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CARDIO PROTECTIVE POTENTIAL OF EMPAGLIFLOZIN AND LINAGLITIN IN EXPERIMENTALLY INDUCED CARDIOTOXICITY IN RATS: A RESEARCH STUDY

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ABSTRACT

Objective: The goal of this research was to evaluate the individual cardio-protective properties of Lignagliptin and Empagliflozin as well as their combination effects on a rat experimental model of cardiotoxicity.

Materials and Methods: Out of the animals, five groups of six each were formed. For 15 days, Group I was given the medium (0.5% carboxy methyl cellulose) and functioned as the standard control group. On days 14 and 15, Isoproterenol (85 mg/kg, s.c.) was given to Group II, which acted as the disease control group. As a drug-treated group, Group III received Isoproterenol (85

mg/kg, s.c.) on the 14th and 15th days as well as Empagliflozin (10 mg/kg/day, p.o.) for 15 days. Linagliptin (5 mg/kg/day, p.o.) for 15 days and isoproterenol on the 14th and 15th days were administered to Group IV as part of their drug treatment regimen. Group V served as the drug-treated group and got isoproterenol (85 mg/kg, s.c.) on the 14th and 15th days along with a 15-day combination of Empagliflozin (10 mg/kg) and linagliptin (5 mg/kg) administered orally.

Result: At the end of the treatment period, animals were anaesthetized with an aesthetic ether after final drugs dose administration and then blood was collected from the retro-orbital plexus for estimation of different biochemical parameters like blood glucose (BG), creatine kinase MB (CK-MB), lactate dehydrogenase (LDH), Aspartate amino transferase, High sensitivity C reactive protein (HSCRP), lipid profile, animals were euthanized and hearts were isolated for Histopathological examination.

Conclusion: The results showed that Empagliflozin and Linagliptin together had better cardio protective benefits along with positive impacts on cardiotoxicity.

Keywords: Cardiotoxicity, Empagliflozin, Linagliptin, Isoproterenol

INTRODUCTION

Damage to the heart muscles known as cardiotoxicity can prevent the heart from properly pumping blood throughout the body. Another undesirable effect of several medications is cardiotoxicity. The typical mechanism causing cardiotoxicity is the release of free radicals, which raises oxidative stress and causes hypoxia. There are two approaches to comprehend a drug's cardio toxic effects **[1]**. Cardiotoxicity can take many distinct forms, including those that are reversible, irreversible, chronic, acute, and late-onset. Life expectancy and overall survival are impacted by cardiotoxicity. Type 1 permanent impairment and Type 2 curable damage are the two categories for direct damage. Type-1 typically results from a cumulative dose, but type-2 is unrelated to a cumulative dose **[2]**. Heart failure has a wide range of additional causes. Radiation and some chemotherapy medications can also cause cardiotoxicity **[3]**. Other forms of cardiotoxicity, such as bradycardia, QT prolongation, myocardial ischemia, and cardiomyopathy, have also been reported. One type of anti-cancer medication may also result in other forms of cardiotoxicity **[4]**. Rapid drug administration causes high blood levels and could cause greater coronary artery disease than if the identical dose was administered gradually. When compared to bigger doses, smaller doses of the treatment

given regularly may reduce toxicity **[5]**. The heart is strongly stimulated by isoproterenol (ISO), which also increases the heart's pace, force, and cardiac output. Because it causes cardiac necrosis, isoproterenol is regarded as a cardio toxic medication. Makes its way of ISO in rats causes typical cardiac gene expression comparable to that seen in pressure-induced ventricular hypertrophy **[6]**. Myocardial hypertrophy and widespread myocardial necrosis were caused by high dosages of ISO in the 85–100mg/kg range. Similar myocardial damage to acute myocardial infraction was brought on by a high dose of ISO in rats. Acute coronary syndrome infraction-induced cardiac failure could be modelled with high doses of ISO **[7]**. On this brand-new family of medications, numerous clinical trials are currently being conducted; among them, the EMPA-REG OUTCOME trial revealed that empagliflozin causes a relative risk. Reduction of 38% in CV mortality over placebo at 3.1 years of median follow-up. The key unfavourable CV outcome of CV mortality, fatal and non - fatal heart attack, and nonfatal stroke was subsequently reduced by 14% as a result of this. Given that the more over 99% of the patients included in EMPA-REG OUTCOME had preexisting CV disease, it is important to interpret this dramatic decrease in CV mortality with caution and to consider the possibility that it may not hold to all T2DM patients. Empagliflozin ability to reduce infarction and cardiac mortality by 14% was demonstrated in a post-marketing clinical investigation involving 7000 patients **[8]**. Numerous extensive clinical tests with DPP-4 inhibitors have been conducted with the goal of evaluating their cardiovascular effects. There have been 20,312 and 13,560 people overall both the comparator and the DPP-4 inhibitors arm of the patient. Comparing DPP-4 inhibitors to a placebo or another form of treatment, a rather significant 31% lower risk of severe cardiovascular events was found **[9]**.

MATERIALS AND METHODS

Drugs and Chemicals

Cyman Chemistry, a firm in Mumbai, India, is where the medication Empagliflozin was obtained. And from Mumbai, India's Clearsynth Labs Pvt. Ltd., Linagliptin was bought. In the whole investigation, only analytical-grade chemicals and reagents were employed. Carboxy methyl cellulose (CMC) was purchased from the Parul Institute of Pharmacy and Research's store along with isoproterenol (ISO) (Sigma hemials, St. Louis, MO, U.S.A.).

Experimental animals

Healthy Albino female adult, Wistar rats averaging 170-225 g were employed.

Polypropylene cages were utilized to hold the rats. Rats were housed in predetermined lab conditions, which included a 24-degree temperature range, a 12-hour cycle of light and darkness, and a moisture range of 35–60%. Rats were given unrestricted access to pellet food and filtered water. The Institutional Animal Ethics Committee (IAEC) of the Pharmacology Department at Parul Institute of Pharmacy and Research, as well as the Committee for the Purpose of Control and Supervision of Experiments on Animals, (CPCSEA)gave their approval to all of the experiments and protocols detailed in the current study (**Protocol No.:984/2019-02**).

Model of cardiotoxicity induction.

Two doses of isoproterenol are subcutaneously (s.c.) provided every 24 hours, and after the last dose, the heart is dissected for additional parameter measurement. Isoproterenol causes heart necrosis when administered at a dose of 85 mg/kg, however other greater doses are also used to cause heart necrosis. On the fourteenth and fifteenth days of the trial, the medication was given to rats to induce cardiotoxicity. Animals were put to death at the conclusion of the treatment after blood samples from the retro orbital plexus were collected. Histopathological research was done on collected hearts.

Design of Experiment

Each of the following groups, each of which had six animals, was given a treatment period of 15 days during the course of the entire study.5% carboxy methyl cellulose was administered as a vehicle to the healthy control group-1 for a period of 15 days. Disease control group 2 received isoproterenol (85 mg/kg, s.c.) on the 14th and 15th study days in addition to 0.5% carboxy methyl cellulose as a carrier for 15 days. Group 3 was given Empagliflozin (10 mg/kg/day, p.o. for 15 days) before being given Isoproterenol. Group 4 was given Linagliptin (5 mg/kg/day, p.o. for 15 days) followed by Isoproterenol. Group 5: Empagliflozin (10 mg/kg/day, orally for 15 days) and Linagliptin (5 mg/kg/day, orally for 15 days) were administered, followed by isoproterenol.

Biochemical parameters

Enzymatic kit was used for in vitro quantitative evaluation of glucose activity in serum (ERBA diagnostic Mannheim, Transasia bio-medicals Ltd. India). In order to isolate the serum, blood samples were taken. Enzymatic kit was applied for in vitro quantitative assessment of Total cholesterol (TC), LDH, HDL, & VLDL activity in serum (ERBA diagnostic Mannheim, Transasia bio-medicals Ltd. India). A kit of Reckon Diagnostics (India) Pvt. Ltd. has been used to

quantify the level of serum triglycerides in vitro. Cardiac troponin I & Creatine kinase (CK-MB) activity in serum was quantitatively evaluated in vitro to use an enzymatic kit (POINTE scientific, Inc. Ltd, India). Enzymatic kit used for in vitro quantitative measurement of High sensitivity C reactive protein (HSCRP) activity in serum (ERBA diagnostic Mannheim, Transasia bio-medicals Ltd, India).

Histological evaluation

Rat hearts that had been removed and kept in 10% formalin with a neutral buffer were from rats that had been put to death after the therapy. The tissues were washed completely in 70% alcohol after 24 hours, then dehydrated in ascending alcohol concentrations (70–100%). The tissues were dehydrated in 100% alcohol, then treated with a 50/50 solution of toluene and xylene, a paraffin wax solution in toluene, and finally 100% paraffin wax (60–62°C), with the tissue subsequently being embedded in wax. On a horizontal plane, pieces between 5 and 15 m thick were successively cut using a Leitz microtome and albumin in a glycerol solution (50% v/v). The staining was then intensified by dipping them in 10% hematoxylin for three to five minutes after colouring them.

Statistical analysis

Every value is expressed as a mean S.E.M. One-way ANOVA was used to examine the statistical significance of differences between more than two groups, and then the one-sample Wilcoxon examine or the unpaired two-tailed student's T-test, as necessary, were performed using a computer-based fitting programme (Prism, Graph Pad 8.1.0). Group II was compared to Group I, while Group III, IV, and V were compared to Group II. When p 0.05 was reached, differences were deemed statistically significant.

RESULTS

Empagliflozin's and Linagliptin's protective effects were determined by examining blood sugar indicators, cardiac biomarker enzymes such as CK-MB, LDH, Troponin I, and HSCRP, as well as the lipid profile (TC, TG, LDL, and VLDL), and histopathology **(Table 1, 2)**.

Effect of Empagliflozin, Linagliptin, and their combination (Empa+Lina) on changes in serum Troponin I level after completion of study in normal and ISO induced cardiotoxicity in rats **(Figure 1)**.

Effect of Empagliflozin, Linagliptin, and their combination (Empa+Lina) on changes in serum HSCRP level after completion of study in normal and ISO induced cardiotoxicity in rats **(Figure 2)**.

The Histopathological Study

Normal structure of the cardiac cells was observed no necrosis in normal control group. Highest necrosis in disease control group. Empagliflozin treatment group showed less necrosis as compared to Linagliptin treated group. Combination of Empagliflozin+Linagliptin showed less necrosis when compared to Empagliflozin and Linagliptin treated group **(Figure 3)**.

Table 1: Effect of Empagliflozin, Linagliptin, and its combination after completion of study

Group	Treatment	Dose	Glucose (mg/dl) Mean± SEM	CK-MB (U/L) Mean± SEM	LDH (U/L) Mean± SEM
I	Normal Control	--	100.5±7.030	18.50±1.190	175±6.445
II	Disease control	85 mg/kg	82.00±3.136	27.50±1.443	220± 12.25
III	Empagliflozin (Treatment Group-1)	10mg/kg	97.60±9.772	21.50±1.323	18.75 ±3.227
IV	Linagliptin (Treatment Group-2)	5mg/kg	96.25±10.06	23.50 ±1.190	190.5± 2.630
V	Empagliflozin + Linagliptin (Treatment Group-3)	10mg/kg + 5mg/kg	90.06 ± 4.262	19.75±0.8539	196.0± 2.739

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**

Table 2: Effect of Empagliflozin, Linagliptin, and its combination on lipid profile after completion of study.

Group	Treatment	Dose	TC (mg/dl) Mean ±SEM	TG (mg/dl) Mean ±SEM	HDL (mg/dl) Mean ±SEM	LDL (mg/dl) Mean ±SEM	VLDL (mg/dl) Mean ±SEM
I	Normal Control	--	103.5±14.01	49.50±2.102	20.50± 2.102	86.75±1.702	20.50±2.104
II	Disease control	--	203.5±14.01	178±24.01	35.25 ±2.056	160.5±2.46	35.83±2.227
III	Empagliflozin (Treatment Group-1)	5mg/kg	151.8±8.64	96.25± 6.169	26.25±3.119	146.8±2.68	26.25±3.595
IV	Linagliptin (Treatment Group-1)	10mg/k g	132.8±6.42	59.58±1.530	25±2.041	146.8±2.68	25±2.232
V	Empagliflozin+ Linagliptin (Treatment Group-1)	5mg/kg+ 50mg/k g	122±7.450	47.84±1.295	23.00±2.852	139.3±2.83	23±2.078

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.**

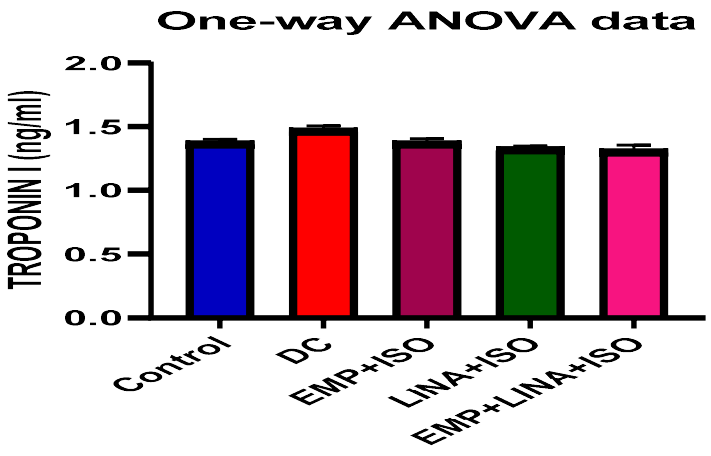

Figure 1: Effect of Empagliflozin, Linagliptin and its combination on Troponin I level in rats
Values are expressed as Mean± SEM (n=6) in the group. ****P<0.0001, ****P<0.0001, ****P<0.0001 considered statistically significant as compared to control group.

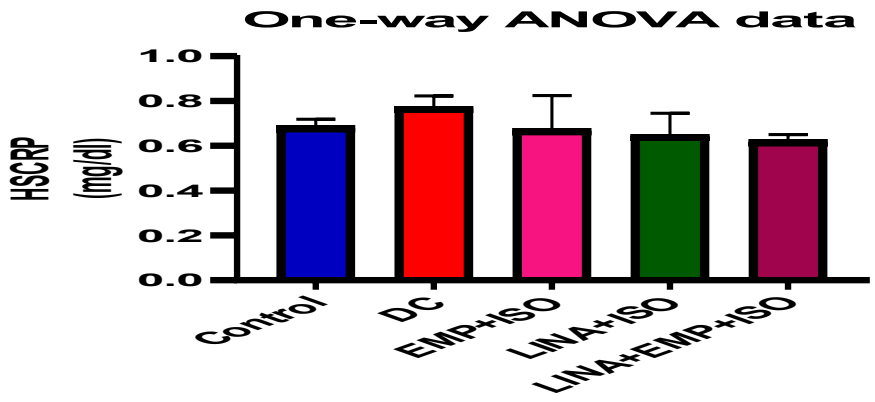

Figure 2: Effect of Empagliflozin, Linagliptin and its combination on HSCRP level in rats
Values are expressed as Mean± SEM (n=6) in the group. **P<0.002, **P<0.006, ****P<0.0001 considered statistically significant as compared to control group.

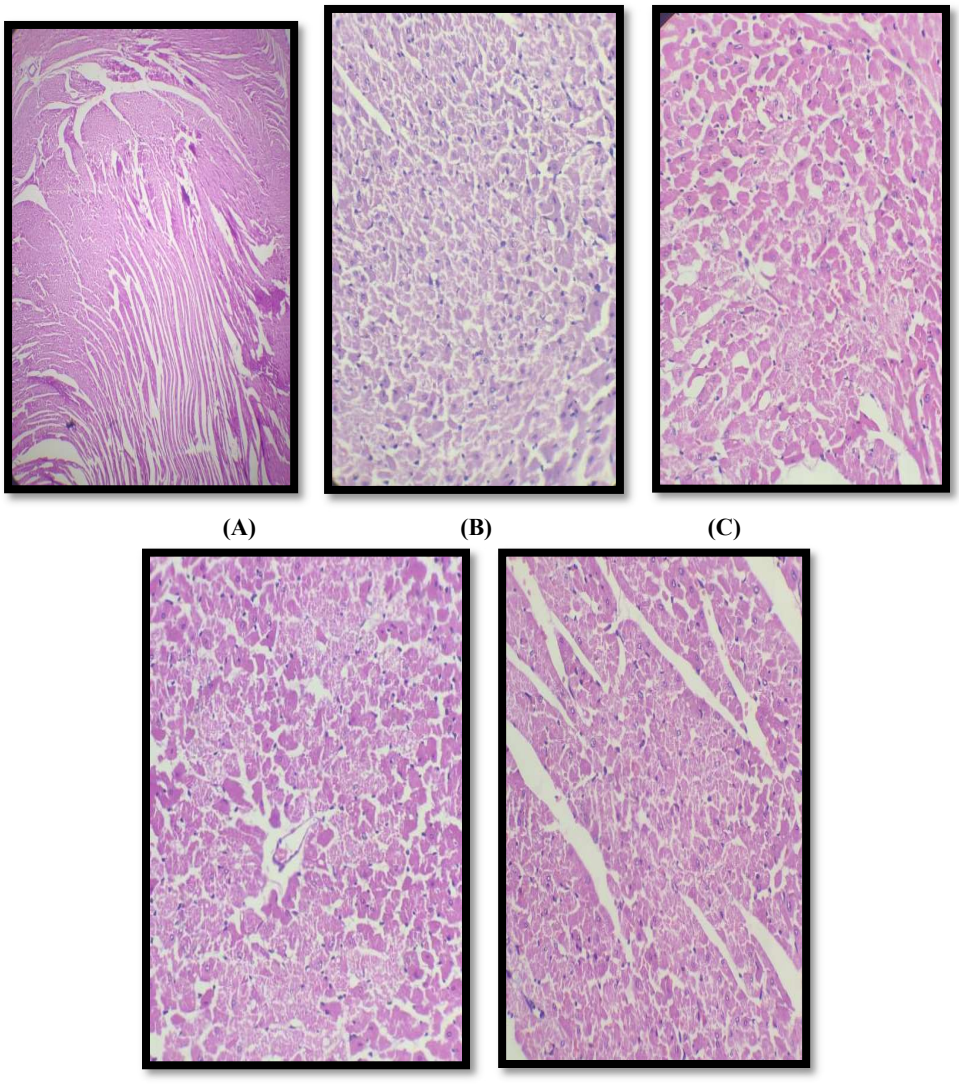

Figure 3: Histopathological study of the Heart, (A) Normal Control group, (B) Disease Control Group, (C) Treatment Group1, Empagliflozin and Isoprenaline treated group(D) Treatment group 2, Linagliptin and isoprenaline treated group (E) Treatment group 3, Combination of Empagliflozin and isoprenaline treated group

DISCUSSION

Cardiotoxicity was created in this investigation by administering isoproterenol twice during a 24-hour period at a dose of 85 mg/kg s.c. on days 14 and 15. After 24 hours after the final dosage of isoproterenol in the treatment groups, rats were divided into three groups and given treatments with Emagliflozin 10 mg/kg, p.o., Linagliptin 5 mg/kg, p.o., and combinations of Empagliflozin+Linagliptin 10+5 mg/kg, p.o. According to our study's findings, both the control group and additional treatment groups had normal blood glucose levels.

Cardiotoxicity results in a rise in cardiac biomarkers such as CK-MB, LDH, Troponin I, HSCRP, and others. Compared to the normal group, the disease control group's CK-MB and LDH levels in this study dramatically increased and lower the raised levels of CK-MB and LDH in all treatment groups, although we noticed different degrees of inhibition in some cases. For example, Empagliflozin and Linagliptin treatments showed less inhibition when compared to the disease control group when it came to inhibiting the elevated level of CK-MB. Comparing the Empagliflozin and Linagliptin combination to the other two groups, more inhibition was seen.

The normal control had troponin I (1.5 ng/ml). Troponin I levels considerably increased during disease management (>1.5 ng/ml). In the therapy groups receiving empagliflozin, ligandliptin, or their combination, there was no troponin I. This data unambiguously shows that empagliflozin (10 mg/kg), lignagliptin (5 mg/kg), and their combination reduce troponin I levels compared to the control group. Blood vessel inflammation causes the body to create HSCRP. Additionally, it can result from artery inflammation, which can cause heart attacks, strokes, and the creation of plagues. In the disease control group, compared to the normal group, the level of HSCRP was considerably higher. On a higher level of HSCRP, treatment groups displayed inhibition.

The benefits of SGLT-2 inhibitors and DPP-4 inhibitors on glycemic control, cardiovascular risk factors, weight loss, blood pressure reduction, and other lipid profile impacts have been demonstrated.

CONCLUSION

Empagliflozin and Linagliptin and combination of both drugs Empagliflozin+Linagliptin were found to be cardio protective and showed cardiac potential in Isoproterenol induced cardiotoxicity in rats. And also decreased the level of different biochemical parameters like cardiac

markers CK-MB, LDH, HSCRP and Troponin-I and lipid profile (TC, TG, LDL and VLDL). In lipid profile there is exception in case of HDL-cholesterol level; it was increased with both Empagliflozin and Linagliptin separately, but it was reduced in combination of Empagliflozin and Linagliptin but the combined treatment with Empagliflozin and Linagliptin was able to improve the cardiotoxicity and decrease the level of CK-MB, LDH, Troponin and lipid profile (TC, TG, LDL and VLDL), with increased level of HDL-cholesterol level even greater than treatment with individual drug. Histopathological study indicates that combination of Empagliflozin and Linagliptin showed less cardiac damage as compared to Empagliflozin and Linagliptin alone.

In conclusion, our study shows that the use of empagliflozin and Linagliptin and their combination proved to be more efficient in reducing the severity of myocardial damage and significantly decreasing oxidative damage during an infarction induced by isoproterenol in rats.

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Conflict of Interest

There are no conflicts of interest

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