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Myocardial Salvaging Effect of Metformin in Isoproterenol Induced Myocardial Injury in Rats

Nadia Sultana[1], Iram Shahriar[2], Sumona Haque[3], Kazi Md Asif Hasnan[4], Tamanna Mahmud Urmi[5], Muqbula Tasrin Aktar[6*]

[1]*Lecturer, Department of Pharmacology, Dhaka Dental College, Dhaka, Bangladesh*

[2]*Professor and Ex- Head, Department of Pharmacology, Sir Salimullah Medical College, Dhaka, Bangladesh*

[3]*Assistant Professor, Department of Pharmacology, Dhaka Dental College, Dhaka, Bangladesh*

[4]*Assistant Surgeon, Department of General Surgery, Chittagong Medical College and Hospital, Chittagong, Bangladesh*

[5]*Senior Lecturer, Department of Anatomy, Bashundhara Ad-din Medical College, Dhaka, Bangladesh*

[6]*Associate Professor, Pharmacology, National Institute of Ophthalmology and Hospital, Dhaka, Bangladesh*

Corresponding Author:** ***Muqbula Tasrin Aktar, *Associate Professor, Pharmacology, National Institute of Ophthalmology and Hospital, Dhaka, Bangladesh.* ***Email:*** *dr.muqbula@gmail.com*

Abstract

Background: *Cardiovascular disease is now-a-days the most common cause of death. Metformin improves vascular function and reduces cardiovascular mortality. It has been shown to have cardioprotective effects beyond its glycemic controls. The present study was designed to observe the cardioprotective effect of metformin, in isoproterenol induced myocardial injury in rats.*

Methods: *Total number of 35 rats were taken for the experiment. Wistar Albino rats were divided into 5 groups (n=7). After grouping, Group III and Group V were induced diabetes by administration of single dose of Alloxan Monohydrate 120mg/kg body weight intraperitoneally. Group I served as healthy control. Group II served as non-diabetic isoproterenol treated group. Group IV which was non diabetic rats received metformin 100 mg/kg/day for 4 weeks (2^{nd}week-5^{th} week). Group V was made diabetic after acclimatization. This group received metformin 100mg/kg/day for 4 weeks (2^{nd} week – 5^{th}week). Isoproterenol 85mg/kg/day was given intraperitoneally 24 to 48 hour prior to scarifice (1^{st} and 2^{nd} day of 6^{th} week) in group II- V. On 3^{rd} day of 6^{th} week, rats were sacrificed. Serum CK-MB and LDH levels were estimated, histopathological examination of heart was done.*

Results: *Mean CK-MB and LDH levels of Isoproterenol treated diabetic group G-III were 7.17±0.28, 913.71±24.2. The levels were 5.13±0.22, 498.57±15.1in G-V. Mean CK-MB, LDH levels were 5.97±0.30, 793.71±70.4, in G–II and levels were 3.49±0.07, 411.57±21.9in G-IV. P (<0.05) value indicates significant difference between G III vs G V and G II vs G IV. Microscopic examination of heart showed myocardial structure disorganization, cardiac muscle fibre separation, necrosis, edema in G II and G III. In G IV and G V microscopic examination showed reduction of necrosis, nearly normal cardiac architecture.*

Conclusion: *Study results showed metformin has cardioprotective effect in isoproterenol induced myocardial injury.*

Keywords: *Metformin, isoproterenol, myocardial injury, cardioprotection.*

1. INTRODUCTION

Cardiovascular Disease (CVD) is a global health problem[1]. Populations most affected are from low and middle income countries like **Bangladesh**. Epidemiological studies have shown a significant increase in its prevalence in **Bangladesh** in the last few decades[2].Among the heart disease, ischaemic heart disease is the leading cause of morbidity and mortality[3]. Myocardial infarction is the most important form of ischaemic heart disease[4].

Isoproterenol induced myocardial infarction serves as the standardized model because pathophysiological changes observed in heart muscle of experimental animal, similar to that in human myocardial infarction. There is an imbalance between oxygen supply and demand from cardiomyocytes which is related to myocardial hyperfunction due to increase both in chronotropism and inotropism. There is also an elevation of Ca++ overcharge inside the cell causes activation of the adenylatecyclase enzyme and the depletion of ATP levels[4]. Isoproterenol also generates free radicals which leads to damage of the structural and functional integrity of the myocardium. Myocyte death or altered membrane permeability causes cytosolic contents to diffuse to the systemic circulation, where they may be detected as markers of the ischemic heart disease[5].

Metformin, an oral anti-diabetic drug from the biguanide, insulin sensitizing class, is primarily used in the management of type 2 diabetes mellitus. Despite the emergence of new glucose lowering drugs, metformin is still reported to be most widely used drug because of its safety record and its various beneficial outcome[6].

AMPK is known to maintain the energy balance in cells during ischemia by increasing ATP levels. Metformin increases AMPK activation in both ischemic and non-ischemic hearts. AMPK also activates endothelial nitric oxide synthase and prevents cellular death[7]. Metformin reduces myocardial infarct size in both the non-diabetic and diabetic heart through preventing the opening of mPTP (mitochondrial permeability transition pore) by activation of phosphatidylinositol-3-kinase (PI3K) pathway[6]. In addition, metformin therapy attenuates postinfarction cardiac remodeling[8].

As cardiovascular disease is a rising health concern in **Bangladesh**[2], metformin may provide a cardioprotective role for patients with cardiovascular risk. The purpose of the study is to evaluate the cardioprotective effect of metformin in isoproterenol (a sympathomimetic) induced myocardial injury in rats.

2. Materials and Methods

The study was carried out in the Department of Pharmacology and Therapeutics of Sir Salimullah Medical College and Mitford Hospital (SSMC), Dhaka in collaboration with Institute of Nutrition and Food Sciences (INFS), University of Dhaka. The total study period was from July 2018 to June 2019.

Study population:

A total number of 35 healthy adult Wistar Albino rats of both sex, weighing approximately 120 to 135 grams, 10-12 weeks of age were purchased from animal house of Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh. The rats were acclimatized in metallic cage in the animal house of Institute of Nutrition and Food Sciences at University of Dhaka for 2 weeks before the actual experiment.

Drugs used:

Tab Metformin (500mg) was obtained from Square pharmaceuticals, Bangladesh. **Alloxan Monohydrate** was supplied by Institution of Nutrition and Food Sciences, University of Dhaka. Alloxan Monohydrate was dissolved in sterile normal saline and administered by single intra peritoneal injection at a dose of 120mg/kg of body weight. **Inj. Isoproterenol-** was procured from Samarth Pharma Life Sciences, India. Inj. Isoproterenol was given intra peritoneally at a dose of 85 mg/kg of body weight.

Experimental schedule:-

The rats were divided into 5 groups, containing 7 rats in each group after acclimatization.

Among the 5 groups, two groups (group III and group V) were kept overnight fasting after acclimatization and then 120 mg/kg body weight of Alloxan Monohydrate was induced intraperitoneally to each of the rats of group III and group V. After 72 hours of alloxan injection, the animals were tail bled and serum blood glucose were estimated to assess glycemic status. Blood glucose level >200mg/dl was considered as diabetes mellitus. Induction of diabetes mellitus in two groups were done at 1st week.

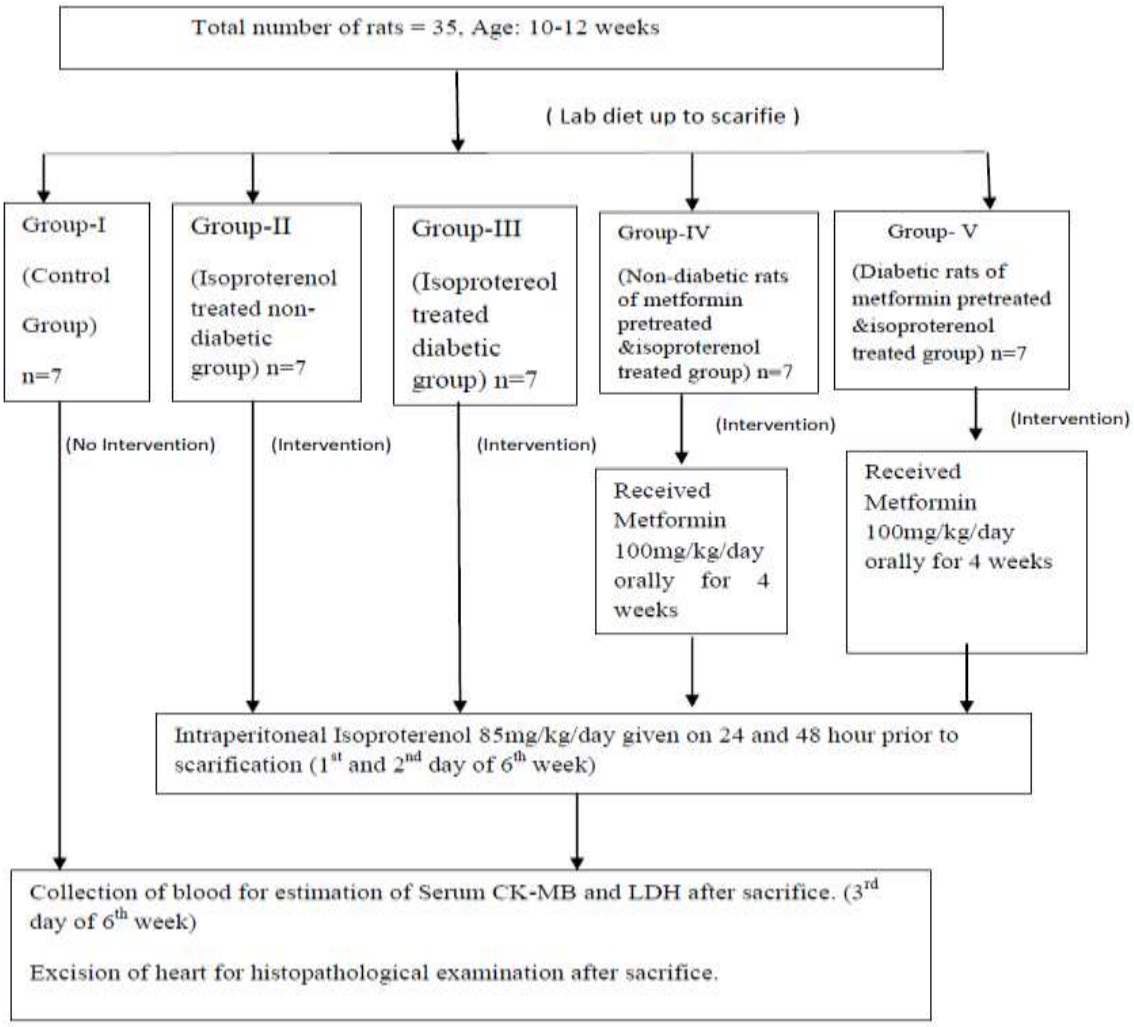

Figure1

Group-I (Control group):- This group of animals were given normal lab diet up to sacrifice.

Group-II (Isoproterenol treated non-diabetic group):-This group of animals received lab diet upto sacrifice. Myocardial infarction was produced by Isoproterenol (85mg/kg body weight) injection intraperitoneally 24 and 48 hour prior to scarifice (1st and 2nd day of 6th week).

Group-III (Isoproterenol treated diabetic group):- The Alloxan Monohydrate (120 mg/kg body weight) was injected intraperitoneally to induce diabetes at 1st week (day 1) and administered with Isoproterenol (85mg/kg body weight) injection intraperitoneally 24 to 48 h prior to scarifice (1st and 2nd day of 6th week).

Group-IV (Non-diabetic rats of metformin pre-treated and isoproterenol treated rats):- This non-diabetic group of rats received metformin (100mg/kg/day, orally) from 2nd week to 5th week (4 weeks). Myocardial infarction was produced by Isoproterenol (85mg/kg body weight) injection intraperitoneally 24 and 48 hour prior to scarifice (1st and 2nd day of 6th week).

Group-V (Diabetic rats of metformin pre-treated and isoproterenol treated group):- The Alloxan Monohydrate (120 mg/kg body weight) was injected intraperitoneally to induce diabetes mellitus at 1st week (day 1). Metformin (100mg/kg/day) was fed orally from 2nd week to 5th week (4weeks). The rats were given with Isoproterenol (85mg/kg body weight) injection intraperitoneally 24 and 48 hour prior to scarifice (1st and 2nd day of 6th week).

Induction of diabetes mellitus:-

Alloxan monohydrate (234mg) was dissolved in 3 ml of Normal saline. A light violet colour clear solution was made in a vial. The drug was

administered by single intraperitoneal injection at a dose of 120mg/kg of body weight (200 micro lit/ rat) in 14 rats of group III and group V at 1st week (day 1).

Experimental dose of metformin: Tablet Metformin was given at a dose of 100mg/kg dissolving in distilled water along with lab diet to the group IV and group V for 2ndweek to 5th week (4 week). The tablets were crushed and dissolved in distilled water and given at a volume of 200 micro lit per rat.

Induction of myocardial injury:-

Myocardial injury was induced in 28 rats (group II-V) by administration of injection isoproterenol intraperitoneally 85mg/kg/day 24 and 48 hour prior to scarifice (1^{st} and 2^{nd} day of 6^{th} week).

Study parameters:-

Serum lactate dehydrogenase was estimated by UV enzymatic method (Kinetic method). Serum CK-MB was estimated by Immunofluorescence Assay.

Sacrifice of animals and collection of blood:-

After completion of experiment (5 weeks), all the rats were anesthetized by chloroform and then sacrificed (3^{rd} day of 6^{th} week) [11]. Approximately 3 to 4 ml of blood were collected from each rat. The blood samples were allowed to clot for 45 minutes and the serum was separated by centrifugation at 2500rpm for 30 minutes and collected by micropipette. After collection, it was transferred to separate eppendrof tubes. Then the serum was kept in refrigerator at -27°c and taken to Biochemistry department of SSMCH to analyze various biochemical parameter.

Excision of hearts for Histopathology:-

After collection of blood sample, chest of the animals were opened and heart was excised. Then heart was preserved in 10% formalin for histopathology .This was taken to Pathology department of SSMCH and then undergo dehydration, clearing, paraffin infiltration, embedding, section cutting and staining for histological examinations.

Statistical analysis:-

The results were expressed as mean ± SD (Standard deviation). Statistical significance of differences between groups was determined by one way **ANOVA** test and **Bonferroni** test. P values of < 0.05 were considered statistically significant. The calculations were performed by using SPSS version 22.

3. RESULTS

Table I. *Body weight (gm) of the rats (n=35)*

Groups	Initial body wt. (gm) Mean±SD	Final body wt. (gm) Mean±SD
Group I (n=7)	127.43±1.81	132.14±2.67
Group II (n=7)	125.14±3.39	126.86±3.76
Group III (n=7)	125.29±3.45	123.00±3.51
Group IV (n=7)	125.14±3.39	124.43±3.31
Group V (n=7)	127.43±2.15	125.00±3.56

Table II. *Fasting Blood Glucose (mmol/L) level in different groups of the rats (n=35)*

Groups	Fasting Blood glucose(mmol/L) Day 1	Fasting Blood glucose(mmol/L) Day 4
Group I (n=7)	4.43±0.39	4.46±0.36
Group II(n=7)	3.87±0.42	3.97±0.32
Group III(n=7)	4.77±0.34	11.99±0.31
Group IV(n=7)	3.94±0.44	4.66±0.39
Group V(n=7)	4.80±0.24	12.00±0.24

Table III (a). *Serum CK-MB, LDH levels in different groups of rats after experiment, 3^{rd} day of 6^{th} week (n=35)*

Groups	Serum CK-MB (ng/ml)	LDH (U/L)
Group-I (n=7)	2.49±0.07	234.14±22.3
Group-II (n=7)	5.97±0.30	793.71±70.4
Group-III(n=7)	7.17±0.28	913.71±24.2
Group-IV(n=7)	3.49±0.07	411.57±21.9
Group-V (n=7)	5.13±0.22	498.57±15.1
p-value	<0.001	<0.001

Results are expressed as **Mean±SD**. One way **ANOVA** followed by **Bonferroni** test was performed to compare between groups.

The test of significance was calculated and p values < 0.05 was accepted as level of significance.

Table III (b). *Comparison of Serum CK-MB and LDH levels among different groups of rats after experiment, 3^{rd} day of 5th week (n=35)*

Groups	P values	
	CK-MB	LDH
I vs II	<0.001**	<0.001**
I vs III	<0.001**	<0.001**
I vs IV	<0.001**	<0.001**
I vs V	<0.001**	<0.001**
II vs III	<0.001**	<0.001**
II vs IV	**<0.001****	**<0.001****
II vs V	<0.001**	<0.001**

III vs IV	<0.001**	<0.001**
III vs V	**<0.001****	**<0.001****
IV vs V	<0.001**	<0.001**

ANOVA was used to analyze the data and comparison between two groups were done by Bonferroni test.

In case of serum CK-MB *indicates significant difference (p value <0.05) between groups I vs II, I vs III, I vs IV, I vs V, II vs III, **II vs IV**,II vs V, III vs IV, **III vs V**,IV vs V.

In case of serum LDH * indicates significant difference (p value < 0.05) between groups I vs II, I vs III, I vs IV, I vs V, II vs III, **II vs IV**,II vs V, III vs IV, **III vs V**,IV vs V

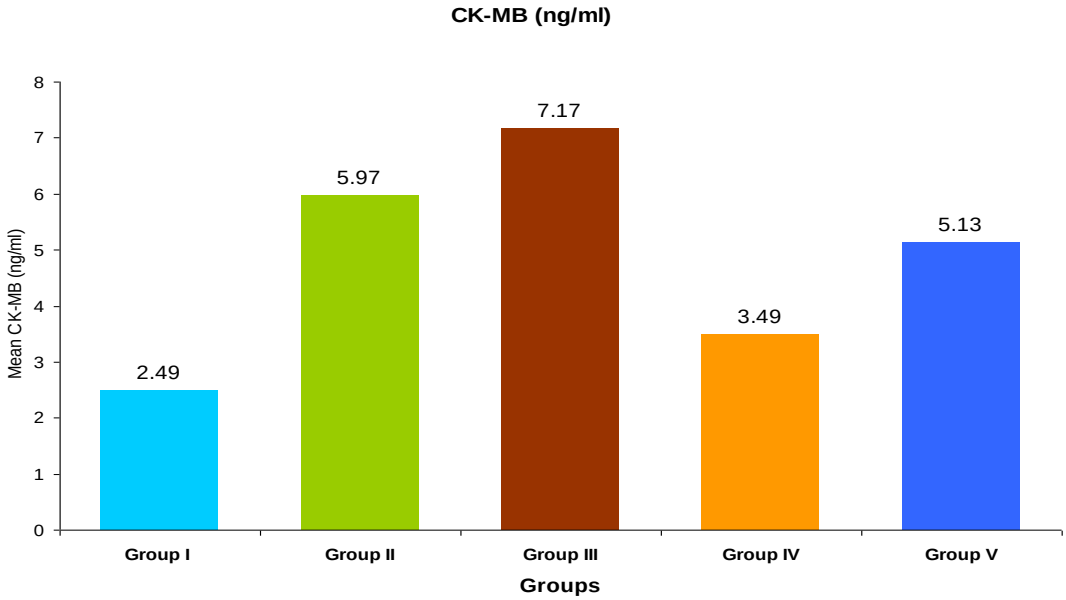

Figure2. *Bar diagram showing the mean CK-MB (ng/ml) in different study groups*

The bar diagram shows Serum CK-MB level is 2.49 ng/ ml, that is within normal range in Group-I. In group II it is 5.97 ng/ml that is higher than normal range and in group III it is 7.17 ng/ml which is highest among all the groups. In group IV it is 3.49ng/ml which is within the normal range and in group V it is 5.13 ng/ml which is above normal range. Significant difference is observed between group **II vs IV** and group **III vs V.**

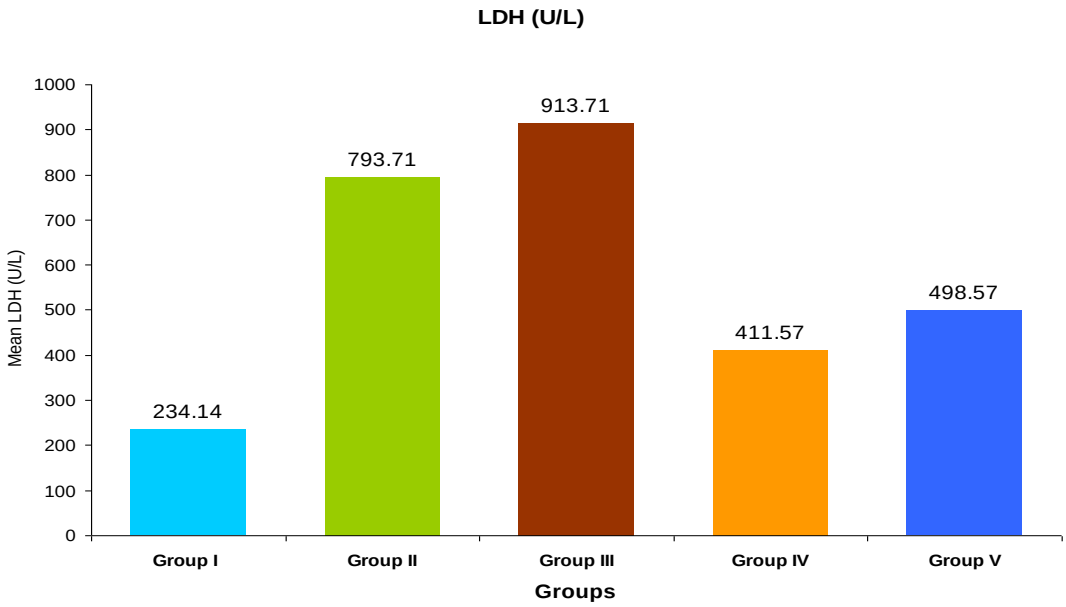

Figure3. *Bar diagram showing the mean LDH (U/L) in different study groups*

The bar diagram shows Serum LDH level is 234.14 U/L, that is within normal range in Group-I. In group II it is 793.71 U/L that is higher than normal range and in group III it is 913.71 U/L which is highest among all the groups. In group IV it is 411.57 U/L which is within normal range and in group V it is 498.57 U/L which is above normal range. Significant difference is observed between group **II vs IV** and group **III vs V.**

Table IV. *Histopathological findings of heart in different groups of rats (n = 35)*

Group	Observation	Result/findings	Score
Group I (n=7) (Normal control rats)	Architecture of - Myocardium - Endocardium - Pericardium	Normal histological finding	0
Group II (n=7) (Isoproterenol treated non-diabetic rats)	-myocardial disorganization -Marked loss of myocardial basement membrane -Marked oedema and necrosis present	Marked histological changes	3-4
Group III (n=7) (Isoproterenol treated diabetic rats)	-Wide spread myocardial disorganization -Marked loss of myocardial basement membrane -Marked oedema and necrosis present	Marked histological changes	3-4
Group-IV (Metformin pre-treated&isoproterenol treated non-diabetic rats)	-Nearly normal cardiac architecture -Scanty necrosis -Partial absence of myocardial basement membrane	Nearly normal	0-1
Group-V (Metformin pre-treated&isoproterenol treated diabetic rats)	-Nearly normal cardiac architecture -Scanty necrosis -Partial absence of myocardial basement membrane	Nearly normal	0-1

4. DISCUSSION

According to **WHO**, CVD is the number one cause of death globally[9]. The present study was carried out to evaluate the cardioprotective effect of metformin. The cardioprotective effects were tested on Wistar Albino rats. As metformin is an anti-diabetic drug, here effect of metformin has been observed in both diabetic and non-diabetic cardiac injured rats[10]. Diabetes mellitus was induced by intraperitoneal injection of alloxan at a dose of 120mg/kg body weight [11] in group III and group V. Here, metformin was given to the group IV and group V of experimental rats at the dose of 100mg/kg body weight[12]. Myocardial injury was induced by intra peritoneal administration of isoproterenol at the dose of 85mg/kg body weight in the study to the group II-group V[7]. Assessment of cardiac function was made by estimating serum CK-MB, LDH and histopathological analysis of heart was done.

Estimation of elevated serum CK-MB serves as a useful guide for necrosis of myocardium[12]. In the present study CK-MB was significantly restored by Metformin pre-treatment in isoproterenol treated group IV and group V. Mean serum CK-MB was 7.17±0.28 in group III. Mean serum CK-MB was 5.97±0.30 in group II. The value was 3.49±0.07 in group IV that is within normal range. It was 5.13±0.22 in group V. p value (<0.001) shows significant difference between group II vs group IV and group III vs group V, which suggests that metformin possesses cardioprotective effect. Similar finding is observed in study done by Borde et al.,2016 who showed that treatment

with metformin 100mg/kg significantly p<0.001 reduced elevated serum CK-MB levels in metformin treated group compared to diabetic isoproterenol control group.

In regards to quantitative estimation of the serum lactate dehydrogenase levels in this study, metformin was found to have a protective effect on the myocardium. Mean serum LDH level in group III was 913.71±24.2, in group II it was 793.71±70.4. In group IV it was 411.57±21.9 which is within normal range and in group V it was 498.57±15.1. p value (<0.001) shows significant difference was found in group II vs group IV and group III vs group V, which suggests that metformin possesses cardioprotective effect. Our results are in agreement with previous study done by Bhave et al., 2016 who showed pre-treatment with metformin 100mg/kg significantly reduced elevated serum LDH levels and protection was statistically similar to that of carvedilol.

Histopathological examination of myocardial tissues in group I revealed clear integrity of the myocardial cell membranes. All of the rat heart showed score "0" in this group. Heart tissues from rats in group II and group III showed myocardial structure disorganization, cardiac muscle fibre separation, necrosis, edema and scoring "3-4". The histopathological findings of the metformin pre-treated myocardial infarcted hearts showed nearly normal cardiac tissue in group IVand group V scoring "0-1". The reduced cardiac muscle fiber architectural damage and necrosis in group IV and group V confirmed the cardioprotective effect of metformin. These observations of the present study coincide with previous similar study done by Borde et al., 2016 (metformin 100mg/kg pre-treated cardiac tissue showed occasional edema and necrosis) and Whittington et al., 2012.

Results of the study showed pre-treatment with metformin significantly reduced cardiac biomarkers associated with ISO-induced myocardial injury. These findings were confirmed by histopathological examination. So, metformin possesses cardioprotective effect in myocardial injury.

5. Summary and Conclusion

From the observations of the study, it shows that pre-treatment by metformin, prior to a subsequent isoproterenol induced myocardial injury, demonstrates a cardioprotective effect. This observation may have clinical relevance, as it can be developed as a new therapeutic approach for cardiovascular risk prevention.

Limitations of the Study

- Sample size was small.
- Duration of study was short.
- Expensive.

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