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Research Article

Combination of Empagliflozin and Liraglutide protects heart against isoproterenol-induced myocardial infarction in rats

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Abstract

Cardiovascular benefit of new anti-hyperglycemic agent such as glucagon like peptide-1 receptor agonist (GLP-1RA) or sodium glucose co-transporter-2 inhibitor (SGLT2i) has been proven, with the proposed-mechanism that might be complementary. We investigated the effects of its combination on blood glucose profile and cardiac biomarkers. The rats were given lipid emulsion for 2 weeks, followed by a single dose of streptozotocin (STZ) 35 mg/kg BW, then treated with empagliflozin and/ liraglutide for 30 days while receiving isoproterenol (ISO) 85 mg/kg on day 29 and 30. The results showed no superior improvement on fasting blood glucose (FBG) and insulin sensitivity (KITT) in the combination group compared to empagliflozin/liraglutide group. However, the combination group showed a higher inhibition in almost all biomarkers, specifically against the elevation of CK-MB compared to one of these agents alone. The histopathological examination using H&E staining even showed a minimal inflammation and gap between cardiomyocytes. These findings may indicate the combination of empagliflozin and liraglutide has a better cardiac protection effect.

Keywords

diabetic cardiomyopathy, GLP1-RA, isoproterenol, myocardial infarction, SGLT2i, type 2 DM

Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia resulting from defects of insulin secretion, function, or both (American Diabetes Association 2010). Based on International Diabetes Federation (2021), T2DM was the type with the most prevalence with more than 90% of world's diabetes cases. Chronic high blood glucose levels often lead to several macro- and microvascular complications in people with diabetes (Skyler et al. 2017). Cardiovascular disease (CVD) is strongly related with DM as the primary cause of its morbidity and mortality due to the increase of stroke and myocardial infarction. This CVD death was also increase along with the other cardiovascular risk factor that often presence in diabetes such as hypertension, obesity, and dyslipidemia (Leon and Maddox 2015). Myocardial infarction mortality in T2DM was higher than people without diabetes. The mortality rates were 15,4% (without prior MI) and 42,0% (with prior MI), while in people without T2DM the mortality rates were 2,1% (without prior MI) and 15,9% (with prior MI) (Haffner et al. 1998). The Framingham Study even suggested that people with diabetes had a higher incidence of reinfarction and heart failure (Kannel et al. 1974), which might be caused by diabetic cardiomyopathy, an abnormal myocardial structure and performance even in the absence of other cardiovascular risk factors such as hypertension, coronary artery disease, and significant valvular disease (Jia et al. 2018).

According to guidelines by American Diabetes Association (ADA), patients with T2DM and established or in high risk of ASCVD are recommended to use one of SGLT2is or GLP-1RAs that have been demonstrated to have cardiovascular benefit, independent of HbA1c and metformin use (ADA 2022). European Society of Cardiology/European Association for The Study of Diabetes (ESC/EASD) guidelines even recommend the use of one of these glucose-lowering classes in newly diagnoses T2DM patient who has established or in high CVD risk as a starting regimen before metformin (Cosentino et al. 2020).

Although the cardiovascular and kidney benefits of both classes are well-established, the molecular mechanism of this benefits are still being elucidated, and whether the combination will provide added risk reduction remains questioned. ADA guidelines only recommend the combination of both classes if the HbA1c above target, although the addition of one of this class after another for patient with or in high risk of ASCVD may be considered because of its proposed mechanism that may provide complementary outcomes (Cheng 2021; ADA 2022). Both classes also have independent effect of risk, thus the concomitant uses are well-tollerated (Cosentino et al. 2020; Lam et al. 2022). Several completed and on-going mechanistic trials to know how these classes reduced cardiovascular outcomes suggested that SGLT2i focusing on hemodynamics, kidney, vascular, and myocardial energetics, while GLP-1RA focusing on metabolic, anti-inflammatory, and possible direct vascular, cardiac, and kidney effects (Lee et al. 2020; Cheng 2021). These different yet complementary mechanisms might be the reason on how some studies suggested other beneficial effect of the combination. In RCT trials, some of GLP-1RAs have shown an additive glucose-lowering benefit in patient treated with SGLT2i (Ludvik et al. 2018; Zinman et al. 2019; Blonde et al. 2020; Das et al. 2020). The combination also demonstrated greater reductions in blood pressure and body weight than to either agent alone (Frias et al. 2016; Das et al. 2020). These findings showed that the combination improved glycemic control and reduced cardiovascular risk factor in T2DM.

Not only on cardiovascular risk factor, some studies may also suggest a direct positive effect of the combination on cardiovascular. One study suggested the benefit of dual therapy on vascular and cardiac function in T2DM patient with high CVD risk as an add-on therapy to metformin which was superior compared to SGLT2i or GLP1-RA alone (Ikonomidis et al. 2020). In other exploratory analyses, GLP-1RA (efpeglenatide) may be showing independent effect on cardiovascular outcome in diabetes patients with existing SGLT2i therapy (Lam et al. 2022). Despite of the supports provided by some studies for adding GLP-1RA on patients receiving SGLT2i or vice versa, there were limitation of clinical retrospective settings such as observational data, potential confounding, limited number of events, and need further randomized controlled study to provide definitive evidence (Lam et al. 2022).

Therefore, in this study we investigated the cardioprotective effect of both agents against several cardiac biomarkers that usually elevated in damaged heart, to suggest whether the combination is able to protect the heart better than SGLT2i and GLP1-RA alone, not only in diabetic rats but also diabetic rats with MI. We also investigated the effect of its combination on blood glucose profile to confirm whether it shows any significant effect in lowering blood glucose level and improving insulin sensitivity. We used agents that has been approved for reduction of cardiovascular event such as liraglutide and empagliflozin. Empagliflozin now is the only SGLT2i that has been approved by FDA for both CVD benefit and heart failure indication (ADA 2022), while liraglutide was one of GLP-1RAs that has been approved by FDA for CVD benefit and proven to be cost-effective in T2DM patient with established CVD or elevated CV risk (Shah et al. 2018; ADA 2022).

Materials and methods

Experimental animals and study design

Thirty male Wistar rats, aged 10–12 weeks, weighing 200–250 g, were purchased from ITB School of Life Sciences and Technology and housed in the animal laboratory at ITB School of Pharmacy in separate cages under controlled environmental condition (12 h light/dark cycle and temperature 24–26 °C) with free access of standard chow and water. The research protocols were approved by the ethics committee for animal research of Bandung Institute of Technology (No.01/KEPHP-ITB/2-2022, 18 February 2022).

The rats were acclimated for 7 days before the study, then randomly divided into 6 groups as follows:

1. Normal control group: nondiabetic rats, given CMC-Na 0.5% via intragastric (i.g.) and NaCl 0.9% subcutaneously (s.c.) for 30 days;
2. DM group: diabetic rats, given CMC-Na 0.5% (i.g.) and NaCl 0.9% (s.c.) for 30 days;
3. DM+ISO group: diabetic rats, given CMC-Na 0.5% (i.g.) and NaCl 0.9% (s.c.) for 30 days, and received isoproterenol (ISO) intraperitoneally (i.p.) at dose 85 mg/kg BW on day 29 and 30 (at 24 hours interval) to induce AMI;
4. GLP-1RA group: diabetic rats, given liraglutide (Victoza, Novo Nordisk, Denmark) at a dose of 0.062 mg/kg BW (dissolved in NaCl 0,9%) via s.c. for 30 days, and received ISO (i.p.) at dose 85 mg/kg BW on day 29 and 30 (at 24 hours interval) to induce AMI (Nair and Jacob 2016; Das et al. 2020);
5. SGLT2i group: diabetic rats, given empagliflozin (Jardiance, Bohringer Ingelheim Pharmaceuticals, USA) at a dose of 1 mg/kg BW (dissolved in CMC-Na 0,5%) via i.g. for 30 days, and received ISO (i.p.) at dose 85 mg/kg BW on day 29 and 30 (at 24 hours interval) to induce AMI (Nair and Jacob 2016; Das et al. 2020);
6. Combination group: diabetic rats, given liraglutide (Victoza, Novo Nordisk, Denmark) at a dose of 0.062 mg/kg BW (dissolved in NaCl 0,9%) via s.c.

and empagloflozin (Jardiance, Bohringer Ingelheim Pharmaceuticals, USA) at a dose of 1 mg/kg BW (dissolved in CMC-Na 0,5%) via i.g. for 30 days, and received ISO (i.p.) at dose 85 mg/kg BW on day 29 and 30 (at 24 hours interval) to induce AMI.

Type 2 DM rat model

T2DM are characterized by the reduction of insulin sensitivity with impaired insulin secretion. Therefore, this study used the combination model to mimic those pathology (Furman 2021). The rats were given fat emulsion (LipomedÒ 20% MCT/LCT; Sanbe Farma, Bandung, WJ, IDN) 20 ml/kg BW via i.g. for 2 weeks to induce insulin resistance, then given a single low dose of STZ (Santa Cruz Biotechnology, Dallas, TX, USA) 35 mg/kg BW via i.p. to reduce pancreatic islet b-cell capacity (Aligita et al. 2018; Hussein et al. 2020; Furman 2021). The reduction of insulin sensitivity was confirmed by intraperitoneal insulin tolerance test (IPITT) with K_{ITT} value that significantly lower than normal control group ($p<0.05$), while the hyperglycemic state was confirmed with FBG >150 mg/dL (Ai et al. 2005; Furman 2021).

Acute myocardial infarction rat model

ISO was obtained from Tokyo-Chemical Industry (Shanghai, China). ISO at dose of 85 mg/kg BW was given twice (i.p.) on day 29 and 30, at 24 hours interval, to induce myocardial infarction. ISO was dissolved in NaCl 0.9% and prepared fresh on the day of induction. The AMI state was confirmed by the elevation of cardiac biomarkers (Halim et al. 2018; Kurniati et al. 2018).

Determination of blood glucose control and insulin sensitivity

The blood glucose control profile was obtained from FBG measurement at the same hour and time-points throughout the study to eliminate the variation of fasting duration of each test. Blood glucose concentration from tail vein was measured using Easy TouchÒ blood glucosemeter. IPITT was performed using insulin (NovorapidÒ) at dose of 0.5 U/kg BW via i.p. (Vinue and Gonzales 2015). The blood glucose levels were measured right before the injection (t=0) and at 15, 30, 45, 60 and 90 minutes after the insulin injection (Vinue and Gonzales 2015; Aligita et al. 2018). The data was plotted into graphic with time as the abscissa and the nature logarithm of blood glucose as the ordinate. The regression coefficient or slope was determined by linear regression and the K_{ITT} value was calculated by multiplying the slope by 100 (Ai et al. 2005).

Measurement of cardiac biomarkers

The blood samples were collected from tail (before MI induction) and from heart (after MI induction, as a terminal procedure), then serum samples were separated for the estimation of cardiac biomarkers. The serum levels of CK, CK-MB, LDH, AST and ALT were measured using commercially kits according to the manufacturer's instructions (DiaSys Diagnostic Systems GmbH, Holzheim, Germany for CK, CK-MB, LDH, and ALT; Glory Diagnostic, Spain for AST). All the biomarkers were assessed using Microlab 300, ELITech Group, France.

Histopathological examination of the heart tissue by H&E

The rat's heart was dissected, washed with 0.9% cold saline, then fixed in 10% neutral buffered formalin. The left ventricle then embedded in paraffin and sectioned at 3 μm thickness using microtome. The tissue specimens were stained using hematoxylin and eosin (H&E). Characteristic of myocytes abnormality such as coagulative necrosis with absent nuclei, hyper eosinophilia of cytoplasm, and an interstitial neutrophil infiltration were observed using light microscope (Olympus BX51) at 400× magnifications (Ghafoor et al. 2020; Hussein et al. 2020).

Statistical analysis

The data obtained in this study were processed using Minitab version 20 and analyzed using one-way ANOVA with Tukey's post hoc test. Considered significantly different at $p<0.05$.

Institutional review board statement

The animal study protocol was approved by the ethics committee for animal research of Bandung Institute of Technology (No.01/KEPHP-ITB/2-2022, 18 February 2022).

Results

Diebetes parameters

Insulin sensitivity profile

Insulin sensitivity was determined at baseline (before the induction), after the induction, and after receiving one or both agents for 4 weeks. The K_{ITT} values are shown in Table 1.

Before the induction, there were no significant difference of K_{ITT} value between groups that indicates similar insulin sensitivity at baseline. After given lipid emulsion for 14 days, all the tested groups showed significantly lower K_{ITT} value compared to normal control and baseline, indicate the reduction of blood glucose disappearance rate from blood (%/min) after given insulin (reduction of insulin sensitivity). The group treated with empagliflozin and/or liraglutide for 4 weeks showed the increase of K_{ITT} value. The K_{ITT} value of all treated group were not significantly different to normal control group. However, only empagliflozin and combination group that significantly higher than DM group, even not significantly different from baseline. Even though these findings may indicate lesser improvement in liraglutide group compared to other two groups, all three treated groups (empagliflozin and/liraglutide alone) were not statistically different ($p>0.05$), showing relatively similar K_{ITT} value.

Table 1. K_{ITT} profile at baseline, after induction, and after treated with SGLT2i and/GLP-1RA.

Groups	K_{ITT} (%/minute)		
	Baseline	After induction	After treated
Normal control	1.01±0.13	1.09±0.21	0.98±0.08
DM	1.14±0.10	0.69±0.08[a,c]	0.61±0.14[a,c]
Liraglutide	0.94±0.04	0.69±0.17[a,c]	0.85±0.06[c]
Empagliflozin	1.00±0.16	0.57±0.15[a,c]	0.93±0.14[b,d]
Combination	1.05±0.27	0.67±0.16[a,c]	0.97±0.10[b]

The data are expressed as mean ± SD. One-way ANOVA with Tukey Post hoc test. The letter shows signifcantly different ($p<0.05$) compared to [a]normal control, [b]DM group, [c]baseline, [d]after induction.

Blood glucose control

Blood glucose control was obtained from FBG measurement at several time-points (before induction, after induction, and every week while treated with one or both agents). The FBG level at each time point are shown in Table 2. At baseline, the FBG were not significantly different between groups, showing similar blood glucose profile. All the three treated groups showed the reduction of FBG that significantly different from DM group even since the first week of treatment. After 4 weeks, the FBG profile were similar in liraglutide, empagliflozin, and combination group ($p>0.05$) and were not significantly different from baseline. This may indicate a well blood glucose control in all treated groups and no superior FBG profile in the combination group compared to empagliflozin/liraglutide alone.

Cardiac biomarkers

Cardiac biomarkers were obtained from serum collected before and after ISO induction to assess the biomarker changes at DM only state and at DM with AMI state. The biomarkers level i.e., CK, CK-MB, LDH, AST, and ALT before AMI are shown in Table 3.

The CK, CK-MB, LDH, and AST levels were significantly elevated in DM group. The elevation of CK and LDH biomarker were not significantly different between liraglutide, empagliflozin, and combination group, and even compared to normal control group, indicate similar inhibition of all three treated group for both parameters. For CK-MB parameter, only empagliflozin and combination group that were not significantly different compared to normal control. However, empagliflozin group was not significantly different from liraglutide group, which may indicate higher inhibition on the elevation of CK-MB level in combination group compared to either these agents alone. As for AST and ALT, these parameters were not significantly elevated in all treated group. The biomarkers level of all treated group then compared to DM group and transformed into percentage of inhibition, shown in Table 4. The combination group showed the highest inhibition for all parameters.

The biomarkers level after ISO induction are shown in Table 5. The CK, CK-MB, and LDH parameters were significantly higher in DM+ISO group compared to DM group. Even ALT, parameter that was not significantly elevated in DM group, started to increase significantly in DM+ISO group compared to normal control group. This may indicate cardiomyocytes damages that were more severe after ISO induction. For CK parameter, all three treated group were significantly lower compared to DM+ISO group, however only empagliflozin and combination group that were not significantly different from normal control. On the other hand, the CK level in liraglutide group was not significantly different from empagliflozin group but significantly higher than combination group.

As for CK-MB and LDH parameter, the levels in all three treated group were significantly lower than DM+ISO group. However, only combination group that was

Table 2. FBG profile at baseline, after induction, and after treated with SGLT2i and/GLP1-RA.

Group	Fasting blood glucose (mg/dL)					
	Baseline	After induction	After treated (Week-)			
			1	2	3	4
Normal control	93.80±7.53	101.00±12.53	91.60±12.10	91.20±13.88	98.00±12.19	89.00±8.28
DM	93.25±3.50	259.00±109.77[a,c]	269.00±93.68[a,c]	312.50±135.93[a,c]	350.25±121.93[a,c]	407.25±89.11[a,c]
Liraglutide	93.00±6.98	266.00±85.81[a,c]	115.25±16.58[b,d]	123.00±24.18[b,d]	122.00±12.94[b,c,d]	106.50±9.18[b,d]
Empagliflozin	97.25±8.77	267.25±75.59[a,c]	112.00±18.67[b,d]	127.75±19.31[b,c,d]	130.50±8.43[b,c,d]	106.5±11.27[b,d]
Combination	94.20±13.52	253.40±26.84[a,c]	96.00±5.66[b,d]	108.60±22.32[b,d]	126.60±22.03[b,d]	95.20±7.09[b,d]

The data are expressed as mean ± SD. One-way ANOVA with Tukey Post hoc test. The letter shows significantly different ($p<0.05$) compared to [a]normal control, [b]DM group, [c]baseline, [d]after induction.

Table 3. Effect of empagliflozin and/liraglutide on cardiac biomarkers in diabetic rats.

Groups	Cardiac biomarkers (U/L)				
	CK	CK-MB	LDH	AST	ALT
Normal Control	123.81±16.16[b]	121.19±10.56[b]	307.93±63.04[b]	99.32±20.97[b]	52.80±20.32
DM	361.64±84.44[a]	244.53±24.60[a]	914.53±196.28[a]	168.98±51.12[a]	101.56±46.82
Liraglutide	192.48±44.06[b]	184.75±24.01[a]	428.22±44.84[b]	148.40±10.34	55.01±18.34
Empagliflozin	149.19±70.24[b]	147.00±18.68[b]	508.97±129.63[b]	142.40±36.57	70.93±7.67
Combination	103.59±30.74[b]	99.07±18.38[b,c]	325.10±103.73[b]	125.32±11.86	53.29±10.76

The data are expressed as mean ± SD. One-way ANOVA with Tukey Post hoc test. The letter shows significantly different ($p<0.05$) compared to [a]normal control, [b]DM group, [c]liraglutide group.

Table 4. Percentage inhibition of serum cardiac biomarkers elevation in treated vs DM group.

Groups	Inhibition of cardiac biomarker serum elevation (%)				
	CK	CK-MB	LDH	AST	ALT
Liraglutide	46.77	23.48	53.18	12.18	45.83
Empagliflozin	58.74	39.11	44.37	15.73	30.16
Combination	71.36	58.97	64.45	25.83	47.53

not significantly different from normal control. The CK-MB level in combination group even significantly lower than both empagliflozin and liraglutide group. As for AST parameter, all three treated group were not significantly different from normal control, however only combination group that was significantly lower than DM+ISO group.

All these findings may indicate better inhibition on the elevation of these biomarkers in combination group. The data then transformed into percentage of inhibition, shown in Table 6. The combination group showed the highest inhibition in almost all parameters, except for ALT parameter which is slightly lower than liraglutide group.

Cardiac histopathological changes

In histopathological examination using H&E staining, regular arrangement of myocardial fibers was shown in normal control group (Fig. 1a), while in DM group irregular arrangement of myocardial fibers and wide gap between cardiomyocytes (black arrow) were observed (Fig. 1b). DM+ISO group (Fig. 1c) also showed wide spacing in heart tissue; massive infiltration of inflammatory cells with the absent of nuclei were also observed (white arrow), indicate coagulative necrosis. In liraglutide group (Fig. 1d) and empagliflozin group (Fig. 1e), hyper eosinophilia and interstitial neutrophilic infiltration were still observed with the space between fibers that was smaller than in DM or DM+ISO group. In combination group (Fig. 1f), myocardial fibers were more regular with minimal inflammation and smaller gap between myocytes (Ghafoor et al. 2020; Hussein et al. 2020).

Discussion

Several in vivo studies have been conducted to confirmed cardiovascular benefit of both classes. Liraglutide, dapagliflozin, and empagliflozin showed cardioprotective effect in diabetic cardiomyopathy model (Zhou and Wu 2017; Lee et al. 2019; Hussein et al. 2020) and heart failure model (Kraker et al. 2020; Withaar et al. 2021). Liraglutide even showed cardioprotective effect to ischemia-reperfusion injury in diabetic ex vivo model (Simanenkova et al. 2021). Not only either this agent alone, some studies also showed cardioprotective effect of its combination in diabetic rats (Trang et al. 2021; El-Shafey et al. 2022). However, there are some limitations of previous studies that the model only used a single high dose of streptozotocin to induce hyperglycemia without the reduction of insulin sensitivity. Thus, these might resemble T1DM more rather than T2DM. The previous studies also only suggested cardioprotective effect in diabetic rats where the alteration of cardiac function such as diabetic cardiomyopathy might occur, while the cardioprotective effect against acute MI as the primary cause of death in diabetes subjects remains unknown. Therefore, in this study we used a model that combined a T2DM and acute MI model to assess the cardioprotective effect in diabetes subjects with and without MI.

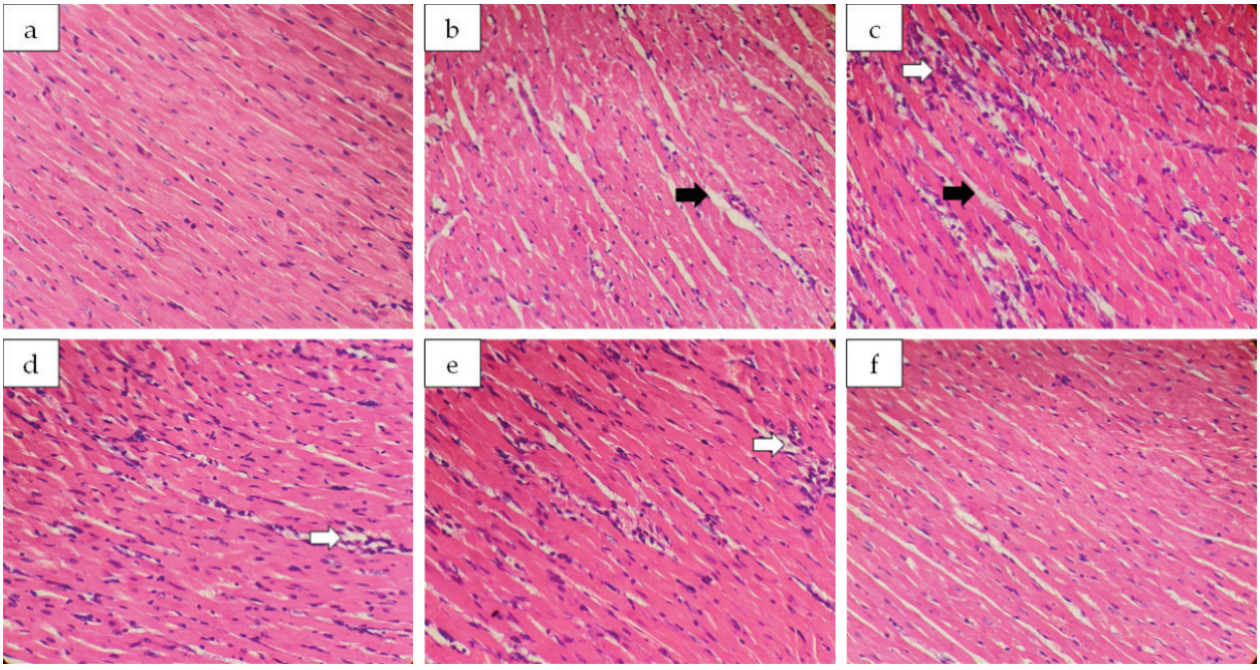

Figure 1. Histopathological examination of heart tissue stained with H&E staining and 400× magnification. **a** Control group; **b** DM group; **c** DM+ISO group; **d** Liraglutide group; **e** Empagliflozin group, and **f** Combination group. The irregular arrangement of myocardial fibers and wide gap between cardiomyocytes (showed in black arrow) may indicate massive cardiomyocytes death. While massive infiltration of inflammatory cells with the absent of nuclei (showed in white arrow) may indicate coagulative necrosis.

Table 5. Effect of empagliflozin and/liraglutide on cardiac biomarkers in diabetic rats induced with AMI.

Groups	Cardiac biomarkers (U/L)				
	CK	CK-MB	LDH	AST	ALT
Normal control	136.85±28.27[b]	134.61±12.87[b]	325.37±90.41[b]	115.79±17.25[b]	51.86±16.89[b]
DM	361.13±103.63[a,b]	263.51±33.41[a,b]	1012.08±191.39[a,b]	189.15±50.86[a]	96.63±41.72
DM+ISO	596.76±28.18[a,c]	438.29±50,79[a,c]	1679.11±255.76[a,c]	247.89±56.45[a]	123.98±59.32[a]
Liraglutide	338.93±26.61[a,b]	238.39±26.33[a,b]	763.63±83.46[a,b]	179.15±21.89	64.48±16.28
Empagliflozin	275.17±145.08[b]	214.28±18.81[a,b]	671.68±102.99[a,b]	180.40±12.19	69.54±19.12
Combination	175.99±35.94[b,*]	125.06±19.32[b,#]	452.60±151.38[b]	145.72±20.84[b]	64.60±7.43

The data are expressed as mean ± SD. One-way ANOVA with Tukey Post hoc test. The letter shows significantly different ($p<0.05$) compared to [a]normal control, [b]DM+ISO group, [c]DM group, [*]liraglutide group, [#]both liraglutide and empagliflozin group.

Table 6. Percentage inhibition of serum cardiac biomarkers elevation in treated vs DM+ISO group.

Groups	Inhibition of cardiac biomarker serum elevation (%)				
	CK	CK-MB	LDH	AST	ALT
Liraglutide	43.21	45.61	54.52	27.73	47.99
Empagliflozin	53.89	51.11	59.99	27.23	43.91
Combination	70.51	71.48	73.05	41.22	47.90

Streptozotocin (STZ) has been shown to cause pancreatic islet b-cell destruction and widely used to induce diabetes in animal study (Furman 2021). STZ alone only cause hyperglycemia with decrease insulin levels without insulin resistance (Reed et al. 2000), while moderate dose of STZ to animal given prior high fat diet cause hyperglycemia, associated with hyperinsulinemia and insulin resistance that mimic the characteristic of most patient with T2DM (Furman 2021). High fat diets were usually used in animal model to disturb lipid metabolism and increase oxidative stress, that leads to insulin resistance (Maithili et al. 2014). However, in this study, it was replaced by lipid emulsion. Some studies supported insulin resistant model using lipid emulsion via intragastric (not by food feeding) to provide better control of fat intake. This method was confirmed to induce insulin resistant after only two weeks administration (Ai et al. 2005; Aligita et al. 2018).

Lipid emulsion given for two weeks was significantly lowering the insulin sensitivity based on K_{ITT} value calculated from IPITT. This lipid emulsion contained triglycerides (TGs) that might increase the TG level in blood which has positive correlation with insulin resistance (Ma et al. 2020). The increase of K_{ITT} value in liraglutide and/empagliflozin groups were due to several mechanisms. GLP-1RA improves insulin resistance by promoting insulin secretion and amplify its signal transduction; increase GLUT-4 expression and its localization, as the important transporter for glucose uptake into cells; modulates lipid metabolism; improves pancreatic beta-cell function; also attenuates ER stress and inflammation-induced insulin resistance through AMPK/mTOR signaling (Yaribeygi et al. 2019). On the other hand, SGLT2i improves insulin sensitivity by inhibiting glucotoxicity through normalizing hyperglycemia near to physiologic ranges; induces caloric deposition by glucosuria, leading to weight loss and lesser lipotoxicity; attenuates inflammatory responses by reducing various process and oxidative stress; also improves beta-cell mass and its function through several pathways related to islets cells' death (Yaribeygi et al. 2020).

After the induction was confirmed and have been treated with either or both classes for 4 weeks, the FBG profile improved significantly even to a normal level that indicates a well-controlled FBG level in all treated group. In this study, one of these agents alone was already sufficient to normalize the FBG level, thus the combination showed no superior effect compared to empagliflozin/liraglutide group. This finding was different to some clinical trials where the additive glucose-lowering benefit were observed. This might be due to different setting and how these agents are combined. In clinical trials, the GLP-1RA was added to patient with inadequately controlled of T2DM, even after receiving SGLT2i therapy (Ludvik et al. 2018; Zinman et al. 2019; Blonde et al. 2020; Das et al. 2020). Even so, the FBG profile of combination group did not show hypoglycemia state, which provided support to use the combination therapy even in a well-controlled blood glucose level. Both classes showed low risk of hypoglycemia due to its mechanism in lowering blood glucose level. The insulinotropic and glucagonostatic effect of GLP-1RAs are glucose-dependent, thus the glucose lowering effect will decrease when the blood glucose level is normalized (Hinnen 2017; Das et al. 2020). On the other hand, the SGTL2is glucosuria effect is independent of insulin secretion and tied to filtered load of glucose, thus its glucose-lowering effect become ineffective in euglycemic state (Horii et al. 2020).

There are few differences in human and animal heart structure, that is challenging to design a good model to mimic pathophysiology of MI in human. In this study, we used isoproterenol (ISO) as an indirect approach to induce MI in diabetic rats. ISO is sympathomimetic agent that stimulates both b-1 and b-2 receptors, causing ischemia due to imbalance of oxygen demand and supply in heart, that further leads to MI (Halim et al. 2018). ISO induction is a relative simple technique, non-invasive, with high successful rate and low mortality risk (Brooks and Conrad 2009; Halim et al. 2018). This agent produces infarct-like lesion that is similar to human MI and also changes in hemodynamic, biochemical, and histopathological parameters (Peer et al. 2008; Halim et al. 2018).

Cardiomyocytes death causing the leak of several proteins into the circulation, thus the level of cardiac enzymes tends to be elevated in the serum after MI (Ali et al. 2016; Kurniati et al. 2018). CK, CK-MB, LDH, AST, and ALT were biomarkers used in this study as they have historical

approaches in the diagnosis of acute coronary syndrome (ACS). AST was the first biomarker found in 1954, followed by LDH, CK, and their isoenzymes (Danese and Montagnana 2016). CK is a dimeric enzyme, predominantly found in tissue that required large amounts of energy. It consists of two subunits M or B, forming three isoenzymes, i.e. CK-MM, CK-BB, and CK-MB. The CK-MB form are found mostly in cardiac muscle and is correlated to infarct size, make it an important predictor for MI prognosis in conjunction with CK level (Lee and Goldman 1986; Aydin et al. 2019). LDH, AST, and ALT are a non-specific biomarkers to heart. LDH is expressed not only in heart muscle but also in many organs such as lungs, kidney, liver, and erythrocytes, while AST and ALT are often used for diagnosis of liver damages. Despite of its non-specificity, these enzymes are also elevated cardiac necrosis and often found in MI patients (Tiwari et al. 2012). The AST and ALT were known to be well-correlated to CK-MB level that may also reflect the severity of infarct (Lofthus et al. 2012).

The biomarker measurement before ISO induction showed significant elevation of almost all parameters (except for ALT) compared to normal control group. These findings were similar to previous in vivo study where cardiomyopathy may occur in diabetes model which leads to myocardial necrosis causing the elevation of several cardiac biomarkers in serum (Hussein et al. 2020; El-Shafey et al. 2022). Clinical study also suggested that diabetes may alter lipid utilization and induce atherogenic dyslipidemia that contribute in the development of MI (Ali et al. 2016). The combination group showed the highest inhibition on the elevation of all parameters. After ISO induction, all the biomarkers in DM+ISO group were elevated compared to DM group, even significantly for CK, CK-MB, and LDH parameters. Moreover, ALT (lesser specific to heart compared to AST) began to significantly higher than normal control group, which was not observed in DM only group. This may indicate more severe myocardial injury after ISO-induced AMI (Lofthus et al. 2012). The combination group showed the greatest inhibition on the elevation of almost all parameters, particularly against CK-MB biomarker which was significantly higher than both liraglutide and empagliflozin group, even up to similar level as normal control group. This may indicate better inhibition against cardiomyocytes death caused by diabetes and AMI.

Glucose-lowering therapies alone were already proven to reduce microvascular event, while it is more challenging to prevent macrovascular complication in T2DM (Ussher et al. 2022). Both GLP-1RA and SGLT2i were known for its beneficial effect on CV risk factors by reducing blood pressure and body weight (Frias et al. 2016; Das et al. 2020). GLP-1RA promoting weight loss due to reduced appetite caused by the reduction of gastric emptying effect, while the SGLT2i promotes glucosuria, thus loss of calories leads to weight loss is expected (Salvatore et al. 2022; Ussher et al. 2022). The increased of glucose excretion was also followed by volume depletion that may be beneficial for blood pressure. SGLT2i did not greatly affect renin-angiotensin-aldosterone (RAA) system, however they often improve circadian blood pressure rhythm (Wilcox 2020; Salvatore et al. 2022). On the other hand, the blood pressure lowering effect of GLP-1RA is due to the increase of ANP released from cardiomyocyte, improvement of endothelial function, and nitric oxide production (Ussher et al. 2022).

Several studies suggested other cardioprotective effects of GLP-1RAs through the attenuation of atherosclerosis, reduced inflammation, and some beneficial action on myocardial function which improved cardiovascular outcomes (Ussher et al. 2022). The anti-atherogenic effect was confirmed in both clinical and preclinical studies, even the reduction of lesion progression was independent of changes in total cholesterol and body weight (Marso et al. 2017; Rakipovski et al. 2018; Ussher et al. 2022). GLP-1RAs minimized and stabilized the development of atherosclerotic plaques through anti-inflammatory mechanisms involving macrophage infiltration, circulating cytokines, NLRP3 inflammasome, inducible nitric oxide synthase (iNOS) expression, and vascular smooth muscle cell proliferation (Sudo et al. 2017; Rakipovski et al. 2018; Ma et al. 2021; Sanada et al. 2021). This agent also promotes cardiomyocytes survival and improve myocardial function that may limit the progression of cardiomyopathy and infarct size. GLP-1RAs reduced and repaired MI via modulating SIRT1/Parkin/Mitophagy, insulin-like growth factor-1/2 and upregulating α-estrogen receptor (Ma et al. 2021). It also improved mitochondrial function via regulating autophagy and inflammatory signaling, also increasing cardiac glucose metabolism and its utilization, thus improved metabolism efficiency and myocardial resistance to ischemia (Giblett et al. 2016; Ma et al. 2021; Ussher et al. 2022).

Even though the SGLT2 is not expressed in heart, its direct cardioprotective effect might be due to the modulation of autophagy. Autophagy is a complex process in response of several stimuli and is important to eliminate defect organelle associated with oxidative stress. Insufficient or excessive activation of autophagy may be harmful in MI. SGLT2i inhibited Na^+/H^+ exchanger 1 (NHE1) on cardiomyocytes that suppress excessive autophagy during ischemia (Jiang et al. 2022). On the other hand, SGLT2i also induce ketogenic nutrient-deprivation pathways involving SIRT1/PGC-1α/FGF21 signaling that promote survival through the attenuation of oxidative stress and modulation of autophagy in heart tissue (Packer 2020).

This study has several limitations, which only focuses on the effect of both agent against several biomarkers that could leaked to circulation when myocardial damage or death occur. The biomarkers used in this study were relatively non- to almost specific to cardiomyocytes. In the future, the measurement of Troponin-I and the isomers of LDH (to calculated LDH-1/LDH-2 ratio), which more specific to cardiomyocyte, might be used. Several oxidative stress markers also haven't been measured yet to support the mechanism or pathway related to cardiomyocyte

survival for each agent and combination therapy. This study also may be limited to liraglutide and empagliflozin, not other drugs in the same class.

Conclusions

This study suggested better cardioprotective effect of liraglutide and empagliflozin combined in diabetic subjects induced with AMI, based on its inhibition against the elevation of several cardiac biomarkers. These results may be used as a consideration for clinical study to provide definitive evidence regarding the additional reduction of cardiovascular outcomes for the combination therapy. GLP-1RAs and SGLT2is are categorized as an expensive antihyperglycemic agents, thus cost-effectiveness study to support the combination to be used earlier in a newly diagnostic T2DM patient with high risk or established ASCVD is needed.

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References

Ai J, Wang N, Yang M, Du ZM, Zhang YC, Yang BF (2005) Development of wistar rat model of insulin resistance.World Journal of Gastroenterology 11(24): 3675–3679. https://doi.org/10.3748/wjg.v11.i24.3675

Ali F, Naqvi SA, Bismillah M, Wajid N (2016) Comparative analysis of biochemical parameters in diabetic and non-diabetic acute myocardial infarction patients. Indian Heart Journal 68(3): 325–331. https://doi.org/10.1016/j.ihj.2015.09.026

Aligita W, Susilawati E, Sukmawati IK, Holidayanti L, Riswanti J (2018) Antidiabetic activities of *Muntingia calabura* L. Leaves water extract in type 2 diabetes mellitus animal models. The Indonesia Biomedical Journal 10(2): 165–170. https://doi.org/10.18585/inabj.v10i2.405

American Diabetes Association (2010) Diagnosis and classification of diabetes mellitus. Diabetes Care 33(Suppl 1): S62–S69. https://doi.org/10.2337/dc10-S062

American Diabetes Association Professional Practice Committee, Draznin B, Aroda VR, Bakris G, Benson G, Brown FM, Freeman R, Green J, Huang E, Isaacs D, Kahan S, Leon J, Lyons SK, Peters AL, Prahalad P, Reusch JEB, Young-Hyman D (2022) 9. Pharmacologic Approaches to Glycemic Treatment: Standards of Medical Care in Diabetes-2022. Diabetes Care 45(Suppl 1): S125–S143. https://doi.org/10.2337/dc22-S009

Aydin S, Ugur K, Aydin S, Sahin I, Yardim M (2019) Biomarkers in acute myocardial infarction: current perspectives. Vascular Health and Risk Management 15: 1–10. https://doi.org/10.2147/VHRM.S166157

Blonde L, Belousova L, Fainberg U, Garcia-Hernandez PA, Jain, SM, Kaltoft MS, Mosenzon O, Nafach J, Palle MS, Rea R (2020) Liraglutide as add-on to sodium-glucose co-transporter-2 inhibitors in patients with inadequately controlled type 2 diabetes: LIRA-ADD2S-GLT2i, a 26-week, randomized, double-blind, placebo-controlled trial. Diabetes, Obesity and Metabolism 22(6): 929–937. https://doi.org/10.1111/dom.13978

Brooks WW, Conrad CH (2009) Isoproterenol-induced myocardial injury and diastolic dysfunction in mice: structural and functional correlates. Comparative Medicine 59(4): 339–343.

Cheng AYY (2021) Why choose between SGLT2 Inhibitors and GLP1-RA when you can use both?: The time to act is now. Circulation 143(8): 780–782. https://doi.org/10.1161/CIRCULATIONAHA.120.053058

Cosentino F, Grant PJ, Aboyans V, Bailey CJ, Ceriello A, Delgado V, Federici M, Filippatos G, Grobbee DE, Hansen TB, Huikuri HV, Johansson I, Jüni Ps, Lettino M, Marx N, Mellbin LG, Östgren CJ, Rocca B, Roffi M, Sattar N, Seferović PM, Sousa-Uva M, Valensi P, Wheeler DC, ESC Scientific Document Group (2020) 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. Eurpean Heart Journal 41(2): 255–323. https://doi.org/10.1093/eurheartj/ehz486

Danese E, Montagnana M (2016) An historical approach to the diagnostic biomarkers of acute coronary syndrome. Annals of Translational Medicine 4(10): 194. https://doi.org/10.21037/atm.2016.05.19

Das SR, Everett BM, Birtcher KK, Brown JM, Januzzi Jr JL, Kalyani RR, Kosiborod M, Magwire M, Morris PB, Neumiller JJ, Sperling LS (2020) 2020 expert consensus decision pathway on novel therapies for cardiovascular risk reduction in patients with type 2 diabetes: A report of the American college of cardiology solution set oversight committee. Journal of The American College of Cardiololgy 76(9): 1117–1145. https://doi.org/10.1016/j.jacc.2020.05.037

El-Shafey M, El-Agawy MSE, Eldosoky M, Ebrahim HA, Elsherbini DMA, El-Sherbiny M, Asseri SM, Elsherbiny NM (2022) Role of dapagliflozin and liraglutide on diabetes-induced cardiomyopathy in rats: Implication of oxidative stress, inflammation, and apoptosis. Frontiers in Endocrinology (Lausanne) 13: 862394. https://doi.org/10.3389/fendo.2022.862394

Frías JP, Guja C, Hardy E, Ahmed A, Dong F, Öhman P, Jabbour SA (2016) Exenatide once weekly plus dapagliflozin once daily versus exenatide or dapagliflozin alone in patients with type 2 diabetes inadequately controlled with metformin monotherapy (DURATION-8): a 28 week, multicentre, double-blind, phase 3, randomised controlled trial. Lancet Diabetes Endocrinology 4(12): 1004–1016. https://doi.org/10.1016/S2213-8587(16)30267-4

Furman BL (2021) Streptozotocin-induced diabetic models in mice and rats. Current Protocols 1(4): e78. https://doi.org/10.1002/cpz1.78

Ghafoor M, Kamal M, Nadeem U, Husain AN (2020) Educational Case: Myocardial Infarction: Histopathology and Timing of Changes, Academic Pathology 7: 5–6. https://doi.rog/10.1177/2374289520976639

Giblett JP, Clarke SJ, Dutka DP, Hoole SP (2016) Glucagon-Like Peptide-1. JACC: Basic to Translational Science 1(4): 267–276. https://doi.org/10.1016/j.jacbts.2016.03.011

Haffner SM, Lehto S, Rönnemaa T, Pyörälä K, Laakso M (1998) Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. The New England Journal of Medicine 339(4): 229–234. https://doi.org/10.1056/NEJM199807233390404

Halim SASA, Ghafar NA, Jubri Z, Das S (2018) Induction of myocardial infarction in experimental animals: A review. Journal of Clinical and Diagnostic Research 12(11): AE01–AE05. https://doi.org/10.7860/JCDR/2018/36997.12221

Hinnen D (2017) Glucagon-like peptide 1 receptor agonists for type 2 diabetes. Diabetes Spectrum 30(3): 202–210 https://doi.org/10.2337/ds16-0026

Horii T, Oikawa Y, Kunisada N, Shimada A, Atsuda K (2020) Real-world risk of hypoglycemia-related hospitalization in Japanese patients with type 2 diabetes using SGLT2 inhibitors: a nationwide cohort study. BMJ Open Diabetes Research and Care 8: e001856. https://doi.org/10.1136/bmjdrc-2020-001856

Hussein AM, Eid EA, Taha M, Elshazli RM, Bedir RF, Lashin LS (2020) Comparative study of the effects of GLP1 analog and SGLT2 inhibitor against diabetic cardiomyopathy in type 2 diabetic rats: Possible underlying mechanisms. Biomedicines 8(3): 43. https://doi.org/10.3390/biomedicines8030043

Ikonomidis I, Pavlidis G, Thymis J, Birba D, Kalogeris A, Kousathana F, Kountouri A, Balampanis K, Parissis J, Andreadou I, Katogiannis K, Dimitriadis G, Bamias A, Iliodromitis E, Lambadiari V (2020) Effects of Glucagon-Like Peptide-1 Receptor Agonists, Sodium-Glucose Cotransporter-2 Inhibitors, and Their Combination on Endothelial Glycocalyx, Arterial Function, and Myocardial Work Index in Patients With Type 2 Diabetes Mellitus After 12-Month Treatment. Journal of The American Heart Association 9(9): e015716. https://doi.org/10.1161/JAHA.119.015716

International Diabetes Federation (2021) IDF Diabetes Atlas 10th edn. International Diabetes Federation, Brussels-Belgium, 135 pp.

Jia G, Hill MA, Sowers JR (2018) Diabetic cardiomyopathy: An update of mechanisms contributing to this clinical entity. Circulation Research 122(4): 624–638 https://doi.org/10.1161/CIRCRESAHA.117.311586

Jiang K, Xu Y, Wang D, Chen F, Tu Z, Qian J, Xu S, Xu Y, Hwa J, Li J, Shang H, Xiang Y (2022) Cardioprotective mechanism of SGLT2 inhibitor against myocardial infarction is through reduction of autosis. Protein Cell 13(5): 336–359. https://doi.org/10.1007/s13238-020-00809-4

Kannel WB, Hjortland M, Castelli WP (1974) Role of diabetes in congestive heart failure: the Framingham study. The American Journal of Cardiology 34(1): 29–34. https://doi.org/10.1016/0002-9149(74)90089-7

Kräker K, Herse F, Golic M, Reichhart N, Crespo-Garcia S, Strauß O, Grune J, Kintscher U, Ebrahim M, Bader M, Alenina N, Heuser A, Luft FC, Müller DN, Dechend R, Haase N (2020) Effects of empagliflozin and target-organ damage in a novel rodent model of heart failure induced by combined hypertension and diabetes. Scientific Reports 10(1): 14061. https://doi.org/10.1038/s41598-020-70708-5

Kurniati NF, Sukandar EY, Pardilah R, Suliska N, Ayuningtyas DK (2018) Cardioprotective potential of ethanol extract of *Sonchus arvensis* L. Leaves on isoproterenol-induced myocardial infarction in rat. Jurnal Ilmu Kefarmasian Indonesia 16(1): 20–24. https://doi.org/10.35814/jifi.v16i1.434

Lam CSP, Ramasundarahettige C, Branch KRH, Sattar N, Rosenstock J, Pratley R, Del Prato S, Lopes RD, Niemoeller E, Khurmi NS, Baek S, Gerstein HC (2022) Efpeglenatide and clinical outcomes with and without concomitant sodium-glucose cotransporter-2 inhibition use in type 2 diabetes: Exploratory analysis of the AMPLITUDE-O trial. Circulation 145(8): 565–574. https://doi.org/10.1161/CIRCULATIONAHA.121.057934

Lee MMY, Petrie MC, McMurray JJV, Sattar N (2020) How do SGLT2 (Sodium-Glucose Cotransporter 2) inhibitors and GLP-1 (Glucagon-Like Peptide-1) receptor agonists reduce cardiovascular outcomes?: Completed and ongoing mechanistic trials. Arteriosclerosis, Thrombosis, and Vascular Biology 40(3): 506–522. https://doi.org/10.1161/ATVBAHA.119.311904

Lee TH, Goldman L (1986) Serum enzyme assays in the diagnosis of acute myocardial infarction. Recommendations based on a quantitative analysis. Annals of Internal Medicine 105: 221–233. https://doi.org/10.7326/0003-4819-105-2-221

Lee TI, Chen YC, Lin YK, Chung CC, Lu YY, Kao YH, Chen YJ (2019) Empagliflozin attenuates myocardial sodium and calcium dysregulation and reverses cardiac remodeling in streptozotocin-induced diabetic rats. International Journal of Molecular Science 20(7): 1680. https://doi.org/10.3390/ijms20071680

Leon BM, Maddox TM (2015) Diabetes and cardiovascular disease: Epidemiology, biological mechanisms, treatment recommendations and future research. World Journal of Diabetes 6(13): 1246–1258. https://doi.org/10.4239/wjd.v6.i13.1246

Lofthus DM, Stevens SR, Armstrong PW, Granger CB, Mahaffey KW (2012) Pattern of liver enzyme elevations in acute ST-elevation myocardial infarction. Coronary Artery Disease 23(1): 22–30. https://doi.org/10.1097/mca.0b013e32834e4ef1

Ludvik B, Frías JP, Tinahones FJ, Wainstein J, Jiang H, Robertson KE, García-Pérez LE, Woodward DB, Milicevic Z (2018) Dulaglutide as add-on therapy to SGLT2 inhibitors in patients with inadequately controlled type 2 diabetes (AWARD-10): a 24-week, randomised, double-blind, placebo-controlled trial. Lancet Diabetes Endocrinology 6(5): 370–381. https://doi.org/10.1016/S2213-8587(18)30023-8

Ma M, Liu H, Yu J, He S, Li P, Ma C, Zhang H, Xu L, Ping F, Li W, Sun Q, Li Y (2020) Triglyceride is independently correlated with insulin resistance and islet beta cell function: a study in population with different glucose and lipid metabolism states. Lipids in Health and Disease 19(1): 121. https://doi.org/10.1186/s12944-020-01303-w

Ma X, Liu Z, Ilyas I, Little PJ, Kamato D, Sahebka A, Chen Z, Luo S, Zheng X, Weng J, Xu S (2021) GLP-1 receptor agonists (GLP-1RAs): cardiovascular actions and therapeutic potential. International Journal of Biological Science 17(8): 2050–2068. https://doi.org/10.7150/ijbs.59965

Maithili Karpaga Selvi N, Sridhar MG, Swaminathan RP, Sripradha R (2014) Curcumin attenuates oxidative stress and activation of redox-sensitive kinases in high fructose- and high-fat-fed male wistar rats. Scientia Pharmaceutica 83(1): 159–175. https://doi.org/10.3797/scipharm.1408-16

Marso SP, Bain SC, Consoli A, Eliaschewitz FG, Jódar E, Leiter LA, Lingvay I, Rosenstock J, Seufert J, Warren ML, Woo V, Hansen O, Holst AG, Pettersson J, Vilsbøll T (2017) Semaglutide and cardiovascular outcomes in patients with type 2 diabetes. The New England Journal of Medicine 376: 891–892. https://doi.org/10.1056/NEJMoa1607141

Nair AB, Jacob S (2016) A simple practice guide for dose conversion between animals and human. Journal of Basic and Clinical Pharmacy 7(2): 27–31. https://doi.org/10.4103/0976-0105.177703

Packer M (2020) Cardioprotective effects of sirtuin-1 and its downstream effectors. Circulation: Heart Failure 13: e007197. https://doi.org/10.1161/CIRCHEARTFAILURE.120.007197

Peer PA, Trivedi PC, Nigade PB, Ghaisas MM, Deshpande AD (2008) Cardioprotective effect of *Azadirachta indica* A. Juss. on isoprenaline induced myocardial infarction in rats. International Journal of Cardiology 126(1): 123–126. https://doi.org/10.1016/j.ijcard.2007.01.108

Rakipovski G, Rolin B, Nøhr J, Klewe I, Frederiksen KS, Augustin R, Hecksher-Sørensen J, Ingvorsen C, Polex-Wolf J, Knudsen LB (2018) The GLP-1 analogs liraglutide and semaglutide reduce atherosclerosis in ApoE-/- and LDLr-/- mice by a mechanism that includes inflammatory pathways. JACC: Basic Translational Science 3: 844–857. https://doi.org/10.1016/j.jacbts.2018.09.004

Reed MJ, Meszaros K, Entes LJ, Claypool MD, Pinkett JG, Gadbois TM, Reaven GM (2000) A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat. Metabolism 49(11): 1390–1394. https://doi.org/10.1053/meta.2000.17721

Sanada J, Obata A, Obata Y, Fushimi Y, Shimoda M, Kohara K, Nakanishi S, Mune T, Kaku K, Kaneto H (2021) Dulaglutide exerts beneficial anti atherosclerotic effects in ApoE knockout mice with diabetes: the earlier, the better. Scientific Reports 11: 1425. https://doi.org/10.1038/s41598-020-80894-x

Salvatore T, Galiero R, Caturano A, Rinaldi L, Di Martino A, Albanese G, Di Salvo J, Epifani R, Marfella R, Docimo G, Lettieri M, Sardu C, Sasso FC (2022) An overview of the cardiorenal protective mechanisms of SGLT2 inhibitors. International Journal of Molecular Science 23(7): 3651. https://doi.org/10.3390/ijms23073651

Shah D, Risebrough NA, Perdrizet J, Iyer NN, Gamble C, Dang-Tan T (2018) Cost-effectiveness and budget impact of liraglutide in type 2 diabetes patients with elevated cardiovascular risk: a US-managed care perspective. Clinicoeconomics and Outcomes Research 10: 791–803. https://doi.org/10.2147/CEOR.S180067

Simanenkova A, Minasian S, Karonova T, Vlasov T, Timkina N, Shpilevaya O, Khalzova A, Shimshilashvili A, Timofeeva V, Samsonov D, Borshchev Y, Galagudza M (2021) Comparative evaluation of metformin and liraglutide cardioprotective effect in rats with impaired glucose tolerance. Scientific Reports 11(1): 6700. https://doi.org/10.1038/s41598-021-86132-2

Skyler JS, Bakris GL, Bonifacio E, Darsow T, Eckel RH, Groop L, Groop PH, Handelsman Y, Insel RA, Mathieu C, McElvaine AT, Palmer JP, Pugliese A, Schatz DA, Sosenko JM, Wilding JP, Ratner RE (2017) Differentiation of diabetes by pathophysiology, natural history, and prognosis. Diabetes 66(2): 241–255. https://doi.org/10.2337/db16-0806

Sudo M, Li Y, Hiro T, Takayama T, Mitsumata M, Shiomi M, Sugitani M, Matsumoto T, Hao H, Hirayama A (2017) Inhibition of plaque progression and promotion of plaque stability by glucagon-like peptide-1 receptor agonist: serial in vivo findings from iMap-IVUS in Watanabe heritable hyperlipidemic rabbits. Atherosclerosis 265: 283–291. https://doi.org/10.1016/j.atherosclerosis.2017.06.920

Tiwari RP, Jain A, Khan Z, Kohli V, Bharmal RN, Kartikeyan S, Bisen PS (2012) Cardiac troponins I and T: Molecular markers for early diagnosis, prognosis, and accurate triaging of patients with acute myocardial infarction. Molecular Diagnosis and Therapy 16(6): 371–381. https://doi.org/10.1007/s40291-012-0011-6

Trang NN, Chung CC, Lee TW, Cheng WL, Kao YH, Huang SY, Lee TI, Chen YJ (2021) Empagliflozin and liraglutide differentially modulate cardiac metabolism in diabetic cardiomyopathy in rats. International Journal of Molecular Science 22(3): 1177. https://doi.org/10.3390/ijms22031177

Ussher JR, Greenwell AA, Nguyen M, Mulvihill EE (2022) Cardiovascular effects of incretin-based therapies: Integrating mechanisms with cardiovascular outcome trials. Diabetes 71(2): 173–183. https://doi.org/10.2337/dbi20-0049

Vinué Á, González-Navarro H (2015) Glucose and insulin tolerance tests in the mouse. Methods in Molecular Biology 1339: 247–254. https://doi.org/10.1007/978-1-4939-2929-0_17

Wilcox CS (2020) Antihypertensive and renal mechanismsof SGLT2 (Sodum-Glucose Linked Transporter 2) inhibitors. Hypertension 75: 894–901. https://doi.org/10.1161/HYPERTENSIONAHA.119.11684

Withaar C, Meems LMG, Markousis-Mavrogenis G, Boogerd CJ, Silljé HHW, Schouten EM, Dokter MM, Voors AA, Westenbrink BD, Lam CSP, de Boer RA (2021) The effects of liraglutide and dapagliflozin on cardiac function and structure in a multi-hit mouse model of heart failure with preserved ejection fraction. Cardiovasc Research 117(9): 2108–2124. https://doi.org/10.1093/cvr/cvaa256

Yaribeygi H, Sathyapalan T, Sahebkar A (2019) Molecular mechanisms by which GLP-1 RA and DPP-4i induce insulin sensitivity. Life Sciences 234: 116776. https://doi.org/10.1016/j.lfs.2019.116776

Yaribeygi H, Sathyapalan T, Maleki M, Jamialahmadi T, Sahebkar A (2020) Molecular mechanisms by which SGLT2 inhibitors can induce insulin sensitivity in diabetic milieu: A mechanistic review. Life Sciences 240: 117090. https://doi.org/10.1016/j.lfs.2019.117090

Zhou Y, Wu W (2017) The Sodium-Glucose Co-Transporter 2 Inhibitor, empagliflozin, protects against diabetic cardiomyopathy by inhibition of the endoplasmic reticulum stress pathway. Cell Physiology and Biochemistry 41(6): 2503–2512. https://doi.org/10.1159/000475942

Zinman B, Bhosekar V, Busch R, Holst I, Ludvik B, Thielke D, Thrasher J, Woo V, Philis-Tsimikas A (2019) Semaglutide once weekly as add-on to SGLT-2 inhibitor therapy in type 2 diabetes (SUSTAIN 9): a randomised, placebo-controlled trial. Lancet Diabetes Endocrinology 7(5): 356–367. https://doi.org/10.1016/S2213-8587(19)30066-X