Original Article

Investigation of Cardioprotective Effect of 2, 4-thiazolidinedione Derivative Using HFD-STZ Fed Rats

Amin Heli H[1,*], Nileshkumar Joitaram Patel[1], Patel Nikita[2], Patel Dolar Govindbhai[1]

[1]Department of Pharmacology, Shree SKPCPER Ganpat University, Kherva, Gujarat, INDIA.
[2]Centre for Management Studies and Research, Ganpat University, Ahmedabad, Gujarat, INDIA.

ABSTRACT

Background: Myocardial infarction is a 2nd most important origin of universal death and after coronary revascularization, cardiovascular events occurs further a lot in subjects through DM: 3.5- as well as 2-fold fatality ranks correspondingly behind percutaneous coronary intervention or coronary bypass graft surgery. **Objectives:** The aim of an attendance research was to discover usefulness of 2, 4- thiazolidinedione derivatives lying on diabetic MI and HFD-STZ diabetic rats. **Materials and Methods:** In *in-vivo* investigation, Type-2 diabetes was elicited by higher fatty meal plus little dosage for streptozotocin and MI was provoked by LAD Coronary artery ligation model. The rats were divided into a variety of groups, including treatment groups (Composite An epalrestat, Composite B pioglitazone). And after 15 days of treatment a variety of factors were evaluated, i.e. serum glucose, total cholesterol, serum triglycerides, lipid profile anti-oxidant enzymes, ECG analysis, and Histopathological examination of heart were done. **Results:** In *in-vivo* investigation, levels of serum glucose, triglycerides, LDL level, LDH level, CK-MB, Troponin I, malondialdehyde (MDA), total cholesterol, infarct area, were considerably decreased in composite A and B treated animals. Whereas the HDL level, antioxidant enzymes, had drastically risen in composite, treated animals balanced to disease control rats. In ECG analysis ST elevation was restored in drug treated animals. Composite treated heart indicated mild congestion and inflammation in histopathological examination. Homogenate of drug treated animals indicated less apoptotic signals and nearer to normal animals. **Conclusion:** The treatment with Composite A and Composite B notably ameliorated the alterations in MI in a dose dependant manner.

Keywords: 2, 4-Thiazolidinedione, Myocardial infarction, Diabetic MI, Type-2 diabetes, LAD, Macrovascular complication.

INTRODUCTION

- The term diabetes mellitus depicts a metabolic ailment of various aetiologies categorized by persistent high blood sugar through turbulence for protein, fat as well as carbohydrate biotransformation ensuing with blemishes in insulin emission over and above insulin action equally. The influence of diabetes mellitus includes stretched–period break, failure and disruption of numerous body parts (WHO 1999).
- Diabetes mellitus may here with distinguishing indications such as blurring of vision, polyuria, thirst, and drop weight.
- More than 371 million people have diabetes In 2012 Death due to diabetes was 4.8 million. Spare than 471 billion USD was depleted on healthcare in favor of diabetes (www.idf.org/diabetesatlas/5e/Update2012).
- The Arcadians among the bulkiest figure of diabetic individuals will be USA China and India beyond 2030.
- Type-2 diabetes mellitus reports for 90-92% of all diabetes.[1]

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Correspondence:
Mrs. Heli H Amin
Department of Pharmacology, Shree SKPCPER Ganpat University, Kherva-384012, Gujarat, INDIA.
E-mail: heliamin8899@gmail.com

www.ijper.org

Diabetic complications

Dysfunction and damage of the cardiovascular system, kidneys, eyes and nerves all are associated with indelible consequences of diabetes with chronic high blood sugar.

- Microvascular (nephropathy, retinopathy, and neuropathy) and.
- Macrovascular (coronary artery disease, stroke, peripheral vascular disease).

MATERIALS AND METHODS

List of Materials

Urethane, Epalrestat, Pioglitazone, 2,3,5-Triphenyl tetrazolium chloride (TTC), Streptozotocin.

List of Instruments

Cooling Centrifuge Machine, UV-Double beam Spectrophotometer-1800, Semi auto analyzer.

(Photometer 5010), Homogenizer High Speed-RQ-127A, Trinocular Microscope, Gel electrophorosis, Powerlab.

Methods

Experimental Animals

Male Wistar rats of weighing 250-300 gm were procured from the Central Animal Facility of Shree S. K. Patel College of Pharmaceutical Education and Research, Ganpat University, Ganpat Vidyanagar. The animals were housed in polypropylene cage lines with husk, maintained in controlled temperature of 24±1°C as well as humidity of 55±5% with 12hr light and dark cycle and with ordinary diet and water provided *ad libitum* (Pranav Agro. Industries, Pune). All animals were acclimatized for a minimum period of 1 week prior to the beginning of the study. The experimental protocol was approved by the Institutional Animal Ethical Committee as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and forest, Government of India, New Delhi.[2]

Initiation of Diabetes in Rats

For diabetes induction, healthy rats (Male Wistar) viewing ordinary blood glucose point within the sort of 80-120 mg/dl were drafted into addition to with nourished of higher fatty food (contents as per Table 1) intended used for 14 days previous to injection of streptozotocin. Diabetes induced by administration of a single dose (35mg/kg, i.p.) of streptozotocin. Blood glucose height was calculated following 2 days of management by streptozotocin. Animals individuals had blood sugar in excess of or equivalent to 250 mg/dl were believed as diabetic and were utilized meant in favor of supplementary lessons. Continuation of high fat diet fed turned over the termination in diabetic animals. Blood glucose was calculated over at before commensing drug treatment to prove unfailing high blood sugar.[3]

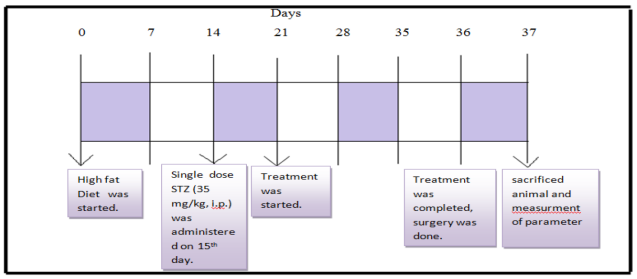
Table 1: Higher Fatty Meal's (HFM) contents.

Components in Meal	Weight (gm/kg)	Components in Meal	Weight (gm/kg)
Powdered NPD	315	Vitamin and minerals	60
Coconut oil	250	Sucrose	50
Casein	250	Fructose	50
Sodium chloride	02	DL-Methionine	03
Cholesterol	20	-	-

Surgical procedure for induction of Myocardial infarction in Rats

With use of pentobarbitone sodium (60 mg/kg, i.p.) male Sprague–Dawley rats were anesthetized. Immediately before coronary artery occlusion, bolus of heparin (30 IU) was administered for prophylaxis beside creation of thrombus just about the capture. With the help of 6-O silk suture left anterior descending coronary artery was ligated after exteriorization of the heart from side to side a 15-mm unwrapping by the side of the fourth-intercostals gap. A handmade bind was fixed more than two parts of suture to hold the flow of blood for 1.5 hr followed by 24 hr reperfusion. With the outer shell of area epicardial cyanosis and elevation of ST-segment, the emergence of ventricular ectopy and whitening of the myocardium ischemia were proved visually. Without coronary artery ligation, identical surgical procedures were performed on the Sham-operated rats. Drugs were directed for 2 weeks and surgery was performed on 37th day. Later than a whole day of surgery, animals were sacrificed and biochemical parameters, survival rate, infarct size, histopathology, and the risk ischemic zone were determined.

Studydesignand Treatmentschedule



RESULTS

- ***In-vivo* Cardioprotective Consequence of 2, 4-Thiazolidinedione Using Hfd-Stz Diabetic Animal Model**

- **TOTAL BODY MASS (Figure 1)**

Based on body weight, grouping of animals was done initially. High fat diet produced a considerable increase body weight (±10% to initial body weight) compared to normal. After the end of the second week, STZ administration was produced an extreme reduction in body weight which had been regained in later stage. After the drug treatment body weight was improved but it was not found noteworthy rise.

- **SERUM GLUCOSE LEVEL (Figure 2)**

As compared to normal control rats (p<0.001) high fat diet and STZ treated rats indicated a considerable rise in serum glucose level. There was a 5 fold inclined in serum glucose level when measured at the end of 6th weeks in diabetic control rats. Composite A (200mg/kg), composite B (80 mg/kg and 200mg/kg) treated animals created an extremely considerable reduction in serum glucose levels as compared to diabetic group.

- **INFARCT MEASUREMENT (Figure 3)**

Comparison of normal control group with diabetic surgery group, diabetic group indicated considerable damaged heart area. The drug treated groups demonstrated appreciably diminish within the damaged part. Higher dose of Composite A and B (200 mg/kg, both) indicated reduction of infarct area compared to diabetic surgery group.

- **Cardiac markers**

- **Creatinine kinase (As per Figure 4)**

Diabetic surgery group indicated considerably increase in CK-MB level as compared to Normal Control Group. Treatment groups indicated considerably reduced levels of serum CK-MB. Higher dose of Composite

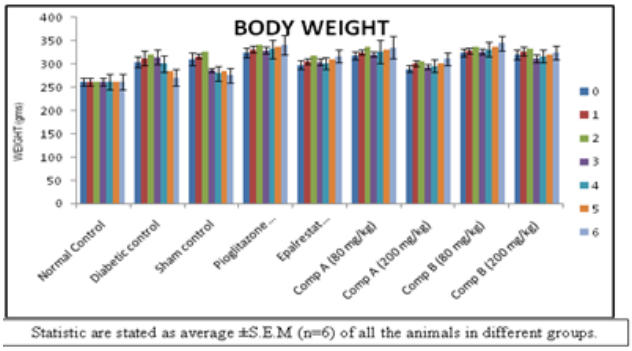


Figure 1: Statistic illustrating Body weight in Normal Control, Diabetic Control, Sham Control, (ordinary-1; 10 mg/kg) Pioglitazone, (ordinary-2; 50 mg/kg) Epalrestat, (80 mg/kg) Composite A, (200 mg/kg) Composite A, (80 mg/kg)Composite B, (200 mg/kg) Composite B treated groups.

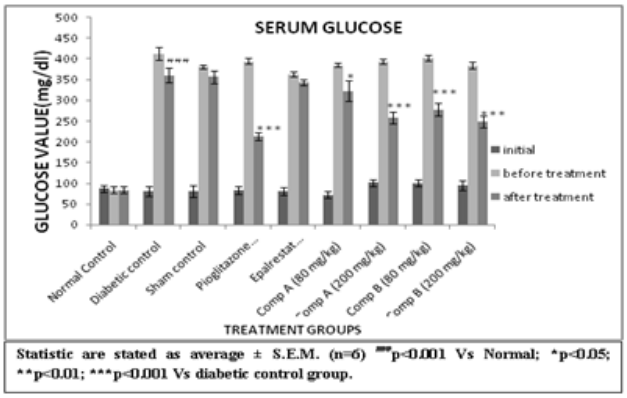


Figure 2: Statistic proving serum glucose rank in Normal Control, Diabetic Control, Sham Control, Pioglitazone (ordinary-1; 10 mg/kg), Epalrestat (ordinary-2; 50 mg/kg), Composite A (80 mg/kg), Composite A (200 mg/kg), Composite B (80 mg/kg), Composite B (200 mg/kg) treated groups.

Table 2: Reagent required for Creatinine kinase test.

Following reagent Pipetted in to centrifuge tube	
Reagent 1	200 µL
Reagent 2	50 µL
Sample	10

B (200 mg/kg, both) indicated considerable lowering of CK-MB level compared to diabetic surgery group (Reagent required for Creatinine kinase test as per Table 2).

- **Troponin I (Figure 5)**

Diabetic surgery group indicated considerably increase in *Troponin I* level as compared to normal Control Group. Treatment groups indicated considerably reduced levels of Troponin I. Higher dose of Composite A and B (200 mg/kg, both) were indicated lower Troponin I level.

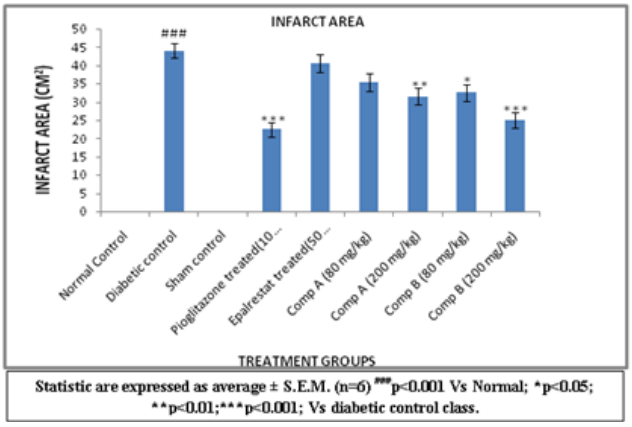
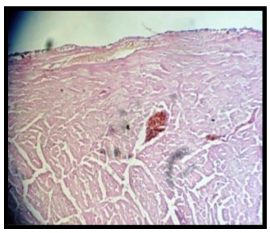
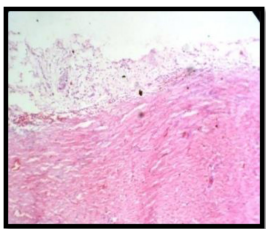
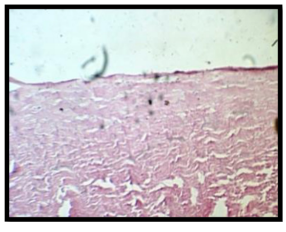
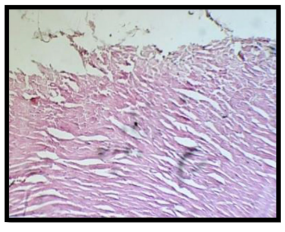
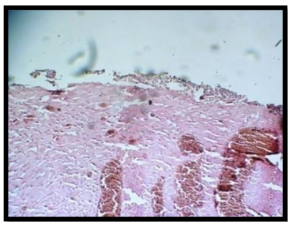
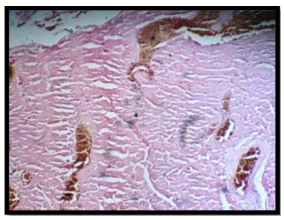
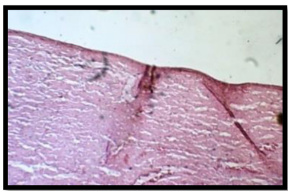
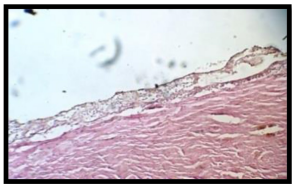
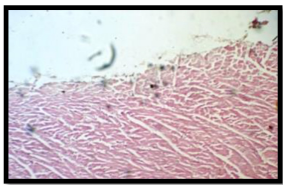


Figure 3: Statistic proving of Infarct area in Normal Control, Diabetic Control, Sham Control, Pioglitazone (ordinary-1; 10 mg/kg), Epalrestat (ordinary-2; 50 mg/kg), Composite A (80 mg/kg), Composite A (200 mg/kg), Composite B (80 mg/kg), Composite B (200 mg/kg) treated classes.

- Histopathological Analysis

Normal control: Normal architecture of heart, over pericardium and cardiac muscle. No marks of erosion and necrosis or extra modify.	**Diabetic control:** Ventricular wall proved infarction on cardiac muscle at multicentral zone. Through incidence of inflammatory cells at central region, pericardium indicated gentle marks of inflammation.
	
Sham control: Common architecture of heart, through cardiac muscle and pericardium. Not a single mark of deterioration and zone of necrosis, swelling as well as extra modification.	**Ordinary 1:**Common architecture of heart, among pericardium and cardiac muscle. Not a single mark of deterioration and zone of necrosis, swelling as well as extra modification.
	
Ordinary 2: Focal region of cardiac muscle degeneration at wall of ventricles. Pericardium indicated gentle symptoms of swelling among attendance of provocative cells at central area. Mild to moderate congestion at ventricular wall.	**Comp A(80mg/kg):** Mild to moderate congestion at ventricular wall**.** Common architecture of heart, with pericardium and cardiac muscle. Not a single mark of deterioration and zone of necrosis, swelling as well as extra modification.

	
Comp A (200mg/kg): Normal architecture of heart, with pericardium and cardiac muscle. Not a single mark of deterioration and zone of necrosis, swelling as well as extra modification.	**Comp B (80mg/kg):** Focal part of cardiac muscle infarction at wall of ventricles. Pericardium indicated gentle symptoms of swelling among attendance of provocative cells at central area.
	
Comp B(200 mg/kg): Common archistructure of heart, through pericardium and cardiac muscle. Not a single mark of deterioration and zone of necrosis, swelling as well as extra modification.	
	

✓ GRAPHICALESTIMATION OF HEART SEGMENTS (Figure 6)

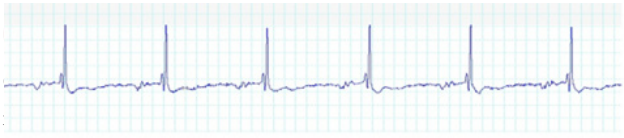
In the normal control group heart damage was found absent. Macroscopical examination of the heart of diabetic surgery group indicated more damaged area in the heart. Less damaged area were observed in similar diabetic surgery groups treated with Composite A (80 mg/kg, 200 mg/kg) and Composite B (80 mg/kg, 200 mg/kg).

ECG Analysis

In Table 3, the drug 2,4-TZDs shows good protective effect against MI.

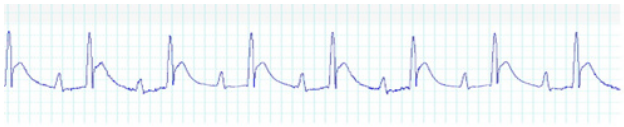
GROUP 1 (NORMAL CONTROL)

Group 1 shows normal pattern of ECG. In that group not show the ST elevation



GROUP 2 (DIABETIC CONTROL)

Group 2 shows ST elevation which indicates myocardial infarction in that graph clearly shows the ST segment elevated because of occlusion of Left anterior descending artery.



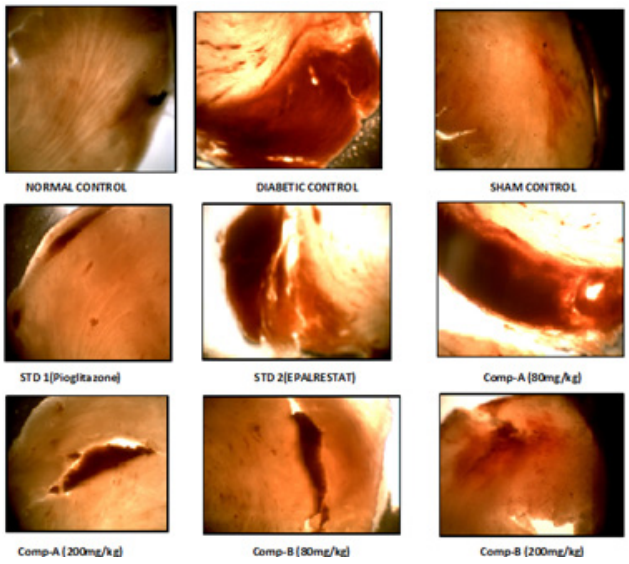


Figure 6: Graphical Estimation of Heart Segments.

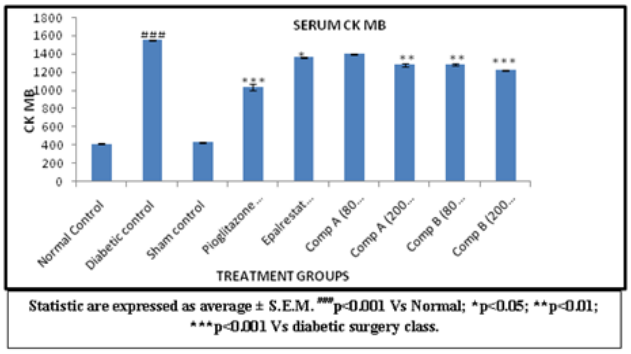


Figure 4: Statistic proving CK-MB level in Normal Control, Diabetic Control, Sham Control, Pioglitazone (ordinary-1; 10 mg/kg), Epalrestat (ordinary-2; 50 mg/kg), Composite A (80 mg/kg), Composite A (200 mg/kg), Composite B (80 mg/kg), Composite B (200 mg/kg) treated classes.

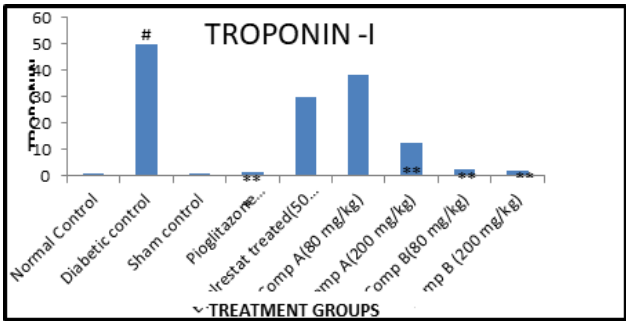
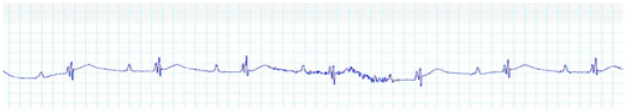


Figure 5: Statistic proving serum Troponin I level in Normal Control, Diabetic Control, Sham Control, Pioglitazone (ordinary-1; 10 mg/kg), Epalrestat (ordinary-2; 50 mg/kg), Composite A (80 mg/kg), Composite A (200 mg/kg), Composite B (80 mg/kg), Composite B (200 mg/kg) treated classes.

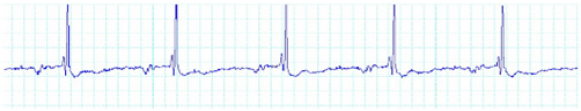
GROUP 3 (SHAM CONTROL)

Group 3 shows mild ST elevation as compared to diabetic group. In that group ST segment is less elevated.



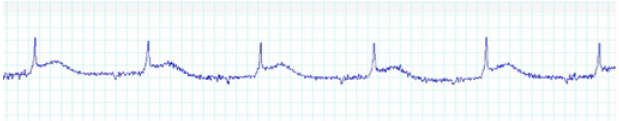
GROUP 4 (PIOGLITAZONE)

Group 4 shows the ST elevation is very less as compared to diabetic group. When compared with diabetic group it shows good effect.



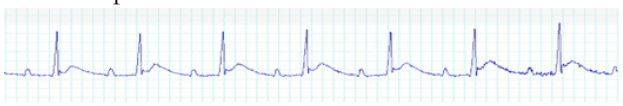
GROUP 5(STD 2 EPALRESTAT)

Group 5 show the ST elevation in graph which indicates the myocardial infarction. When compared with diabetic group not show good effect.



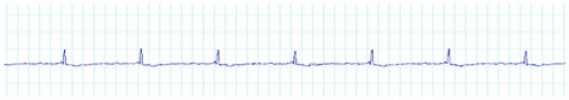
GROUP 6 (COMP A (80MG/KG))

Group 6 shows ST elevation which indicates myocardial infarction. When compared with diabetic group not show the protective effect.



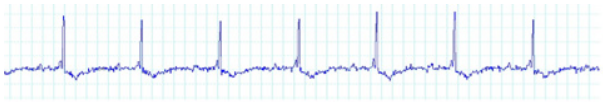
GROUP 7 (COMP A (200MG/KG))

Group 7 show mild ST elevation when compared with diabetic group it shows good protective effect.



GROUP 8 (COMP B (80MG/KG))

Group 8 shows less moderate effect on ST elevation when compare with diabetic group it shows good protective effect.



GROUP 9 (COMP B (200 MG/KG))

Group 9 shows very little ST elevation. When compared with diabetic group it indicated good protective effect.

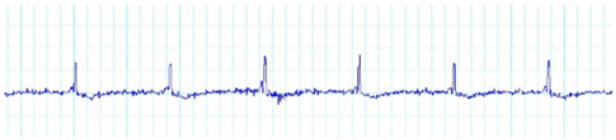


Table 3: ECG Analysis of different groups.

Group of Animals	Results	Conclusion
Group 1 (Normal Control)	No ST elevation	Normal
Group 2 (Diabetic Control)	ST elevation	MI
Group 3 (Sham Control)	Less ST elevation	No MI
Group 4 (Pioglitazone)	Less ST elevation	No MI
Group 5 (Std 2 Epalrestat)	ST elevation	MI
GROUP 6 (COMP A (80mg/Kg))	ST elevation	MI
GROUP 7 (COMP A (200mg/Kg))	Less ST elevation	No MI
GROUP 8 (COMP B (80mg/Kg))	Less moderate ST elevation	No MI
GROUP 9 (COMP B (200 Mg/Kg))	Little ST elevation	No MI

DISCUSSION

- DM is an adjustable risk factor for Myocardial infarction. Macrovascular complication of diabetes mellitus is MI. Death as of coronary artery ailment is 3 period more regular in diabetic patients matched up to with no diabetic subjects matched by factors such as sex and age. Throughout the first-month fatality is more than 42%, in patients with diabetes and acute myocardial infarction, whereas it is less than 20% in subjects without diabetes. The rate of CVDs is lower in diabetic men than into diabetic women.
- 2, 4-Thiazolidinediones have been patented or accounted once aldose reductase inhibitors after the innovation of glitazones like oral antidiabetic drugs, several of which are in addition gifted over antihyperglycemic result.[4,5]
- The current learning was thus aimed towards investigating the effectiveness of these composites alongside myocardial infarction-a macrovascular complication of diabetes.
- Later than the supervision of 35 mg/kg of STZ next to higher fatty meal consequences in catabolic defeat on total mass distinguishing of type I diabetic within difference to the incline in total mass depicts type II diabetes.[6] For the reason that consequence of PPAR-γ agonist like body weight, weight gain was enhanced slowly after drug treatment.
- A link between PPARγ and disease including hypertension,[7] inflammation,[8,9] atherosclerosis[10,11] and cancer[12] has been suggested. Many chemical composites have been developed as PPARγ agonists. For the reason that many studies of Thiazolidinediones have indicated that PPARγ agonists are effective against the insulin resistance syndrome or metabolic syndrome.
- The potential of the thiazolidinedione derivatives to operate for a ligant of PPAR-γ is fit confirmed along with the ensuing function of PPAR-γ effect on triglycerides and cholesterol and to lower blood glucose as well as decrease insulin resistance is also [Lee, 2003].[13] In this study, owing to the

administration of STZ, the composites were proficient to combat the increase in blood glucose levels.

- Inside diabetic MI rats the infract magnitude was radically enlarged. During MI stipulation blood supply is decreased and enhanced in O_2 demand in the heart cell as well as declined in the ATP plane that construct apoptosis, mitochondrial injury and gangrene.[13,14] TZD has proven considerably diminish in the infarct range of dose reliant relative approach here diabetic MI rats.
- Histopathological changes and multi focal zone of cardiac brawn infarction was monitored at ventricular wall were observed on normal MI rats. In diabetic MI rats pericardium mild swelling through the existence of incendiary cells at the central zone. Within diabetic MI rats, 2, 4 TZD was helpful for important dwindle into central area and inflammation within dose- dependent manner.
- Area of infarction involving sham and normal control had not confirmed any noteworthy variation other than inferior into normal MI over diabetic MI rats may have thanks to a diabetic state before surgery. This wealth necrosis of myocardium hassles by high blood sugar.
- Electrocardiograph-aberrations are the chief criteria for the conclusion of myocardial infarction. In enduring by acute myocardial ischemia ST-segment elevation was observed. The characteristic findings were reductions in the QT interval and prolongation, R-R intervals, QRS complex, P wave intensity of the cardiac cycle. We also examined an increase in heart rate and major increase in the ST segment. These modifications could be due to the consecutive loss of cell membrane in injured myocardium.[15] Several of the indicative signs of ischemia are the appearance of ST segment elevation and Q wave. The repeated failure of cellular membrane spoils owing to oxidative stress might be categorized by ST elevation.[16]
- Biochemical beliefs pointed out that the composites (A and B) beneath examination could fruitfully hold-up the succession of diabetic MI.

CONCLUSION

In-vivo study

- Under examination of these composites explained optimistic results beside inhibition of PPAR-gamma agonist and act as an aldose reductase inhibitor hindrances the expansion of Myocardial infarction.
- With the analysis of biochemical our composites besides demonstrated notably protection against the enhanced body weight, elevated blood sugar level.
- The composite also indicated considerable protection against Cardiac biomarkers like Troponin I, CK-mb.
- This composite also indicated also a considerable effect on Electrocardiogram.
- Composite B was set up to be higher valuable in terms beyond factors than Composite A, from the two composites under examination. By averages of the doses of two picked pronounced things were monitored at superior dose, i.e. 200 mg/kg as balanced to 80 mg/kg dose proposing dose reliant exploit of in cooperation the composites.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

2,4-TZD: 2,4-Thiazolidinedione; **CK:** Creatinine Kinase; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **CVD:** Cardiovascular disease; **DM:** Diabetes Mellitus; **ECG:** Electrocardiogram; **HDL:** High density lipoprotein; **HFM:** High fat meal; **LAD:** Left Anterior Descending; **LDH:** Lactate dehydrogenase; **LDL:** low-density lipoprotein; **MDA:** Malonaldehyde; **MI:** Myocardial infarction; **PPAR:** Peroxisome proliferate activated receptor; **STZ:** Streptozotocin; **WHO:** World health organization.

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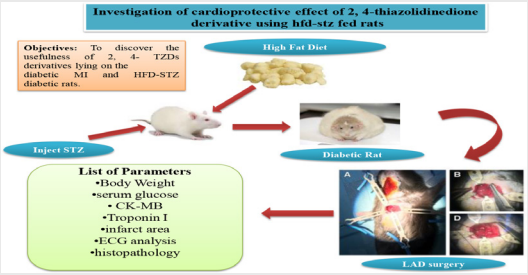
PICTORIAL ABSTRACT



SUMMARY

Myocardial infarction is a 2nd most important origin of universal death and after coronary revascularization, cardiovascular events occurs further a lot in subjects through DM: 3.5- as well as 2-fold fatality ranks correspondingly behind percutaneous coronary intervention or coronary bypass graft surgery. In in-vivo investigation, levels of serum glucose, CK-MB, Troponin I, infarct area, were considerably decreased in composite A & B treated animals. In ECG analysis ST elevation was restored in drug treated animals. Composite treated heart indicated mild congestion and inflammation in histopathological examination. Homogenate of drug treated animals indicated less apoptotic signals and nearer to normal animals.

About Authors

Ms. Heli H Amin: She has done M. Pharmacy (Pharmacology) from Shree SKPCPER Ganpat University, under the supervision of Dr. Nilesh J Patel. Presently, she is working as Assistant Professor in pharmacology department at Sankalchand Patel University. Her area of interest is cardiovascular pharmacology and preclinical experiments.

Dr. Nileshkumar Joitaram Patel: More than 16 years of Academic and Research experience in the field of Pharmacology, Toxicology and Clinical Research. Nilesh is passionate translational Pharmacologist by profession and has been putting his expertise and experience to develop biologics for patients suffering from some debilitating illnesses like cancer, infection, autoimmune diseases and inflammatory conditions.

Dr. Nikita Patel is program coordinator of MBA Pharmaceuticals and MBA Healthcare and Hospital Management at Centre for management studies and research, Ganpat University. She is also a Chairperson of Ganpat University Shalby Centre for Healthcare Management and Research. She is Six sigma green belt certified and also a trainer for Six sigma training modules. She has published more than 7 research paper and case studies in recognized journals. Her area of interest is quality management, strategic management, supply chain management, entrepreneurship and project management, manufacturing planning and control, project planning and control, service quality and operations management.

Mr. Dolar Govindbhai Patel: He has done M. Pharmacy (Pharmacology) from Shree SKPCPER Ganpat University, under the supervision of Dr. Nilesh Kanzaria.

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