INTERNATIONAL JOURNAL OF
PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

EVALUATION OF CARDIO PROTECTIVE EFFECT OF LINAGLIPTIN A NOVEL DPP-4 INHIBITOR IN NORMAL AND STREPTOZOTOCIN INDUCED TYPE-2 DIABETIC RATS BY ISCHEMIC REPERFUSION INJURY METHOD

B. ANITHA[1], K. NAGA RANI[1], P. VENKATESWARA RAO[2]

1. Department of Pharmacology, St.Mary's Group of Institutions Guntur, Chebrole (V&m), Guntur, Andhra Pradesh India - 522212
2. Department of Pharmaceutical Chemistry, St.Mary's Group of Institutions Guntur, Chebrole (V&m), Guntur, Andhra Pradesh India – 522212

Accepted Date: 20/08/2017; Published Date: 27/08/2017

Abstract: Evaluation of the cardio protective effect of Linagliptin a novel DPP-4 inhibitor which is used for the treatment of diabetes mellitus by the method ischemic reperfusion injury in normal and streptozotocin induced rats. To evaluate the cardio protective effect of Linagliptin in normal and type 2 diabetic rats by ischemic reperfusion injury using the following parameters. Evaluation of percentage left ventricle necrosis. Estimation of tissue antioxidant parameters like SOD, Catalase and GSH. Estimation of blood parameters like SGOT, CK, CK-MB and LDH. The Cardio protective effect of Linagliptin (5 mg/kg bd. wt p.o) was observed in normal rats by comparing the percentage of necrosis of left ventricle with normal I/R group, by using Left descending coronary artery ligation. Microscopical examination of the left ventricle proves that the Linagliptin having the cardio protective effect by protecting the myocytes against the oxidative stress. Finally Cardio protective effect of Linagliptin was noticed at acute levels of study.

Keywords: Linagliptin, Diabetes mellitus, Cardio protective action, Left ventricle necrosis.

Corresponding Author: MS. B. ANITHA

Access Online On:

www.ijprbs.com

How to Cite This Article:

B. Anitha, IJPRBS, 2017; Volume 6(4): 191-204

PAPER-QR CODE

INTRODUCTION

Diabetes is a group of metabolic disorders characterized by abnormal energy metabolism, which results most notably in hyperglycemia and dyslipidemia, due to defects in insulin secretion, insulin action, or both. It is recognized as a major health problem affecting millions of people and predisposing to micro and macro-vascular complications including coronary heart disease. (pittas *et al.,* 2003)[1] . It is a chronic disease without a cure, and it is associated with significant morbidity and mortality. Acute complications are due to severe hyperglycemia. Chronic complications are characterized by damage, dysfunction, and eventual failure of various organs, especially the eyes, kidneys, nerves, heart, and brain. The common denominator is vascular damage. A tight glycaemic control reduces the morbidity and mortality associated to type2 diabetes, but has proven challenging and is usually not sustained.

2. MATERIALS AND METHODS

ANIMALS:

Male Wister rats weighing 200-250 g were housed in groups of 4-5 on a 12-hour light-dark cycle with standard rat chow and water available ad libitum. The animals were subjected to sham-surgery or left coronary artery ligation. All experiments were carried out after approval of the Institutional Animal Ethical Committee (IAEC) 1362/C/10/CPCSEA for the use of experimental animals and conform to the Guide for Care and Use of Laboratory Animals.

DRUGS AND CHEMICALS:

Linagliptin purchased from Boehringer Ingelheim Mumbbai, STZ obtained from Sigma Aldrich, Germany. Nicotinamide obtained from Sigma Aldrich, Germany. All other reagents and chemicals used were of analytical grade were procured locally.

INSTRUMENTS USED:

- Rat Ventilator
- Teflon homogenizer
- Cooling centrifuge (Remi)
- Bio analyser (Eco prietest)
- UV/Vis spectro photo meter (Labindia UV 3092)
- Weighing balance (Dhona 200D)

MATERIALS USED:

- Tuberculin syringes
- Insulin syringes
- Rat oral feeding tube
- Suture needles no-18
- Surgical blades no-11
- Silk thread no-30
- Cotton threads no-40

3. RESULTS

Table-1: Effect of linagliptin on % Left ventricle necrosis

s.no	Treatment groups	% LV necrosis
1	Normal sham control group	3±0.09
2	Normal I/R group	64±0.13
3	Normal + Treatment I/R group	5±0.17
4	Diabetic sham control group	10±0.11
5	Diabetic I/R group	70±0.19
6	Diabetic + treatment I/R group	9±0.23

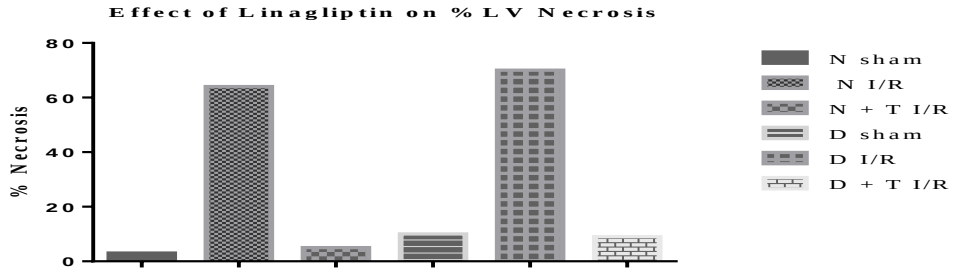


Fig-1 : Effect of Linagliptin on %LV Necrosis

Table 2: Effect of linagliptin on SOD levels

s.no	Treatment groups	SOD
1	Normal sham control group	17.5±0.07
2	Normal I/R group	12.33±0.13
3	Normal + Treatment I/R group	17.88±0.31
4	Diabetic sham control group	25.92±0.26
5	Diabetic I/R group	11.64±0.19
6	Diabetic + treatment I/R group	21.60±0.25

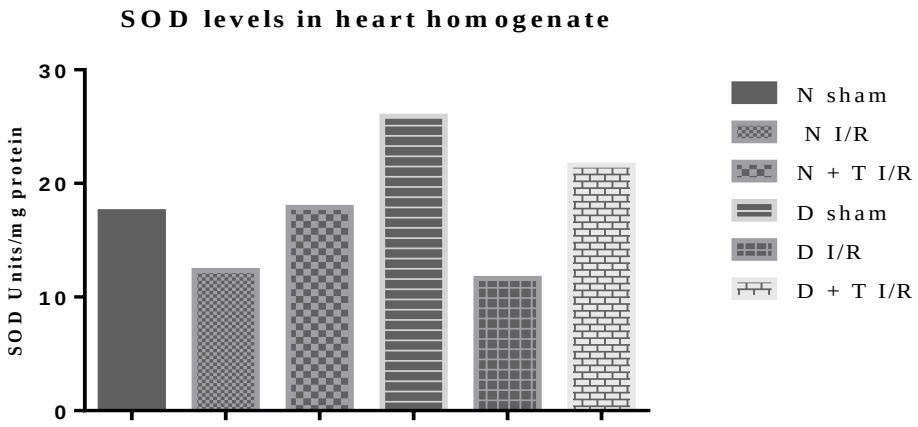


Fig 2: Effect of Linagliptin levels on SOD

Table 3: Effect of linagliptin on Catalase levels

s.no	Treatment groups	Catalase
1	Normal sham control group	0.019±0.03
2	Normal I/R group	0.008±0.15
3	Normal + Treatment I/R group	0.031±0.19
4	Diabetic sham control group	0.029±0.05

5	Diabetic I/R group	0.007±0.11
6	Diabetic + treatment I/R group	0.048±0.07

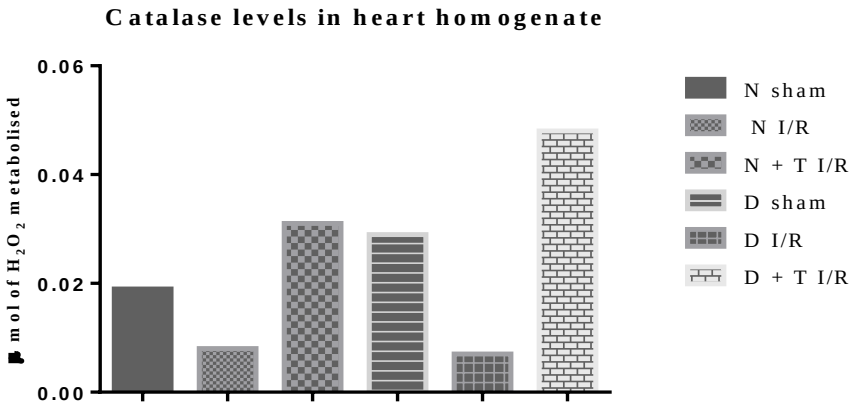


Fig 3: Effect of Linagliptin on Catalase

Table 4: Standard values of GSH

s.no	Concentration of GSH (µg/ml)	Absorbance
1	100	0.61
2	200	1.25
3	300	1.86
4	400	2.51
5	500	3.20
6	600	3.79

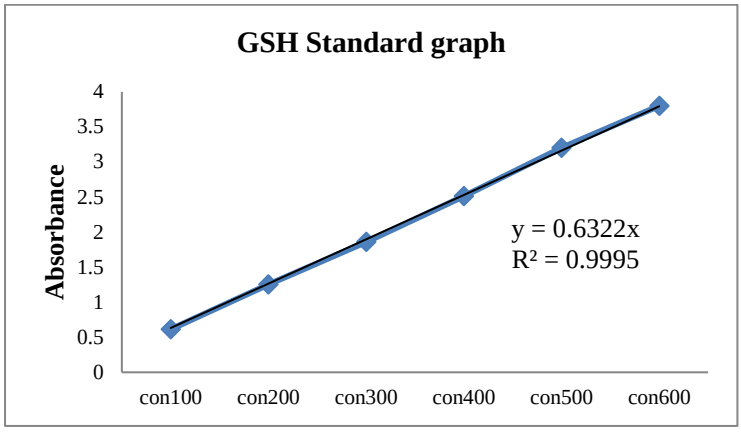


Fig 4: GSH standard graph

Table 5: Effect of linagliptin on GSH

s.no	Treatment groups	GSH (µg/ml)
1	Normal sham control group	100±0.25
2	Normal I/R group	55±0.37
3	Normal + Treatment I/R group	140±0.29
4	Diabetic sham control group	115±0.31
5	Diabetic I/R group	61±0.18
6	Diabetic + treatment I/R group	165±0.22

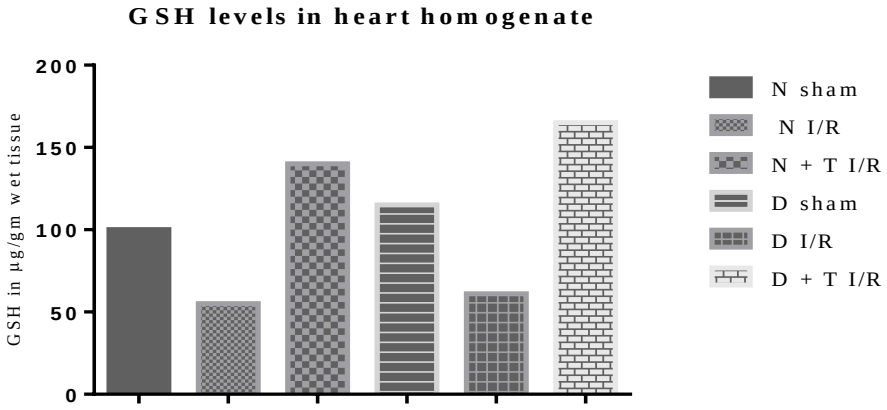
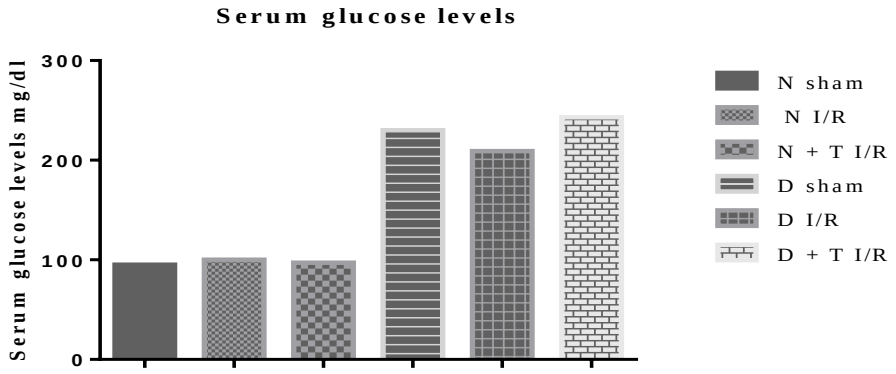


Fig 5: Effect of Linagliptin on GSH

Table 6 : Serum glucose levels in normal and diabetic animals

s.no	Treatment groups	Glucose(mg/dl)
1	Normal sham control group	95±0.05
2	Normal I/R group	100±0.07
3	Normal+ Treatment I/R group	97±0.03
4	Diabetic sham control group	230±0.12
5	Diabetic I/R group	209±0.09
6	Diabetic+ treatment I/R group	243±0.17



Blood glucose levels

Fig 6: Effect of Linagliptin on serum glucose levels

Table 7: Effect of linagliptin on serum SGOT levels

s.no	Treatment groups	SGOT(U/L)
1	Normal sham control group	33±0.09
2	Normal I/R group	327±0.04
3	Normal + Treatment I/R group	146±0.07
4	Diabetic sham control group	67±0.15

5	Diabetic I/R group	358±0.06
6	Diabetic+ treatment I/R group	109±0.05

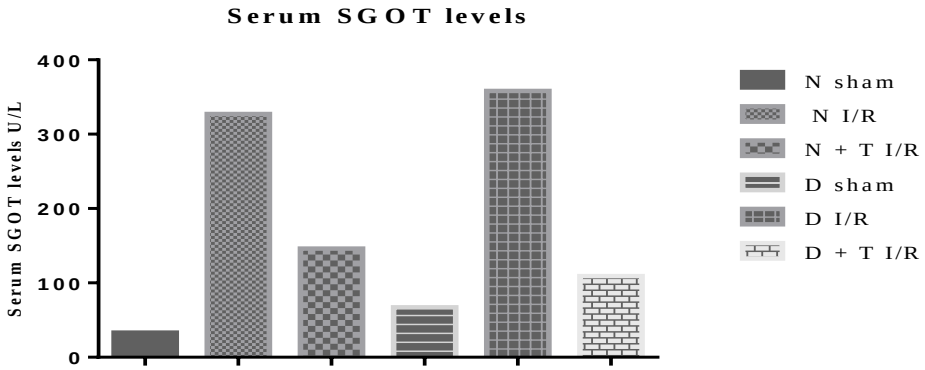


Fig 7: Effect of linagliptin on serum SGOT levels

Table 8 : Effect of linagliptin on serum CK levels

s.no	Treatment groups	CK(U/L)
1	Normal sham control group	189±0.07
2	Normal I/R group	1490±0.13
3	Normal + Treatment I/R group	988±0.21
4	Diabetic sham control group	279±0.17
5	Diabetic I/R group	1968±0.29
6	Diabetic + treatment I/R group	576±0.19

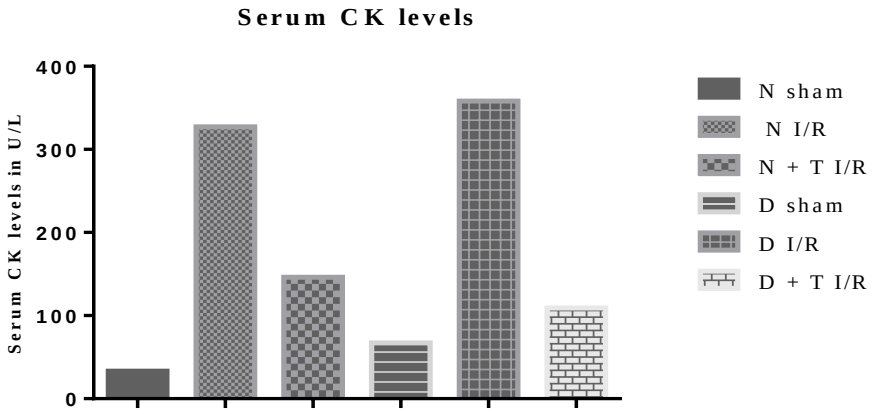


Fig 8: Effect of Linagliptin on serum CK levels

Table 9: Effect of linagliptin on serum CK-MB levels

s.no	Treatment groups	CK-MB(U/L)
1	Normal sham control group	26.5±0.13
2	Normal I/R group	1900±0.27
3	Normal + Treatment I/R group	463±0.19
4	Diabetic sham control group	56±0.16
\5	Diabetic I/R group	2106±0.39
6	Diabetic + treatment I/R group	459±0.09

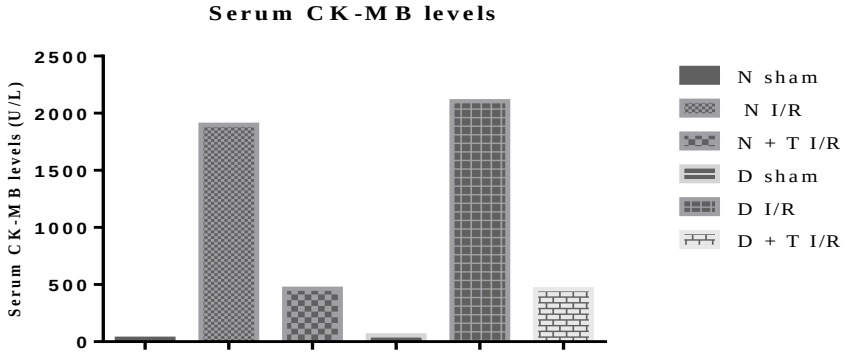


Fig 9: Effect of Linagliptin on serum CK-MB levels

Table 10: Effect of linagliptin on serum LDH levels

s.no	Treatment groups	LDH(U/L)
1	Normal sham control group	306±0.17
2	Normal I/R group	2519±0.23
3	Normal + Treatment I/R group	1004±0.29
4	Diabetic sham control group	459±0.17
5	Diabetic I/R group	2753±0.36
6	Diabetic + treatment I/R group	737±0.31

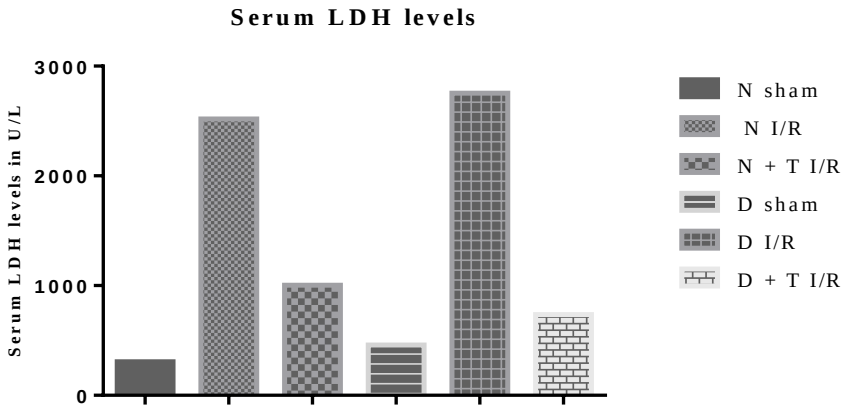
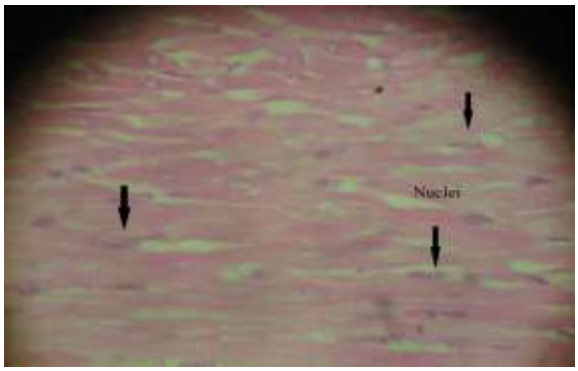
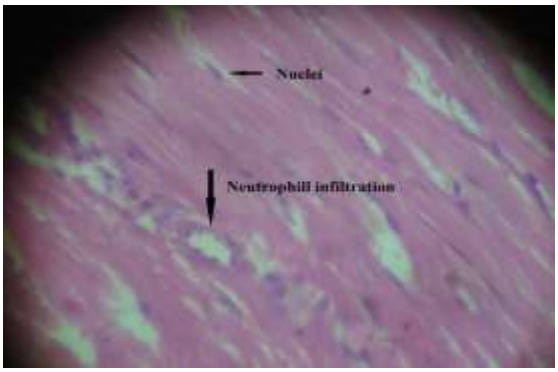


Fig 10 : Effect of Linagliptin on serum LDH levels





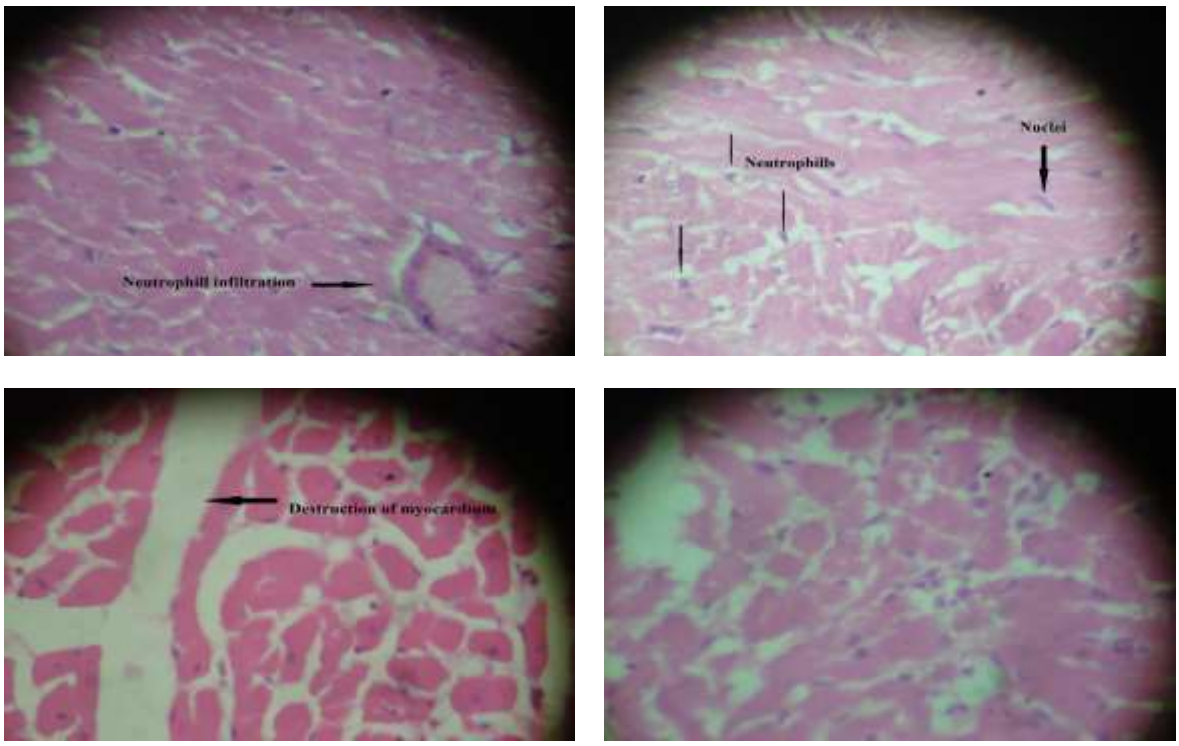


Fig 11: Microscopical examination of left ventricle

TTC stained sections of left ventricle: (A) represents the Normal sham control group showing normal anatomy of myocardium. (B) Represents the Normal I/R group showing the neutrophil infiltration into blood vessels significantly. (C) Represents the Normal + treatment I/R group which was also showing neutrophil infiltration but markedly less when compared with normal I/R group. (D) Represents diabetic sham control group showing neutrophils outside the nuclei of myocytes. (E) Represents diabetic I/R group showing the complete destruction of myocardium. (F) Represents diabetic + treatment I/R group in which linagliptin shows the moderate protection of myocardium.

4. DISCUSSION

Linagliptin is a new DPP-4 inhibitor which is used for the treatment of type-2 diabetes. In general, diabetic patients are more prone to the cardiovascular comorbidities and other micro and macro vascular complications. Despite its benefits on glycaemic control, the long term cardiovascular safety of DPP-4 inhibitor is to be evaluated. I investigated the effect of DPP-4 inhibitor Linagliptin in Acute myocardial infarction (I/R method) in normal and diabetic rats.

In the present investigation, a significant increase of infarct size in the normal control I/R group, compared with the sham control and treatment I/R groups as well as in diabetic groups. The results clearly show the presence of cytotoxic free radical activity because there is a loss of

membrane integrity, with disintegration of polyunsaturated fatty acids in the membrane bilayer, which exerts unfavourable effects on the heart structure and function.

Endogenous antioxidant enzymes such as SOD, CAT, and GSH are the first-line cellular defence against oxidative stress, decomposing oxygen and Hydrogen Peroxide(H_2O_2) before they interact to form the more reactive hydroxyl radical (OH). The equilibrium between these enzymes is an important process for the effective removal of oxygen stress in intracellular organelles. SOD and CAT are important antioxidant enzymes in mitigating free radical-induced cell injury.

A decrease in the activity of SOD and CAT can result in the decreased removal of superoxide ion and Hydrogen Peroxide (H_2O_2) radicals that brings about a number of reactions, which are harmful to myocardium. Superoxide is inactivated by SOD, the only enzyme known to use a free radical as a substrate..

An increase in SOD activity is beneficial in the event of increased free radical generation. However, it has been reported that a rise in SOD activity, without a concomitant rise in the activity of CAT, may be detrimental because SOD generates Hydrogen Peroxide H_2O_2 as a metabolite, which is more cytotoxic than oxygen radicals, and must be scavenged by CAT (Yim et al., 1990). Thus, a simultaneous increase in CAT activity is essential for an overall beneficial effect of an increase in SOD activity (Harman 1991)[91]. In the present study, during ischemia-reperfusion injury, the levels of endogenous antioxidant enzymes (SOD, CAT, and GSH) in heart tissue were decreased significantly compared with normal and diabetic animals which are treated with Lingliptin. In the treatment groups, Linagliptin antagonized the decrease in endogenous antioxidant enzyme levels and produced beneficial effects.

Cytosolic enzymes leak into the intracellular space, where there is cell membrane damage (Mueller et al., 1977)[92] and (Lombardini 1980)[93]. Increased levels of SGOT, CK, CK-MB and LDH are well-known diagnostic markers of myocardial infarction. In the present study, marked elevations in the level of these enzymes in the serum of the control ischemia-reperfusion group suggest the occurrence of considerable membrane damage compared with the sham control group. Treatment with Linagliptin resulted in a significant reduction in the levels of these enzymes toward near normal levels compared with the control ischemia-reperfusion group.

In microscopical examination of the left ventricle we observed that the Linagliptin shows the protection against the destruction of the myocardium in normal and diabetic I/R group and treatment I/R group because the review literature shows that Linagliptin having the antioxidant property so that it protects the myocytes against the oxidative stress.

The present findings suggest that Linagliptin, a well-known Anti diabetic drug, possess cardio protection against ischemia-reperfusion-induced myocardial injury. Reduction in infarct size clearly indicates that Linagliptin reduced the cytotoxic free radical effect during ischemia-reperfusion injury, thereby protecting against the loss of membrane integrity and stabilizing the membrane. Hence, Linagliptin protects myocytes from oxidative stress. This is further confirmed by the investigations on antioxidant enzymes (SOD, CAT, and GSH) and serum marker enzymes (SGOT, CK, CK-MB and LDH), which leak out from tissues to plasma when degenerative changes develop in myocardial cell membranes.

5. CONCLUSION

The Cardio protective effect of Linagliptin (5 mg/kg bd.wt p.o) was observed in normal rats by comparing the percentage of necrosis of left ventricle with normal I/R group, by using Left descending coronary artery ligation. The same effect was observed in STZ induced diabetic rats treated with Linagliptin by comparing the percentage of necrosis of left ventricle with Diabetic control group by using Left descending coronary artery ligation. In this part of study it was observed that an increase in the levels of anti oxidant enzymes like Super Oxide Dismutase (SOD), Catalase, and reduced glutathione levels in normal and diabetic animals when treated with Linagliptin. This is useful for preventing the tissue damage of organs in diabetic conditions. The SGOT, CK, CK-MB and LDH levels were decreased in diabetic rats when treated with Linagliptin indicating the evidence for tissue protection. Microscopical examination of the left ventricle proves that the Linagliptin having the cardio protective effect by protecting the myocytes against the oxidative stress. Finally Cardio protective effect of Linagliptin was noticed at acute levels of study. Long term evaluation of Cardio protective effect of Linagliptin in diabetes is to be continued.

6. REFERENCES

1. Pittas AG and Greenberg AS. Contemporary Diagnosis and Management of Diabetes. Handbooks in Health Care Co, Newtown, PA. 2003.
2. Hélène Duez, Bertrand Cariou and Bart Staels. DPP-4 inhibitors in the treatment of type 2 diabetes. Biochemical Pharmacology 2012; 83(7): 823-32.
3. A Ramachandran, AK Das, SR Joshi, CS Yajnik, S Shah, KM Prasanna Kumar. Current Status of Diabetes in India and Need for Novel Therapeutic Agents. Supplement To JAPI 2010 ; 58: 6-9.
4. Ward WK, Beard JC, Porte D. Clinical aspects of islet B cell function in non-insulin dependent diabetes mellitus. Diabetes Metab Rev 1986; 2:297–313.
5. Leahy JL. Natural history of B-cell dysfunction in NIDDM. Diabetes Care 1991; 13:992–1010.
6. Porte D. B cells in type 2 diabetes mellitus. Diabetes 1991; 40:166–180.
7. Malaisse W. Metabolic signaling of insulin secretion. Diabetes Rev1996; 4:145–159.

8. O'Rahilly S, Turner RC, Matthews DR. Impaired pulsatile secretion of insulin in relatives of patients with non-insulin dependent diabetes mellitus. N Engl J Med 1988; 318: 1225–1230.
9. DeFronzo RA, Binder C, Wahren J, Felig P, Ferrannini E, Faber O. Sensitivity of insulin secretion to feedback inhibition by hyperinsulinemia. Acta Endocrinol (Copenh) 1981; 98:81–84.
10. Unger RH, Grundy S. Hyperglycemia as an inducer as well as a consequence of impaired islet cell function and insulin resistance: implications for the management of diabetes. Diabetologia 1985; 28:119–125.
11. Arner P, Pollace T, Lithell H. Different etiologies of type 2 (non-insulin dependent) diabetes mellitus in obese and non-obese subjects. Diabetologia 1991; 34:483–487.
12. Kissebah A, Freedman D, Peiris A. Health risk of obesity. Med Clin North Am 1989; 73: 111–138.
13. Bjorntop P. Abdominal obesity and the development of non-insulin dependent diabetes mellitus. Diabetes Metab Rev 1988; 4:615–622.
14. Ferrannini E, Stern MP. Primary insulin resistance: a risk syndrome. In: Leslie RDG, Robbins DC, eds. Diabetes: clinical science in practice. Cambridge: Cambridge University Press 1995; 200–220.