NAME (NOS inhibitor) (10^{-12}, 10^{-9}, 10^{-6}, and 10^{-3} M) was investigated to evaluate the engagement of nitric oxide synthase (NOS) in the CANA-induced relaxing response on the isolated rat hearts.

Transmission electron microscopy (TEM) study. Left ventricle segments were collected for morphological examination of the tissue collected from all study groups. Tissues were preserved in phosphate buffered 3 % glutaraldehyde (pH 7.4) and 2 % osmium tetroxide followed by dehydration in increasing ethanol concentrations, and submerged in Araldite resin. Ultrathin sections were stained by uranyl acetate saturated in 70 % ethanol and lead citrate, and examined using a JEOL transmission electron microscope (JEM-100 CX, Japan). Moreover, freshly isolated ventricular cardiomyocytes were subjected to 3 mmol/l CANA or vehicle (0.02 % DMSO [vol. /vol.]) for 30 min. The concentration was based on the maximum plasma concentration of the drug found *in vivo* at clinically relevant doses (Scheen, 2014; Uthman *et al*., 2018).

Statistical Analysis. Analysis of collected data was done by Statistical Package for Social Science software computer program version 26 (SPSS, Inc., Chicago, IL, USA. The Shapiro-Wilk test was performed to determine if the data was distributed normally. The mean and standard deviation were used to display quantitative parametric data, whereas the median and interquartile range (IQR) were used to represent quantitative nonparametric data. Student's t-test(paired) was used for comparing two related groups of parametric data, while one-way analysis of variance (ANOVA) followed by post-hoc Tukey provided a comparison of more than two different groups of parametric data. Kruskal Wallis followed by post-hoc Dunn's was used for comparing more than two different groups of non-parametric data. P-value less than 0.05 was considered statistically significant.

Ethical Approval. The research protocol with animal experimentation was approved by ZU-IACUC Committee (Approval number:ZU-IACUC/3/F/43/2021)

RESULTS

Dose-dependent effect of CANA on spontaneous contractions of the isolated rat hearts: The amplitude and frequency of spontaneous cardiac contractions of isolated rat hearts showed a significant suppression induced by different doses of CANA (10^{-12}, 10^{-9}, 10^{-6}, 10^{-2}, and 10^{-3} M), which was proportional to the administrated dose (Fig. 1, Tables I and II). The CANA (10^{-6} M) application significantly lowered the spontaneous cardiac contraction amplitude by 37.55 % ± 8.27 % and the frequency by 11.15 % ± 2.42 %. This cardiac depressant effect of CANA (10^{-6} M) was not prevented by atropine.

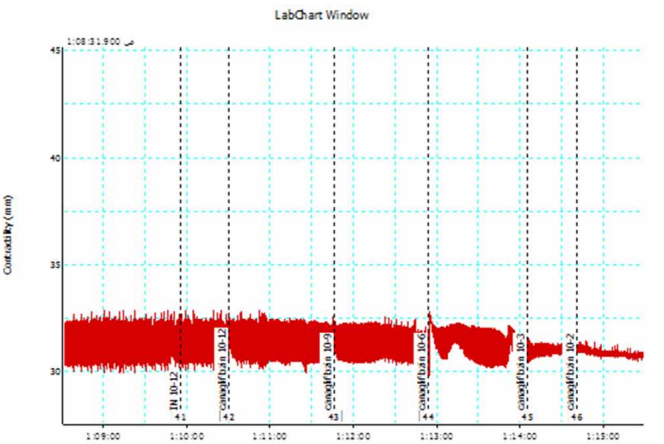

Fig. 1. Dose-dependent effect of canagliflozin on amplitude and frequency of cardiac contractility on the isolated rat hearts.

Table I. Effect of CANA on the amplitude of cardiac contractility on the isolated rat hearts in various doses.

	10^{-12}	10^{-9}	10^{-6}	10^{-3}	10^{-2}	P value
Before	1.10±0.09	1.10±0.09	1.10±0.09	1.10±0.09	1.10±0.09	1.00
After	0.95±.10#	0.88±.08#	0.68±.08ab#	0.45±0.10 abc#	0.45±0.14 abc#	<0.001
Percent of change	-13.16	-19.52	-37.55 %±8.27 % ab	-56.42 %±6.98 % abc	-58.42 %±10.21 % abc	<0.001

Data displayed as mean± SD, P: Probability, *: significance <0.05 . Test used:1- One-way ANOVA followed by *post-hoc* Tukey (a: significance *vs* 10^{-12}, b: significance *vs* 10^{-9}, c: significance *vs* 10^{-6}, d: significance *vs* 10^{-3}). 2- Student's t-test (Paired) (#: significance between after *vs* before)

Table II. Effect of CANA on the frequency of cardiac contractility on rat isolated hearts in different doses.

	10^{-12}	10^{-9}	10^{-6}	10^{-3}	10^{-2}	P value
Before	350.00±35.78	353.33±35.59	346.67±28.05	341.67±27.87	350.00±23.66	0.97
After	338.33±34.30#	340.00±33.47#	308.33±30.61#	275.00±18.71ab#	270.00±20.98ab#	<0.001*
Percent of change	-3.29 %±2.73 %	-3.74 %±1.26 %	-11.15 %±2.42 %ab	-19.35 %±4.20 %abc	-21.80 %±4.19 %abc	<0.001*

Data displayed as mean± SD, *: significance <0.05 . Test used:1- One-way ANOVA followed by *post-hoc* Tukey (a: significance vs 10^{-12}, b: significance vs 10^{-9}, c: significance vs 10^{-6}, d: significance vs 10^{-3}). 2- Student's t-test (Paired) (#: significance between after *vs* before)

The impact of CANA on isoproterenol-induced contractions in isolated rat hearts: The amplitude of spontaneous cardiac contractions increased significantly by 135.42 % and the frequency to 27.33 % after adding isoproterenol (10^{-5} M) to the rat isolated heart in the bath. However, the effect of isoproterenol (10^{-5} M) on both the amplitude and the frequency of pretreated cardiac preparation with CANA (10^{-6} M) was masked (p>0.05). Application of CANA on top of isoproterenol-evoked contraction significantly decreased the amplitude of cardiac contractions by 51.32 % and the frequency by 30.94 %. However, the application of CANA and isoproterenol successively and repeatedly did not affect the amplitude or the frequency of isolated heart (p>0.05) (Fig. 2, Tables III and IV).

Table III. Effect of isoproterenol alone, CANA on top of isoproterenol, isoproterenol following CANA and isoproterenol accompanied by CANA on the frequency of cardiac contractility of the isolated rat heart.

	Isoproterenol	CANA on top of isoproterenol	isoproterenol following CANA	isoproterenol accompanied by CANA	P value
Before	301.67±40.70	380.00±42.43[a]	201.67±14.72[a,b]	233.33±25.82[a,b]	<0.001*
After	380.00±42.43[#]	263.33±39.83[a,#]	206.67±13.66[a,b]	232.33±25.14[a]	<0.001*
Percent of change	27.33 % (21.88 %-28.57 %)	-30.94 % (-34.37 %- -26.32 %)[a]	2.27 % (0 %-5.26 %)[b]	-0.61 % (-0.83 %-0 %)[a]	<0.001*

Data presented as mean± SD & Median (IQR), SD: standard deviation, IQR: interquartile range, *: significance <0.05. Test used: 1- One-way ANOVA followed by *post-hoc* Tukey for data expressed as mean± SD & Kruskal Wallis followed by *post-hoc* Dunn's (a: significance *vs* isoproterenol, b: significance *vs* CANA on top of isoproterenol, c: significance *vs* isoproterenol following CANA). 2- Student's t-test (Paired) (#: significance between after *vs* before)

Table IV. Effect of isoproterenol alone, CANA on top of isoproterenol, isoproterenol following CANA and isoproterenol accompanied by CANA on the amplitude of cardiac contractility of the isolated rat heart.

	Isoproterenol	CANA on top of isoproterenol	isoproterenol following CANA	isoproterenol accompanied by CANA	P value
Before	0.87±0.12	1.95±0.19[a]	0.98±0.25[b]	0.80±0.09[b]	<0.001*
After	1.98±.15[#]	0.98±0.25[a,#]	1.02±0.24[a]	0.85±0.08[a]	<0.001*
Percent of change	135.42 % (120.36 %-137.50 %)	-51.32 % (-52.94 %- -47.62 %)[a]	0 % (0 %-10.00 %)[ab]	6.25 % (0 %-12.50 %)[ab]	<0.001*

Data presented as mean± SD & Median (IQR), SD: standard deviation, IQR: interquartile range, P: Probability, *: significance <0.05. Tests used: 1- One-way ANOVA followed by *post-hoc* tukey for data expressed as mean± SD & Kruskal Wallis followed by *post-hoc* Dunn's (a: significance *vs* isoproterenol, b: significance *vs* CANA on top of isoproterenol, c: significance *vs* isoproterenol following CANA). 2- Student's t-test (Paired) (#: significance between after *vs* before).

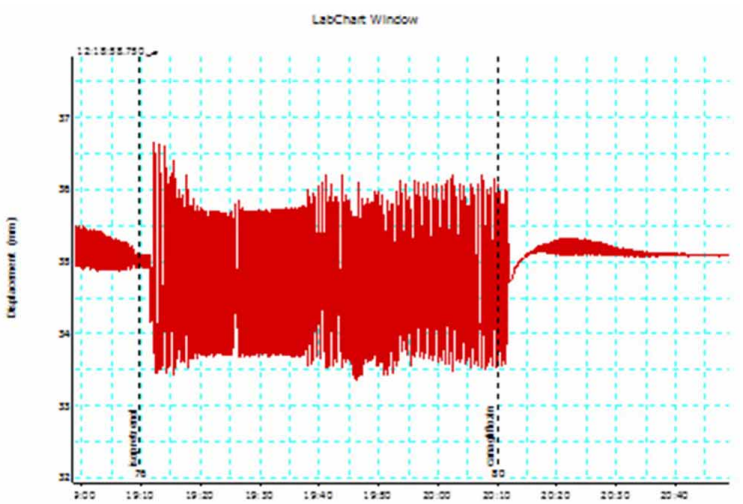

Fig. 2. The concomitant impact of canagliflozin and isoproterenol on amplitude and frequency of cardiac contractility in isolated rat hearts.

The impact of CANA on $CaCl_2$-induced contractions in rat isolated hearts: The amplitude of spontaneous cardiac contractions was significantly increased by 100 % and the frequency by 23.79 % after administration of $CaCl_2$ (10^{-6} M) to the bath. While, the application of CANA (10^{-6} M) on top of $CaCl_2$ (10^{-6} M) reduced the amplitude and the frequency of $CaCl_2$-induced contractions by 20.95 %, and 10.93 % respectively (p>0.05) (Fig. 3, Tables V and VI).

The impact of CANA on the contractility of L-NAME (NOS inhibitor) pre-treated isolated hearts: The concomitant application of CANA (10^{-6} M) with variable doses of L-NAME 10^{-12} M to 10^{-3} M showed a significant decline of the CANA- relaxant effect on cardiac tissue. However, CANA (10^{-6} M) administration after L-NAME (10^{-6} M) showed non-significant effect on the amplitude and the frequency of spontaneous cardiac contractility. (Fig. 4, Tables VII and VIII).

Table V. Effect of $CaCl_2$ alone and CANA on top of $CaCl_2$ on the frequency of cardiac contractility on the isolated rat hearts

	$Cacl_2$	CANA on top of $Cacl_2$	P value
Before	295.00±32.71	365.00±48.48	<0.001*
After	365.00±48.48	324.66±41.09	<0.001*
Percent of change	23.79 % (19.23 %-25.71 %)	-10.93 % (-13.14 %- -9.32 %)	0.002*

Data displayed as mean± SD & Median (IQR), SD: standard deviation, IQR: interquartile range, P: Probability, *: significance <0.05. Test used: Student's t-test (Paired) for data expressed as mean±SD & Mann Whitney for data expressed as Median (IQR)

Table VI. Effect of $CaCl_2$ alone, CANA on top of $CaCl_2$ on the amplitude of cardiac contractility on the isolated rat hearts.

	$Cacl_2$	CANA on top of $Cacl_2$	P value
Before	0.87±.12	1.95±.19	<0.001*
After	1.95±.19	1.54±.18	<0.001*
Percent of change	100.00 % (90.00 %-112.50 %)	-20.95 % (-22.63 %-18.84 %)	<0.001*

Data displayed as mean± SD & Median (IQR), SD: standard deviation, IQR: interquartile range, P: Probability, *: significance <0.05 Test used: Student's t-test (Paired) for data expressed as mean± SD & Mann Whitney for data expressed as Median (IQR)

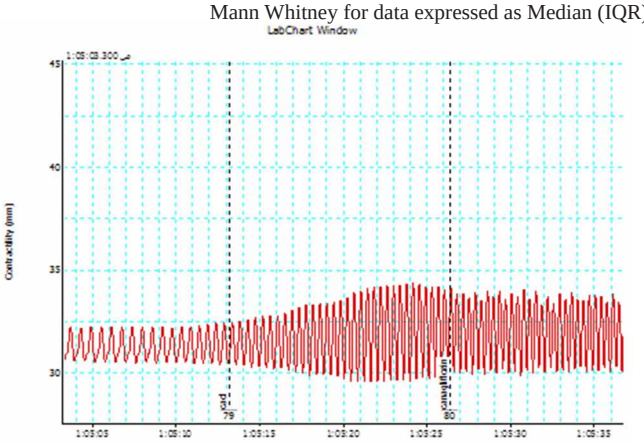

Fig. 3. The impact of canagliflozin on amplitude and frequency of cardiac contractility in the presence of Calcium chloride in isolated rat hearts.

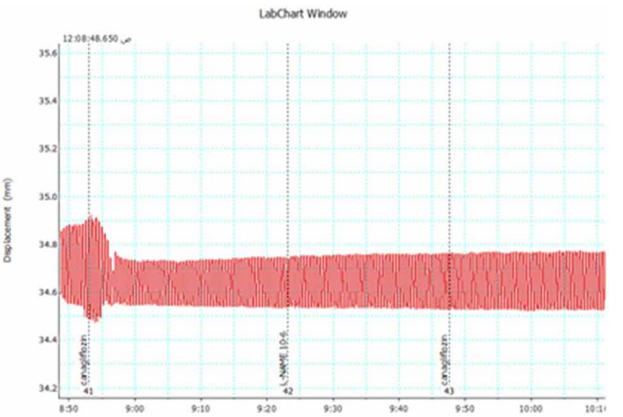

Fig. 4. The impact of canagliflozin on amplitude and frequency of cardiac contractility with L-NAME in isolated rat hearts.

Table VII. Impact of CANA (10-6 M) on the cardiac contractility frequency of the isolated rat hearts in the presence of variable L-NAME doses.

	10^{-12}	10^{-9}	10^{-6}	10^{-3}	P value
Before	320.00±26.08	308.33±24.83	316.67±25.82	306.67±17.51	0.72
After	243.33±30.77#	290.00±28.28 a#	315.17±25.27 a	306.33±17.33 a	0.001*
Percent of change	-23.36 % (-28.12 %- -20.59 %)	-5.12 % (-9.37 % - -3.23 %)	-0.29 % (-0.59 %-0 %) ab	0 % (-0.30 %-0 %) ab	<0.001*

Data displayed as mean± SD & Median (IQR), SD: standard deviation, IQR: interquartile range, P: Probability, *: significance <0.05. Tests used: 1- One-way ANOVA followed by *post-hoc* Tukey for data expressed as mean± SD & Kruskal Wallis followed by post-hoc Dunn's (a: significance vs 10^{-12}, b: significance vs 10^{-9}, c: significance vs 10^{-6}). 2- Student's t-test (Paired) (#: significance between after vs before)

Table VIII. Impact of CANA (10-6 M) on the cardiac contractility amplitude of rat isolated hearts in the presence of variable L-NAME doses.

	10^{-12}	10^{-9}	10^{-6}	10^{-3}	P value
Before	1.10±0.09	1.10±0.09	1.17±0.16	1.10±0.09	0.66
After	0.45±0.10#	0.93±0.08 a#	1.13±0.16 ab	1.07±0.08 a	<0.001*
Percent of change	-57.27 % (-66.67 %- -54.55 %)	-16.67 % (-18.18 %- -10.00 %)	0 % (-7.69 %-0 %) ab	0 % (-8.33 %-0 %) ab	<0.001*

Data displayed as mean± SD & Median (IQR), SD: standard deviation, IQR: interquartile range, *: significance <0.05. Tests used: 1- One-way ANOVA followed by post-hoc Tukey for data expressed as mean± SD & Kruskal Wallis followed by post-hoc Dunn's (a: significance vs 10^{-12}, b: significance vs 10^{-9}, c: significance vs 10^{-6}). 2- Student's t-test (Paired) (#: significance between after vs before).

Transmission electron microscopy (TEM): TEM examination of the left ventricular cardiac myocytes in the control (Fig. 5) revealed normally branched striated myofibrillar structure, and preserved continuity of regularly arranged repetitive sarcomeres with areolar connective tissue in between myofibrils. Additionally, TEM shows normal intercalated disc as delineated by interdigitating desmosome, and gap cell junctions. Moreover, mitochondria were abundant, normally shaped, and interspersed between myofibrils. There was no evidence of sarcomere-specific localization.

TEM examination of cardiomyocytes excised from the left ventricle of CANA-treated rat hearts (Fig. 6) showed intact myofibrils demarcated by their parallel continuous arrangement with branching. A mild swelling of myofibrils was noticed secondary to dilated endoplasmic reticulum. Mitochondria (M) showed increased density and folding between myofibrils, and sarcomeres of the treated hearts showed considerable broadening in various sections, as denoted by the distance between two subsequent Z lines (Z). Widening of the intercalated disc (ID) was also seen.

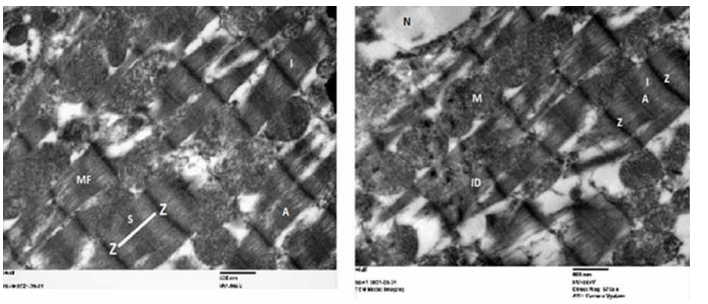

Fig. 5. Ultrastructure observations of Transmission electron microscopy (TEM) of the cardiac myocytes of normal control rat left ventricle showing no ultrastructure abnormalities, normal shape of the nucleus (N), with normal endoplasmic reticulum, the mitochondria (M) are homogenously distributed between myofibrils (MF). Z line (Z) is a series of dark lines, which represent the boundaries of the sarcomere unit (S). I band (I) is pale band across the striated muscle fiber that consists of actin, is situated between two A bands. A band (A): is the region of a striated muscle sarcomere that contains myosin thick filaments. Intercalated disc (ID): Junctions that connect cardiomyocytes together.

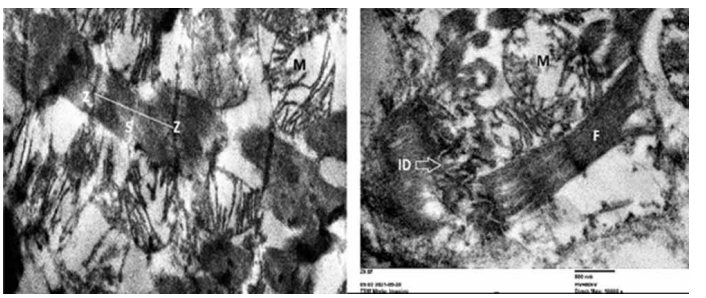

Fig. 6. Ultrastructure observations of Transmission electron microscopy (TEM) of the cardiac myocytes of treated rat left ventricle with CANA showing the sarcomere of the treated heart with significant widening of the distance between the two Z lines (Z) in addition to the presence of mitochondria (M) with increased folds. Widening of intercalated disc (ID, arrow).

DISCUSSION

The SGLT-2inhibitor CANA that was approved for the management of type 2 diabetes mellitus (T2D), has recently been investigated in several studies for the prevention and treatment of heart failure in patients with or without Diabetes Mellitus (Budoff & Wilding, 2017). There are many mechanisms suggested for the cardioprotective effects of CANA, vasodilation, natriuresis, diuresis, improving ventricular loading secondary to decrease in preload, increase cardiac energy supply, inhibiting cardiac sodium/hydrogen (Na^+/H^+) exchanger, that prevent remodeling, and improving diastolic function (Lam *et al*., 2019; Verma, 2019). All these protective effects improve cardiac performance by either increasing cardiac index and fuel availability or decreasing resistance of peripheral blood vessels (Nakagawa & Kuwahara, 2020). Additionally, In preclinical studies, it was reported that CANA activates adenosine monophosphate (AMP)-activated protein kinase (AMPK) *in vitro* (Hawley *et al*., 2016), directly inhibits sodium–hydrogen exchanger (NHE) in non-diabetic healthy hearts (Uthman *et al*., 2018) and has a direct vasodilatory effect under diabetic conditions (Han *et al*., 2015; El-Daly *et al*., 2018).

To the best of our knowledge, despite all these suggested mechanisms to prevent or treat heart failure in diabetic and non-diabetic patients, there were no studies have done to elucidate the direct effect of CANA on cardiac contractility. So we have conducted this work aiming to show if CANA has positive inotropic or cardiac depressant effects on the isolated healthy adult rat hearts. In a dose-response curve, CANA surprisingly led to a significant cardiac depressant effect compared to the normal as shown by the decrease in both amplitude and frequency of cardiac contractions. The submaximal dose of CANA used in all other curves of this work was 10^{-6} M. The cardiac depressant effect of CANA (10^{-6} M) was not prevented by blocking muscarinic or nicotinic receptors. However, the interesting cardiac depressant effects of CANA (10^{-6} M), could be attributed partially to beta-antagonistic actions, since CANA was able to antagonize the stimulant actions of isoproterenol (10^{-5}) on the amplitude and frequency of cardiac contractions. In addition to the beta-blocking

action, CANA seems to have calcium channel blocking action because it could partially antagonize the cardiac stimulant effect of Calcium chloride (10^{-6}), preventing the rise of intracellular calcium levels.

Moreover, the cardiac depressant effect of CANA (10^{-6} M) was partially prevented by Nitric oxide synthase inhibitor, L-NAME (10^{-9} M), which decreases Nitric oxide level in the cardiac cells. The abolished CANA-induced cardiac depression by L-Name suggests that CANA may elicit its depressant effect through the accumulation of NO in the hearts. It has been reported that NO may elicit a biphasic effect on cardiac contractile function with a potentiation at lower NO levels and an attenuation at higher NO levels (Méry *et al*., 1993; Kelly *et al*., 1996). NO was found to prevent Ca^{+2} influx and the rise of intracellular Ca^{+2} (Norby & Ren, 2002) and this means that when CANA when increases intracellular NO, it consequently decreases intracellular Ca^{+2} level. Hence, CANA decreases Ca^{+2} inside cardiac cells by two mechanisms; the first is blocking Ca^{+2} channels, and the second by accumulating NO. The decrease in intracellular Ca^{+2} level was proved also but for different mechanisms in which CANA inhibits Na^{+}/H^{+} exchanger leading to decreased intracellular Na+ and hence Ca^{+2} level (Uthman *et al*., 2018).

This cardiac depressant action may allow CANA to work synergistically with its diuretic, and vasodilator in reducing the overall pre-load, after-load, and energy expenditure in the heart, and all of these mechanisms will help prevention and treatment of heart failure. In addition, this cardiac depressant action may help to understand the CANA-mediated increases in end-diastolic & stroke volumes observed in other studies (Baker *et al*., 2019).

CONCLUSIONS

Data from this study demonstrate that CANA has a significant cardiac depressant effect in isolated hearts of healthy adult rats by different mechanisms including beta-blocking action and prevention of rising of intracellular Ca^{+2} level through Ca^{+2} channel blocking action and accumulation of Nitric oxide inside cardiac cells. Findings from this study highlight the therapeutic potential for CANA to help its other cardiac protective mechanisms in the prevention and treatment of diabetic and non-diabetic patients with heart failure and demonstrate the need for further investigations to both elucidate the mechanisms of cardiac depressant action and to extend this study into *in vivo* experiments to examine if this *in vitro* cardiac depressant effect will occur *in vivo* or not.

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RESUMEN: Los inhibidores del cotransportador de sodio-glucosa 2 (SGLT2i) representan una clase única de fármacos dirigidos a los riñones que disminuyen la glucosa. El SGLT2i Canagliflozin (CANA) es un fármaco antihiperglucémico que reduce varios resultados cardiovasculares y renales en pacientes con diabetes mellitus tipo 2. Este estudio tuvo como objetivo explorar los efectos potenciales de CANA en corazones aislados de ratas adultas sanas para indicar si CANA tiene efectos inotrópicos o depresores cardíacos positivos mediante el análisis de la amplitud y la frecuencia de las contracciones cardíacas. En corazones aislados de ratas adultas normales, se examinaron los efectos de CANA sobre la contractilidad cardíaca. En una curva de dosis-respuesta, CANA condujo a un efecto depresor cardíaco significativo de manera dependiente de la dosis. Este efecto depresor cardíaco de CANA (10^{-6} M) no fue impedido por la atropina. Sin embargo, este efecto depresor cardíaco fue parcialmente antagonizado tanto por el isoproterenol (10^{-5} M) como por el cloruro de calcio (10^{-6} M), lo que sugiere acciones bloqueadoras de los receptores beta adrenérgicos y de los canales de calcio. Además, el efecto depresor cardíaco de CANA (10^{-6} M) fue mitigado en parte por el inhibidor de la sintasa de óxido nítrico, L-NAME, lo que sugiere que su acción probablemente depende en cierta medida de la acumulación de óxido nítrico, lo que disminuye el aumento de calcio intracelular. Los datos de este estudio demuestran que CANA tiene un efecto relajante cardíaco significativo en corazones aislados de ratas adultas sanas por diferentes mecanismos posibles. Este efecto inhibitorio sobre la contractilidad cardíaca puede ayudar a mejorar el llenado ventricular diastólico proporcionando un potencial terapéutico para ayudar a los otros mecanismos cardioprotectores de CANA en la prevención y tratamiento de la insuficiencia cardíaca.

PALABRAS CLAVE: Canagliflozina; Isoproterenol; L-NAME; Contractilidad del corazón; Ratas.

REFERENCES

Baker, H. E.; Kiel, A. M.; Luebbe, S. T.; Simon, B. R.; Earl, C. C.; Regmi, A.; Roell, W. C.; Mather, K. J.; Tune, J. D. & Goodwill, A. G. Inhibition of sodium-glucose cotransporter-2 preserves cardiac function during regional myocardial ischemia independent of alterations in myocardial substrate utilization. *Basic Res. Cardiol., 114(3)*:1-13, 2019.

Budoff, M. J. & Wilding, J. P. Effects of canagliflozin on cardiovascular risk factors in patients with type 2 diabetes mellitus. *Int. J. Clin. Pract., 71(5)*:e12948, 2017.

Cherney, D. Z.; Perkins, B. A.; Soleymanlou, N.; Maione, M.; Lai, V.; Lee, A.; Fagan, N. M.; Woerle, H. J.; Johansen, O. E.; Broedl, U. C.; *et al*. Renal hemodynamic effect of sodium-glucose cotransporter 2 inhibition in patients with type 1 diabetes mellitus. *Circulation, 129(5)*:587-97, 2014.

El-Daly, M.; Venu, V. K. P.; Saifeddine, M.; Mihara, K.; Kang, S.; Fedak, P. W.; Alston, L. A.; Hirota, S. A.; Ding, H.; Triggle, C. R.; *et al*. Hyperglycaemic impairment of PAR2-mediated vasodilation: Prevention by inhibition of aortic endothelial sodium-glucose-co-Transporter-2 and minimizing oxidative stress. *Vascul. Pharmacol., 109*:56-71, 2018.

Han, Y.; Cho, Y. E.; Ayon, R.; Guo, R.; Youssef, K. D.; Pan, M.; Dai, A.; Yuan, J. X. J. & Makino, A. SGLT inhibitors attenuate NO-dependent vascular relaxation in the pulmonary artery but not in the coronary artery. *Am. J. Physiol Lung Cell. Mol. Physiol, 309(9)*:L1027-36, 2015.

Hawley, S. A.; Ford, R. J.; Smith, B. K.; Gowans, G. J.; Mancini, S. J.; Pitt, R. D.; Day, E. A.; Salt, I. P.; Steinberg, G. R. & Hardie, D. G. The Na+/ glucose cotransporter inhibitor canagliflozin activates AMPK by inhibiting mitochondrial function and increasing cellular AMP levels. *Diabetes, 65(9)*:2784-94, 2016.

Kelly, R. A.; Balligand, J. L. & Smith, T. W. Nitric oxide and cardiac function. *Circ. Res., 79(3)*:363-80, 1996.

Lam, C. S. P.; Chandramouli, C.; Ahooja, V. & Verma, S. SGLT-2 inhibitors in heart failure: current management, unmet needs, and therapeutic prospects. *J. Am. Heart Assoc., 8(20)*:e013389, 2019.

Li, H. Y.; Bian, J. S.; Kwan, Y. W. & Wong, T. M. Enhanced responses to 17beta-estradiol in rat hearts treated with isoproterenol: involvement of a cyclic AMP-dependent pathway. *J. Pharmacol. Exp. Ther., 293(2)*:592-8, 2000.

Méry, P. F.; Pavoine, C.; Belhassen, L.; Pecker, F. & Fischmeister, R. Nitric oxide regulates cardiac Ca2+ current. Involvement of cGMP-inhibited and cGMP-stimulated phosphodiesterases through guanylyl cyclase activation. *J. Biol. Chem., 268(35)*:26286-95, 1993.

Nakagawa, Y. & Kuwahara, K. Sodium-Glucose Cotransporter-2 inhibitors are potential therapeutic agents for treatment of non-diabetic heart failure patients. *J. Cardiol., 76(2)*:123-31, 2020.

Neal, B.; Perkovic, V.; Mahaffey, K. W.; De Zeeuw, D.; Fulcher, G.; Erondu, N.; Shaw, W.; Law, G.; Desai, M.; Matthews, D. R.; *et al*. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N. Engl. J. Med., 377(7)*:644-57, 2017.

Norby, F. L. & Ren, J. Anisodamine inhibits cardiac contraction and intracellular Ca(2+) transients in isolated adult rat ventricular myocytes. *Eur. J. Pharmacol., 439(1-3)*:21-5, 2002.

Reddy, R. P. M. & Inzucchi, S. E. SGLT2 inhibitors in the management of type 2 diabetes. *Endocrine, 53(2)*:364-72, 2016.

Sayour, A. A.; Korkmaz-Icöz, S.; Loganathan, S.; Ruppert, M.; Sayour, V. N.; Oláh, A.; Benke, K.; Brune, M.; Benko˝, R.; Horváth, E. M.; *et al*. Acute canagliflozin treatment protects against *in vivo* myocardial ischemia-reperfusion injury in non-diabetic male rats and enhances endothelium-dependent vasorelaxation. *J. Transl. Med., 17(1)*:127, 2019.

Scheen, A. J. Evaluating SGLT2 inhibitors for type 2 diabetes: pharmacokinetic and toxicological considerations. *Expert Opin. Drug Metab. Toxicol., 10(5)*:647-63, 2014.

Uthman, L.; Baartscheer, A.; Bleijlevens, B.; Schumacher, C. A.; Fiolet, J. W. T.; Koeman, A.; Jancev, M.; Hollmann, M. W.; Weber, N. C.; Coronel, R.; *et al*. Class effects of SGLT2 inhibitors in mouse cardiomyocytes and hearts: inhibition of Na +/H + exchanger, lowering of cytosolic Na + and vasodilation. *Diabetologia, 61(3)*:722-6, 2018.

Vasilakou, D.; Karagiannis, T.; Athanasiadou, E.; Mainou, M.; Liakos, A.; Bekiari, E.; Sarigianni, M.; Matthews, D. R. & Tsapas, A. Sodium-glucose cotransporter 2 inhibitors for type 2 diabetes: a systematic review and meta-analysis. *Ann. Intern. Med., 159(4)*:262-74, 2013.

Verma, S. Potential mechanisms of sodium-glucose co-transporter 2 inhibitor-related cardiovascular benefits. *Am. J. Cardiol., 124 Suppl. 1*:S36-S44, 2019.

Whiting, D. R.; Guariguata, L.; Weil, C. & Shaw, J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Res. Clin. Pract., 94(3)*:311-21, 2011.

Williams, R. S.; Dukes, D. F. & Lefkowitz, R. J. Subtype specificity of alpha-adrenergic receptors in rat heart. *J. Cardiovasc. Pharmacol., 3(3)*:522-31, 1981.

Wu, J. H. Y.; Foote, C.; Blomster, J.; Toyama, T.; Perkovic, V.; Sundström, J. & Neal, B. Effects of sodium-glucose cotransporter-2 inhibitors on cardiovascular events, death, and major safety outcomes in adults with type 2 diabetes: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol, 4(5)*:411-9, 2016.

Zelniker, T. A. & Braunwald, E. Cardiac and renal effects of sodium-glucose co-transporter 2 inhibitors in diabetes: JACC state-of-the-art review. *J. Am. Coll. Cardiol., 72(15)*:1845-55, 2018.

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