Research Article **CODEN: AJPCFF** **ISSN: 2321 – 0915**

Asian Journal of Phytomedicine and Clinical Research

Journal home page: www.ajpcrjournal.com

PROTECTIVE EFFECT OF EMPAGLIFLOZIN ALONE AND ITS COMBINATION WITH METFORMIN IN EXPERIMENTALLY INDUCED MYOCARDIAL INFARCTION IN DIABETIC RATS

Jagdish Kakadiya*[1], Anteneh Tamirat[1], Utsav Shah[1], Anas Jamsa[1], Snigdha Mandal[1], Shivkumar Rathod[1]

[1*]Department of Pharmacology, Parul Institute of Pharmacy and Research, Parul University, Vadodara, Gujarat, India.

ABSTRACT

The objective of the present study was to evaluate effect of Empagliflozin alone and its combination with Metformin on cardiovascular complications in Streptozotocin-Nicotinamide induced diabetic rats. Animals were divided into following groups, each group containing 6 animals and the treatment period for whole study was 4 weeks. Group 1: Non-diabetic control [0.5 % hydroxy ethyl cellulose] as vehicle for 4 weeks and (ND-CON)] and normal saline subcutaneously on 29th and 30th day. Group 2: STZ-NIC diabetic control [0.5 % hydroxy ethyl cellulose] as vehicle for 4 weeks (D-CON)] and received ISO (85 mg/kg, s.c.) on 29th and 30th day in normal saline. Group 3: Diabetic rats treated with Empagliflozin (5mg/kg p.o for 4 weeks) followed by Isoproterenol. Group 4: Diabetic rats treated with Empagliflozin (10mg/kg p.o for 4 weeks) followed by Isoproterenol. Group 5: Diabetic rats treated with Empagliflozin (5mg/kg) + Metformin (50mg/kg) p.o for 4 weeks followed by Isoproterenol. At the end of the treatment period, rats were anaesthetized with anaesthetic and blood was collected from the retro-orbital plexus for estimation of different biochemical parameters like Creatine kinase, lactate dehydrogenase, aspartate aminotransferase, cardiac troponin I, lipid serum profile, total cholesterol, triglycerides, high density lipoprotein, low density lipoprotein, very low density lipoprotein and histopathology of heart. The combined treatment with empagliflozin 5 mg/kg and metformin was able to improve the hyperglycemia and decrease the level of CK-MB, LDH, AST, Troponin and lipid profile (TC, TG, LDL and VLDL), with increased level of HDL-cholesterol level even greater than treatment with individual drug.

KEYWORDS

Type 2 Diabetes, Myocardial Infarction, Empagliflozin and Metformin.

Author for Correspondence:

Jagdish Kakadiya,

Department of Pharmacology,

Parul Institute of Pharmacy and research,

Parul University, Vadodara, Gujarat, India.

Email: Jagdishkakadiya@gmail.com

INTRODUCTION

Type 2 Diabetes mellitus (T2DM) is characterized by an increase in blood glucose level due to the resistance of pancreatic hormone insulin action and secretion[1]. T2DM is a multi-decade progressive disease with complex interconnected pathophysiology.

Cardiovascular disease (CVD) and T2DM have

attained epidemic proportions worldwide, becoming increasingly alarming public health problems. The prevalence of T2DM ranges from 6.9% to 10.2% in developed countries, and exceeds 7% in developing countries[2,3]. CVD accounts for 17.5 million deaths annually; an estimated 31% of all deaths worldwide[4]. A recent report has revealed that this number is projected to exceed 23.6 million by 2030[5]. A growing body of evidence suggests that there is a strong links or relationship between these three disease states T2DM, CVD and hypertension.

Firstly, T2DM is known to be a major risk factor for cardiovascular (CV) morbidity and mortality[6]. In addition to microvascular complications (nephropathy, retinopathy and neuropathy), T2DM is also associated with macrovascular complications including CVD, cerebrovascular disease and peripheral artery disease. Secondly, the majority of patients with T2DM are overweight or obese, an additional risk factor for both CVD and hypertension[7]. Finally, hypertension is a common co-morbidity in both CVD and T2DM. A substantial proportion of people with T2DM have concomitant hypertension (20–60%, depending on age, sex, ethnicity and body mass index). Hypertension almost doubles the risk of all-cause mortality, and increases the risk of coronary artery disease threefold[8,9]. In addition, hypertension enhances the progression of diabetic complications such as nephropathy, retinopathy and neuropathy.

Given the relationship between CV risk and T2DM, and the uncertainty surrounding the CV risk of some glucose-lowering therapies, the US FDA and EMA require evaluation of CV risk for new compounds being developed as therapies for T2DM to reduce the risk of CV complication and increase the positive outcome for patients[10,11].

The reason behind this is the existence of great correlation between diabetes mellitus and cardiovascular disease occurrences due to this every drug coming into the market for management of diabetes should be screened for cardiovascular risk. Hyperglycemia is the main pathogenic factor that leads to diabetic complications including coronary heart disease, retinopathy, nephropathy, and neuropathy. Although there remains great controversy concerning the impact of glucose lowering on CV outcomes; it is conceivable that glycaemic control plays an important role in this process, as suggested by epidemiological studies[12]. Thus, and in light of the multiple CV risk factors beyond hyperglycaemia that exist in most patients with T2DM, a multifactorial approach like control of blood pressure (BP) and lipids, weight management, smoking cessation and, when indicated, anti-platelet therapy are recommended but, it is known to be difficult for most patients in clinical practice to reach their therapeutic goals.

Sodium–glucose co transporter-2 (SGLT-2) is a new molecular target to directly induce glucose excretion and to safely normalize plasma glucose in type 2 diabetes. SGLT-2 inhibitors have a unique mechanism of action, which is independent of insulin secretion and action. This unique mechanism of action, in addition to lowering plasma glucose, corrects a number of metabolic and hemodynamic abnormalities that are risk factors for cardiovascular disease (CVD). Most guidelines recommend SGLT-2 inhibitors as add-on therapy to metformin although monotherapy is potentially appropriate. Use of this class of drugs has continued to increase yearly since market introduction because of their many positive effects, including low risk of hypoglycemia; compelling degrees of HbA1c reduction; osmotic renal excretion of glucose and the corresponding caloric loss; and modest blood pressure reduction. All patients with type 2 diabetes with a history of significant coronary artery disease, myocardial infarction, stroke, peripheral vascular disease, and A1C >7% should be considered for

treatment with an SGLT-2 inhibitor, the evidence is very strong for Empagliflozin. Canagliflozin likely has benefit but has important side effects as well (increased amputations and bone fractures), and the evidence for Dapagliflozin is from observational studies such as CVD Real Nordic with all the limitations of such studies.

MATERIAL AND METHODS

Drug and Chemical

All chemicals, reagent and diagnostic kits were procured from Span Diagnostics Ltd., India. The STZ and NIC were purchased from SIGMA, St. Louis, MO, USA and Isoproterenol from Sigma Chemicals, St. Louis, U.S.A. All the reagents and chemicals used in the entire study were of analytical grade.

Experimental Animals

Healthy adult female Sprague Dawley rats weighing 180-220 gm. were used in the study. Rats were housed in polypropylene cages, maintained under standardized condition (12-hr light/dark cycle, 24°C, 35 to 60% humidity) and provided free access to pelleted CHAKKAN diet (Nav Maharashtra Oil Mills Pvt. Ltd., Pune) and purified drinking water ad libitium.

All experiments and protocols described in the present study were approved by the Institutional Animal Ethics Committee (IAEC) of department of pharmacology, Parul institute of pharmacy and research and with permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

EXPERIMENTAL METHODS

Type 2 Diabetes model

Type 2 Diabetes was induced in rats by a single intraperitoneal (i.p) injection of Streptozotocin (65 mg/kg, STZ) in overnight fasting rats followed by the i.p administration of Nicotinamide (120 mg/kg, NIC) after 15 minutes. STZ was dissolved in citrate buffer (pH 4.5) and NIC was dissolved in normal saline. After 7 days following STZ and NIC administration, blood was collected from retro-orbital puncture and serum samples were analysed for blood glucose. Blood samples after collecting from rats from retro-orbital plexus under light ether anaesthesia without any anticoagulant and allowed for 10 minutes to clot at room temperature. It was then centrifuged at 2500 rpm for 20 min. The serum obtained was kept at 4ºC until used. Animals showing fasting blood glucose higher than 300 mg/dL were considered as diabetic and used for the further study.

Myocardial infarction model (Isoproterenol induced)

Experimental Procedure

In this model isoproterenol (85 mg/kg, s.c) in suitable dose is subcutaneously administered twice at 24 hour intervals and after 24 hour of last dose the heart is studied for selected parameters. 85 mg/kg ISO dose was selected for the study as this dose offered significant alteration in biochemical parameters along with moderate necrosis in heart after trying various doses. However other researchers opted for still higher dose to produce severe necrosis for investigating cardioprotective activities of test substances which was selected for the study. The injury was induced by two subcutaneous injections of isoproterenol (85 mg/kg at 24 hr interval). A treatment drug was administered for 28 days in diabetic rats and after isoproterenol induced myocardial infarction in rats on 29^{th} and 30^{th} day.

At the end of experimental period (i.e. on the day 31) blood samples were collected and animals were euthanized. A heart tissue sample of each rat was collected and carried out for further estimations were carried out.

Treatment Groups of Animals

The details of groups made for the study of effect of various drugs on ISO model are given below.

Animals were divided into following groups, each group containing 6 animals and the treatment period for whole study was 4 weeks.

Group 1

Non-diabetic control [0.5 % hydroxy ethyl cellulose] as vehicle for 4 weeks and (ND-CON)]

and normal saline subcutaneously on 29th and 30th day.

Group 2

STZ-NIC diabetic control [0.5 % hydroxy ethyl cellulose] as vehicle for 4 weeks (D-CON)] and received ISO (85 mg/kg, s.c.) on 29th and 30th day in normal saline.

Group 3

Diabetic rats treated with Empagliflozin (5mg/kg p.o for 4 weeks) followed by Isoproterenol.

Group 4

Diabetic rats treated with Empagliflozin (10mg/kg p.o for 4 weeks) followed by Isoproterenol.

Group 5

Diabetic rats treated with Empagliflozin (5mg/kg) + Metformin (50mg/kg) p.o for 4 weeks followed by Isoproterenol.

After completed treatment blood was collected by retro-orbital puncture. Serum and tissue was separated and analysed for the following biochemical parameters for all group.

Biochemical parameter for assessment of diabetes

- Glucose mg/dL (GOD-POD Method)

Serum marker enzymes for cardiac functions

- Creatine kinase (CK-MB) IU/L
- Lactate dehydrogenase (LDH) IU/L (Mod. IFCC method)
- Aspartate aminotransferase (AST) IU/L (2, 4-DNPH, Reitman and Frankel method)
- Cardiac Troponin I

Lipid Serum Profile

- Total Cholesterol (TC) IU/L
- Triglycerides (TG) IU/L
- High density lipoprotein (HDL) IU/L
- Low density lipoprotein (LDL) IU/L
- Very low density lipoprotein (VLDL) IU/L

STATISTICAL ANALYSIS

All the values are expressed as mean ± S.E.M. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Bonferroni multiple comparisons test or unpaired two-tailed student's t-test as appropriate using computer based fitting program (Prism, Graphpad 5). Comparisons were made between a) Group II vs Group I b) Group III, IV, V vs Group II c) Group V vs Group III, IV. Differences were considered to be statistically significant when $p<0.05$.

RESULTS

Protective effects of Empagliflozin with two different doses (5mg/kg and 10mg/kg) and in combination with Metformin was established by observing, diabetic marker, cardiac biomarker enzymes, ECG recording and histopathology.

Diabetic marker

Glucose level

Group II animals injected with STZ-NIC in a dose of 65-120mg/kg once, i.p and isoproterenol 85mg/kg s.c in a two consecutive doses at an interval of 24hr in 29th and 30th day exhibited significant ($P<0.001$) increase in serum glucose level (281.3±4.201) when compared with Group I animals (87.83±4.167). Group III, IV and V animals showed significant ($P<0.001$) inhibition to an elevated serum glucose level when compared to Group II animals. Group IV animals exhibited significant ($P\leq0.01$) reduction in serum glucose level as compared to Group III animals. Group V animals exhibited significantly ($P<0.001$) and non-significantly lower serum glucose level when compared with Group III and Group IV animals respectively. Results are shown in Table No.1.

Values are expressed as Mean±SEM (n=6) in the group. # Significantly different from Normal control group at $P<0.001$. *$P\leq0.05$, **$P\leq0.01$, ***$P\leq0.001$ considered statistically significant as compared to control group.

Cardiac markers profile

CK-MB activity

Group II animals injected with STZ-NIC in a dose of 65-120mg/kg once, i.p and isoproterenol 85mg/kg s.c in a two consecutive doses at an interval of 24hr in 29th and 30th day exhibited significant ($P<0.001$) increase in serum CK-MB level (114.5±4.552) when compared with Group I animals (40.93±0.5357). Group III, IV and V animals showed significant ($p<0.001$) inhibition to

an elevated serum CK-MB level when compared to Group II animals.

Group V animals exhibited significantly ($p<0.001$) and ($p<0.05$) lower serum CK-MB level when compared with Group III and Group IV animals respectively. Results are shown in Figure No.2.

LDH activity

Group II animals injected with STZ-NIC in a dose of 65-120mg/kg once, i.p and isoproterenol 85mg/kg s.c in a two consecutive doses at an interval of 24hr in 29th and 30th day exhibited significant ($P<0.001$) increase in serum LDH level (251.7±3.989) when compared with Group I animals (183.7±0.8819). Group IV and V exhibited non-significant association with Group I. Group III, IV and V animals showed significant ($P<0.001$) inhibition to an elevated serum LDH level when compared to Group II animals. Group V animals exhibited significantly ($P<0.001$) and non-significantly lower serum LDH level when compared with Group III and Group IV animals respectively. Results are shown in Figure No.2.

AST activity

Group II animals injected with STZ-NIC in a dose of 65-120mg/kg once, i.p and isoproterenol 85mg/kg s.c in a two consecutive doses at an interval of 24hr in 29th and 30th day exhibited significant ($P<0.001$) increase in Serum AST (47.33±1.892) when compared with Group I animals (12.17±0.9458). Group III, IV and V showed significant ($P<0.001$), ($P<0.05$) and non-significant association with Group I animals respectively. Group V animals exhibited significantly ($P<0.001$) and non-significantly lower serum AST level when compared with Group III and Group IV animals respectively. Results are shown in Figure No.3.

Cardiac Troponin I (cTIn)

Group II animals injected with STZ-NIC in a dose of 65-120mg/kg once, i.p and isoproterenol 85mg/kg s.c in a two consecutive doses at an interval of 24hr in 29th and 30th day showed a positive test result of troponin I (≥1.5ng/ml); whereas Group I animals showed negative test result that is less than 1.5ng/ml (<1.5ng/ml). Group III, IV and V animals showed the same result as that of Group I animals; from this we can see that both doses of Empagliflozin and its 5mg/kg combination with Metformin have their own ability to make the test result negative. Results are shown in Table No.2.

Lipid Profile

Total cholesterol (TC)

Group II animals injected with STZ-NIC in a dose of 65-120mg/kg once, i.p and isoproterenol 85mg/kg s.c in a two consecutive doses at an interval of 24hr in 29th and 30th day exhibited significant ($P<0.001$) increase in serum total cholesterol level (194.8±4.468) when compared with Group I animals (100.0±0.9661). Group III, IV and V animals exhibited significant ($P<0.001$), ($P<0.01$) and non-significant increase in serum total cholesterol level when compared with Group I animals respectively; however in case of Group II animals all the three groups showed significant ($P<0.001$) inhibition of elevated serum total cholesterol level. Group V animals exhibited significantly ($P<0.001$) and non-significantly lower serum total cholesterol level when compared with Group III and Group IV animals respectively. Results are shown in Figure No.4.

Triglycerides (TG)

Group II animals injected with STZ-NIC in a dose of 65-120mg/kg once, i.p and isoproterenol 85mg/kg s.c in a two consecutive doses at an interval of 24hr in 29th and 30th day exhibited significant ($P<0.001$) Increase in serum triglycerides level (183.2 ±4.600) when compared with Group I animals (46.55±1.786). Group III, IV and V animals exhibited significant ($P<0.001$), non-significant and non-significant increase in serum triglycerides level when compared with Group I animals respectively. Group IV animals exhibited significant ($P<0.001$) inhibition of elevated serum triglycerides level as compared to Group III animals. Group V animals exhibited significantly ($P<0.001$) and non-significantly lower serum triglycerides level when compared with Group III and Group IV animals respectively. Results are shown in Figure No.5.

High-density Lipoprotein (HDL)

Group II animals injected with STZ-NIC in a dose of 65-120mg/kg once, i.p and isoproterenol 85mg/kg s.c in a two consecutive doses at an interval of 24hr in 29th and 30th day exhibited significant (P<0.001) Decrease in serum HLD level (27.67±1.453) when compared with Group I animals (52.83±0.6009). Group III, IV and V animals exhibited significant (P<0.001), (P<0.05) and non-significant increase in serum HDL cholesterol level when compared with Group I animals respectively. Group IV animals exhibited significant (P<0.001) inhibition of elevated serum HDL cholesterol level as compared to Group III animals. Group V animals exhibited significantly (P<0.001) and (P<0.05) lower serum high-density Lipoprotein level when compared with Group III and Group IV animals respectively. Results are shown in Figure No.6.

Low-density Lipoprotein (LDL)

Group II animals injected with STZ-NIC in a dose of 65-120mg/kg once, i.p and isoproterenol 85mg/kg s.c in a two consecutive doses at an interval of 24hr in 29th and 30th day exhibited significant (P<0.001) increase in serum LDL level (133.5±2.335) when compared with Group I animals (82.50±0.7638). Group III, IV and V animals exhibited significant (P<0.001), (P<0.05) and non-significant increase in serum LDL cholesterol level when compared with Group I animals respectively. Group IV animals exhibited significant (P<0.05) inhibition of elevated serum LDL cholesterol level as compared to Group III animals. Group V animals exhibited significantly (P<0.001), and (P<0.05) lower serum low-density lipoprotein level when compared with Group III and Group IV animals respectively. Results are shown in Figure No.7.

Very low-density Lipoprotein (VLDL)

Group II animals injected with STZ-NIC in a dose of 65-120mg/kg once, i.p and isoproterenol 85mg/kg s.c in a two consecutive doses at an interval of 24hr in 29th and 30th day exhibited significant (P<0.001) increase in serum very LDL level (39.83±2.227) when compared with Group I animals (15.00±0.5774). Group III, IV and V animals exhibited significant (P<0.001), non-significant and non-significant increase in serum VLDL cholesterol level when compared with Group I animals respectively; these same groups showed significant (P<0.05), (P<0.001) and (P<0.001) inhibition of elevated serum VLDL cholesterol level when compared with Group II animals respectively. Group V animals exhibited significantly (P<0.01) and non-significantly lower serum very low-density lipoprotein level when compared with Group III and Group IV animals respectively. Results are shown in Figure No.8.

DISCUSSION

Cardiovascular disease (CVD) and T2DM have attained epidemic proportions worldwide, becoming increasingly alarming public health problems. The prevalence of T2DM ranges from 6.9% to 10.2% in developed countries, and exceeds 7% in developing countries[2,3]. CVD accounts for 17.5 million deaths annually; an estimated 31% of all deaths worldwide[4]. A recent report has revealed that this number is projected to exceed 23.6 million by 2030[5]. A growing body of evidence suggests that there is a strong links or relationship between these three disease states T2DM, CVD and hypertension.

Firstly, T2DM is known to be a major risk factor for cardiovascular (CV) morbidity and mortality[6]. In addition to microvascular complications (nephropathy, retinopathy and neuropathy), T2DM is also associated with macrovascular complications including CVD, cerebrovascular disease and peripheral artery disease. The majority of patients with T2DM are overweight or obese, an additional risk factor for both CVD and hypertension[7]. Hypertension is a common co-morbidity in both CVD and T2DM. A substantial proportion of people with T2DM have concomitant hypertension (20–60%, depending on age, sex, ethnicity and body mass index). Hypertension almost doubles the risk of all-cause mortality, and increases the risk of coronary artery disease threefold[8,9]. In addition, hypertension enhances the progression of diabetic

complications such as nephropathy, retinopathy and neuropathy.

In the present study, type-2 diabetes induced using streptozotocin-Nicotinamide with a dose of (65-120 mg/kg); i.p single dose and those rats having fasting plasma glucose of greater than 250 mg/dl were selected for further studies. Treatment with Empagliflozin two different doses that means 5 mg/kg and 10 mg/kg and combination of 5 mg/kg Empagliflozin with Metformin 50 mg/kg were given to rats in different groups. After 24 hr. of final dose in the respective treatment groups and diabetic control group Isoproterenol 85 mg/kg was given for two consecutive doses in an interval of 24 hr.

The relationship between CV risk and T2DM, and the uncertainty surrounding the CV risk of some glucose-lowering therapies, the US FDA and EMA require evaluation of CV risk for new compounds being developed as therapies for T2DM to reduce the risk of CV complication and increase the positive outcome for patients[10,11]. Hyperglycemia is the main pathogenic factor that leads to diabetic complications including coronary heart disease, retinopathy, nephropathy, and neuropathy. Although there remains great controversy concerning the impact of glucose lowering on CV outcomes; it is conceivable that glycaemic control plays an important role in this process, as suggested by epidemiological studies[12]. Thus, multiple CV risk factors beyond hyperglycemia that exist in most patients with T2DM, a multifactorial approach like control of blood pressure (BP) and lipids, weight management, smoking cessation and, when indicated, anti-platelet therapy are recommended but, it is difficult for most patients in clinical practice to reach their therapeutic goals.

The result of our study exhibited that there was a significant increase in blood glucose level in STZ-NIC induced diabetic control group when compared to normal control group. This result was consistent with many studies. All the three treatment groups i.e Empagliflozin 5 mg/kg alone, Empagliflozin 10 mg/kg alone and 5 mg/kg of Empagliflozin combination with Metformin 50 mg/kg showed a significant decrease in blood glucose level when compared to diabetic control group. Since combination of Empagliflozin 5 mg/kg and Metformin shows significant decrease in blood glucose when compared to Empagliflozin 5 mg/kg alone, but when compared to Empagliflozin 10 mg/kg alone it is not significantly associated; even though the result favors combination treatment. Synergistic effect is expected for decrease in blood glucose level when combination of two drugs is used in diabetic complications like myocardial infarction.

Cardiac biomarkers like CK-MB, LDH, Troponin etc. increases in cardiovascular diseases condition, obesity, diabetes mellitus, and hyperlipidemia together develop myocardial infraction in which cardiac markers raise. In our study the level of CK-MB and LDH increased significantly in diabetic control group when compared to normal group. Inhibition of elevated serum CK-MB and LDH were seen in case of all treatment groups, but there is a difference in the level of inhibition like it favors to combination and Empagliflozin 10 mg/kg alone when compared to Empagliflozin 5 mg/kg alone. Group EMP-5 mg/kg + MET-50 mg/kg showed significant decrease in CK-MB level when compared to diabetic control and even more as compared to animals treated with EMP-5 mg/kg and EMP-10 mg/kg alone. Empagliflozin-5 mg/kg has less effect in lowering LDH level as compared to both EMP-10 mg/kg and combination. The qualitative analysis of Troponin I showed that troponin was absent (<1.5ng/ml) in normal control. Troponin was present (≥1.5ng/ml) in diabetic control only; otherwise troponin was absent (<1.5ng/ml) in Group EMP-5, EMP-10 and EMP-5 + MET. From these data it is clear that both lower and higher dose of Empagliflozin and combination are lowering troponin level towards (<1.5ng/ml) as like that of normal control group.

AST, ALT, ALP enzymes are increased in liver and cardiac diseases. They are mainly increased in liver injury but they are also present in cardiac cells and skeletal muscles. Thus in cardiac diseases AST, ALT, ALP level increases. AST level was

significantly increased in diabetic control group when compared to normal control. This result was consistent with many studies. Group EMP-5 and EMP-10 showed significant inhibition on elevated level of AST as compared to diabetic control. Group EMP-5 + MET showed significant decrease in AST level compared to diabetic control and even more significant decrease in elevated level of AST as compared to rats treated with EMP-5 alone, but not EMP-10 alone treated group. The data shows that Group EMP-5 and MET exhibits synergistic effect of both drug in lowering elevated level of AST when compared to diabetic control rats.

Many risk factors for type 2 diabetes include lifestyle decisions that can be reduced or even cut out entirely with time and effort. Men are also at slightly higher risk of developing diabetes than women; this may be more associated with lifestyle factors. It is stated that dyslipidemia is a recognized risk factor for CVD and DM. It was found that the levels of TC, TG, LDL and VLDL were significantly increased in diabetic control as compared to the normal control. On the other hand, the level of HDL was significantly reduced in diabetic control as compared to the normal control. This result was consistent with studies. Group EMP-5 showed a significant decrease in TC, TG and LDL levels but there is less significant decrease in VLDL level when compared to diabetic control group. Group EMP-10 rats showed significant decrease in TC, TG, LDL and VLDL levels when compared to diabetic control group. Treatment with combination of Empagliflozin-5 and Metformin also significantly decreased TC, TG, LDL and VLDL levels even more as compared to rats treated with Empagliflozin 5 mg/kg and 10 mg/kg alone. Group EMP-5 and EMP-10 showed significant increase in HDL level when compared to diabetic control. Group EMP-5 + MET showed significant increase in HDL level even more when compared to diabetic control, Group EMP-5 and EMP-10.

Table No.1: Effect of Empagliflozin + Metformin on Glucose level in diabetic rats

Group	Treatment	Dose	Glucose (mg/dl) Mean± SEM
I	Normal Control	--	87.83±4.167
II	Diabetic-Myocardial	--	281.3±4.201$^{\#}$
III	Empagliflozin	5mg/kg	125.0±2.875***
IV	Empagliflozin	10mg/kg	103.7±2.404***
V	Empagliflozin + Metformin	5mg/kg + 50mg/kg	96.50±2.156***

Table No.2: Effect of Empagliflozin + Metformin on Troponin I level in diabetic rats

Group	Treatment	Dose	Test result	Expected Troponin I level (ng/ml)
I	Normal Control	--	Negative	< 1.5
II	Diabetic Myocardial	--	Positive	≥ 1.5
III	Empagliflozin	5mg/kg	Negative	< 1.5
IV	Empagliflozin	10mg/kg	Negative	< 1.5
V	Empagliflozin + Metformin	5mg/kg + 50mg/kg	Negative	< 1.5

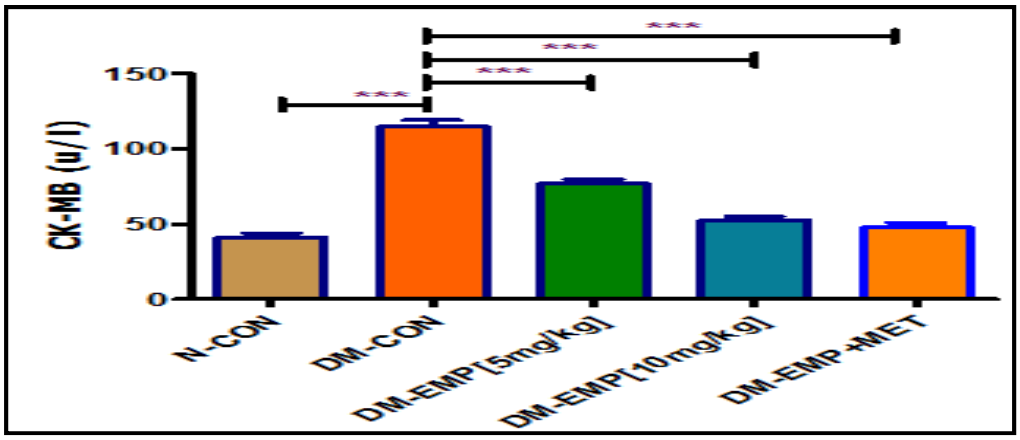

Figure No.1: Effect of Empagliflozin + Metformin on changes in serum CK-MB level after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats. Values are expressed as Mean SEM (n=6) in the group. *P 0.05, **P 0.01, ***P 0.001 considered statistically significant as compared to control group

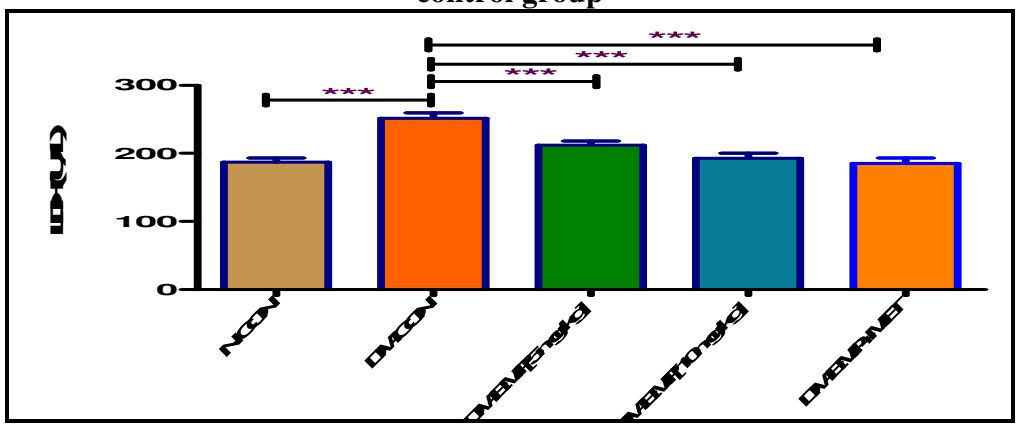

Figure No.2: Effect of Empagliflozin + Metformin on changes in serum LDH level after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats. Values are expressed as Mean± SEM (n=6) in the group. *P<0.05, **P<0.01, ***P<0.001 considered statistically significant as compared to control group

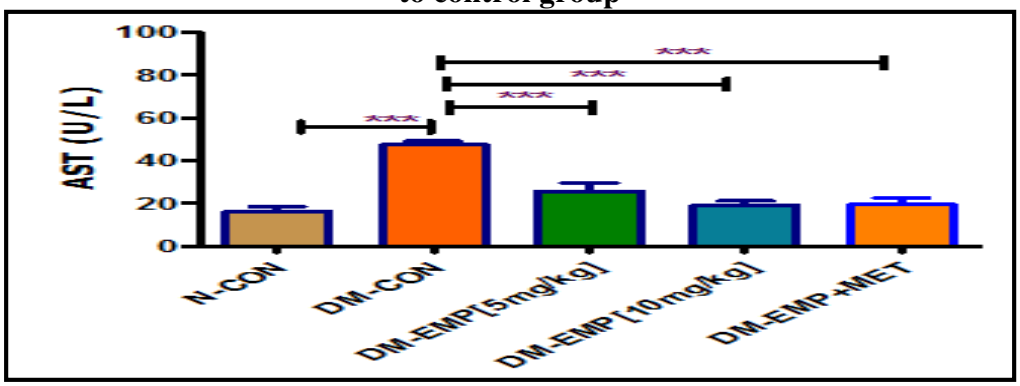

Figure No.3: Effect of Empagliflozin + Metformin on changes in serum AST level after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats. Values are expressed as Mean±SEM (n=6) in the group. *P<0.05, **P<0.01, ***P<0.001 considered statistically significant as compared to control group

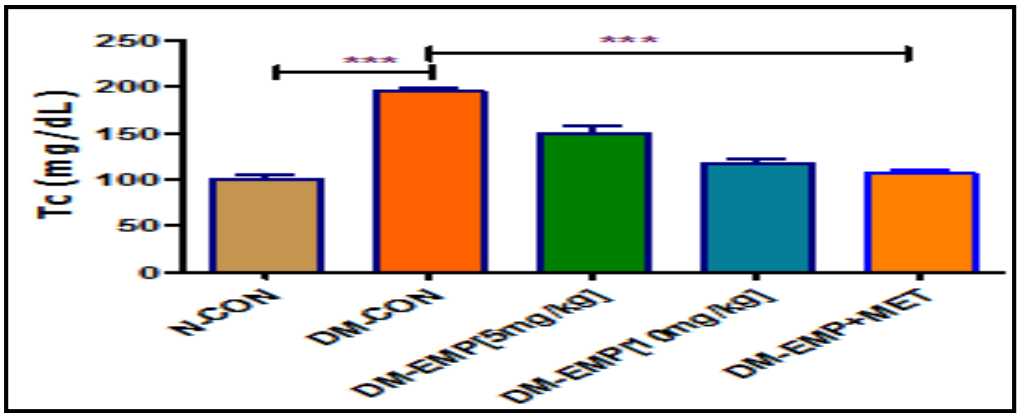

Figure No.4: Effect of Empagliflozin + Metformin on changes in serum total cholesterol level after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats. Values are expressed as Mean ± SEM (n=6) in the group. *P<0.05, **P<0.01, ***P<0.001 considered statistically significant as compared to control group

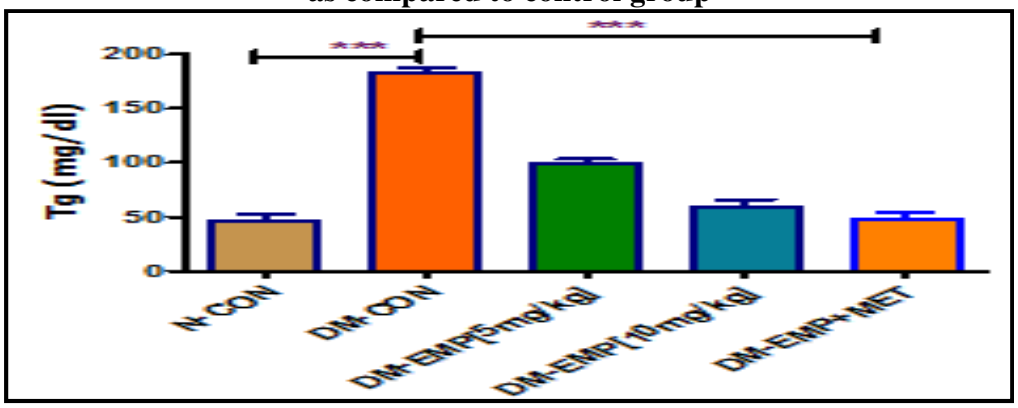

Figure No.5: Effect of Empagliflozin + Metformin on changes in serum triglycerides level after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats. Values are expressed as Mean ± SEM (n=6) in the group. *P<0.05, **P<0.01, ***P<0.001 considered statistically significant as compared to control group

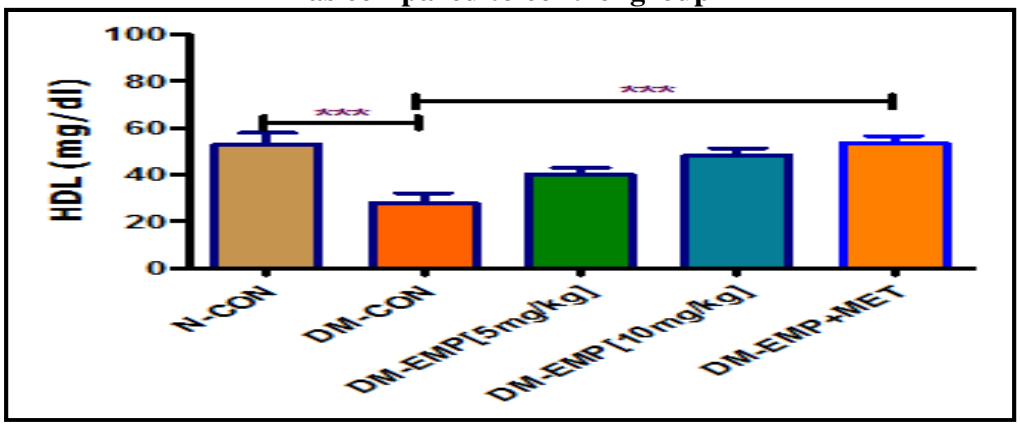

Figure No.6: Effect of Empagliflozin + Metformin on changes in serum HDL level after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats. Values are expressed as Mean ± SEM (n=6) in the group. *P<0.05, **P<0.01, ***P<0.001 considered statistically significant as compared to control group

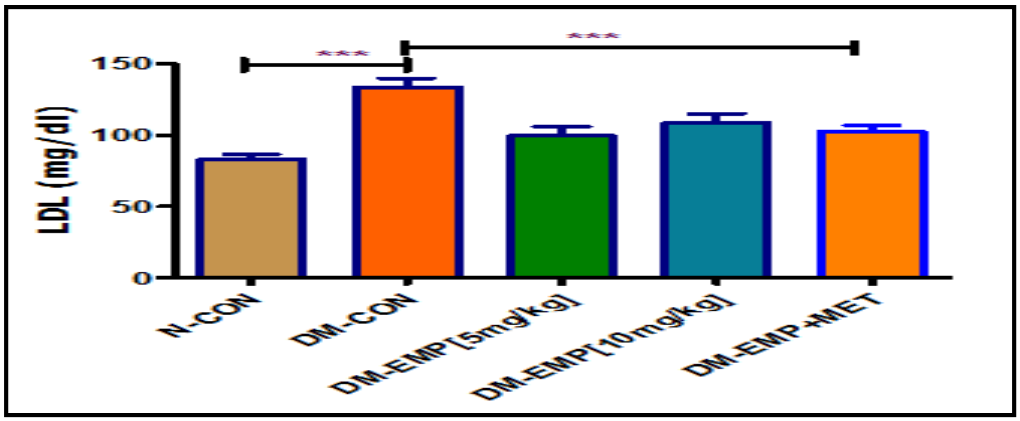

Figure No.7: Effect of Empagliflozin + Metformin on changes in serum LDL level after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats. Values are expressed as Mean ± SEM (n=6) in the group. *P<0.05, **P<0.01, ***P<0.001 considered statistically significant as compared to control group

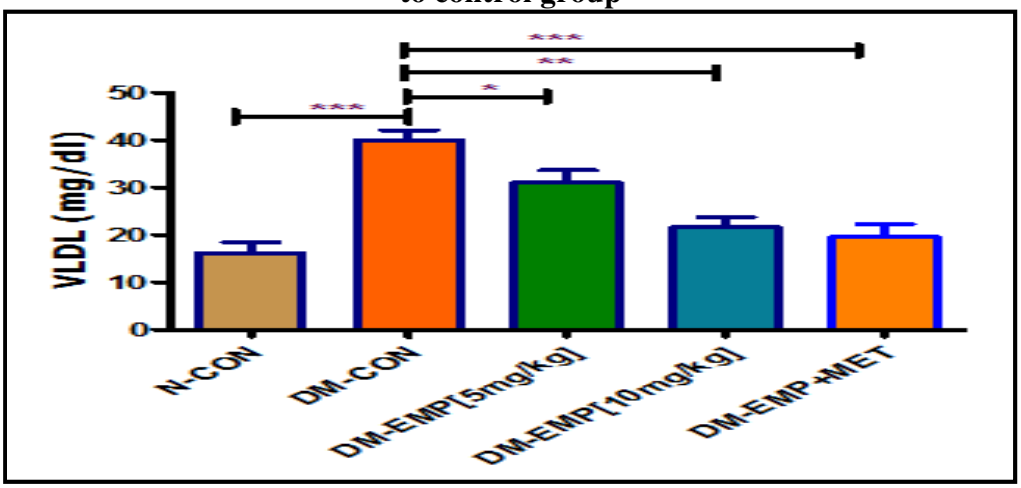

Figure No.8: Effect of Empagliflozin + Metformin on changes in serum VLDL level after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats. Values are expressed as Mean ± SEM (n=6) in the group. *P<0.05, **P<0.01, ***P<0.001 considered statistically significant as compared to control group

CONCLUSSION

Our results demonstrate that treatment with two different doses of Empagliflozin that means both lower and higher dose of 5 mg/kg and 10 mg/kg respectively were able to improve hyperglycemia and decrease the level of different biochemical parameters like CK-MB, LDH, AST and Troponin from cardiac markers and lipid profile (TC, TG, LDL and VLDL). From lipid profile there is exception in case of HDL-cholesterol level; it was increased with both doses of Empagliflozin. We have seen that Empagliflozin 10 mg/kg was more effective in both improving hyperglycemia and decreasing different biochemical parameters, but both doses had shown the same result in case of Troponin I which is negative (<1.5 ng/ml) as normal control rats.

But the combined treatment with Empagliflozin 5 mg/kg and Metformin was able to improve the hyperglycemia and decrease the level of CK-MB, LDH, AST, Troponin and lipid profile (TC, TG, LDL and VLDL), with increased level of HDL-cholesterol level even greater than treatment with individual drug.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Department of Pharmacology, Parul Institute of Pharmacy and Research, Parul University, Vadodara, Gujarat, India for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Rajesh R, Patel Naren, *et al.* SGLT-2 inhibitors A new sword for the treatment of T2DM, *International Journal of Pharma Sciences and Research,* 1(2), 2010, 139-147.
2. Mancia G, Fagard R, Narkiewicz K, Redán J, Zanchetti A, Böhm M, Christiaens T, Cifkova R, De Backer G, Dominiczak A, Galderisi M, Grobbee DE, Jaarsma T, Kirchof P, Kjeldsen S E, Laurent S, Manolis A J, Nilsson P M, Ruilope L M, Schmieder R E, Sirnes P A, Sleigh. ESH/ESC Task Force for the Management of Arterial Hypertension, *J Hypertens,* 31(10), 2013, 1925-1938.
3. Shaw J E, Sicree R A, Zimmet P Z. Global estimates of the prevalence of diabetes for 2010 and 2030, *Diabetes Res Clin Pract,* 87(1), 2010, 4-14.
4. World Health Organization (WHO), *Cardiovascular disease,* 2016.
5. Go A S, Mozaffarian D, Roger V L, *et al.* Heart disease and stroke statistics--2014 update: a report from the American Heart Association, *Circulation,* 129(3), 2014, 28-29.
6. Martin-Timon I, Sevillano Collantes C, Segura-Galindo A, *et al.* Type 2 diabetes and cardiovascular disease: have all risk factors the same strength? *World J Diabetes,* 5(4), 2014, 444-470.
7. Aylsworth A, Dean Z, Van Norman C, *et al.* Dapagliflozin for the treatment of type 2 diabetes mellitus, *Ann Pharmacother,* 48(9), 2014, 1202-1208.
8. Sowers J R, Epstein M, Frohlich E D. Diabetes, hypertension, and cardiovascular disease an update, *Hypertension,* 37(4), 2001, 1053-1059.
9. Adeniyi O V, Yogeswaran P, Longo-Mbenza B, *et al.* Uncontrolled hypertension and its determinants in patients with concomitant type 2 diabetes mellitus in rural South Africa, *PLoS One,* 11(3), 2016, 764-768.
10. Guidance for industry: diabetes mellitus - evaluating cardiovascular risk in new antidiabetic therapies to treat type 2 diabetes, 2008, *Food and Drug Administration (Center for Drug Evaluation and Research),* 2014.
11. Guideline on clinical investigation of medicinal products in the treatment or prevention of diabetes mellitus, *European Medicines Agency,* 2014.
12. Johansen O E. Cardiovascular disease and type 2 diabetes mellitus: a multifaceted symbiosis, *Scand J Clin Lab Invest,* 67(8), 2007, 786-800.

Please cite this article in press as: Jagdish Kakadiya *et al.* Protective effect of empagliflozin alone and its combination with metformin in experimentally induced myocardial infarction in diabetic rats, *Asian Journal of Phytomedicine and Clinical Research,* 7(2), 2019, 88-99.