Life Sciences 225 (2019) 79–87

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Temporal dynamics of pre and post myocardial infarcted tissue with concomitant preconditioning of aerobic exercise in chronic diabetic rats☆

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ARTICLE INFO

Keywords:
Diabetes mellitus
Ischemia-reperfusion injury
Preconditioning
Aerobic exercise
Myocardial infarction
Renal abnormalities

ABSTRACT

The different ailments of heart including myocardial infarction (MI) and ischemic heart diseases are the foremost trigger of high mortality across the world which is instigated by sedentary life style, chronic hyperglycaemia and atherosclerosis. Albeit strenuous exercise itself induces temporary hypoxia which causes myocardial damage and this vitiosus circulus is poorly understood and has been assumed difficult to break. Present investigation targets temporal dynamics of aerobic exercise treatment induced preconditioning against diabetes associated pre- and post- myocardial injury. The persisting high blood sugar level leads to several biochemical alterations at pre- and post-MI phase. Here, we present the assessment of temporal expression of cardiac biomarkers (CKMB, LDH, cTnI and serum nitrite/nitrate), oxidative stress (myocardial TBARS and reduced NBT), inflammatory cytokines (IL-6, TNF-α and IL-10), renal biomarkers (BUN, serum creatinine and microproteinuria) and structural alterations of cardio-renal tissue. Aerobic exercise preconditioning significantly downregulate the pathological events or biomarkers and upsurge the physiological biomarkers at both pre- and post-MI phase. The attenuation or returning of pathological makers to lowest level at different time points endorses the therapeutic management of aerobic exercise against diabetic MI. Furthermore, the temporal expression of various cardio-renal biomarkers pattern elucidates that aerobic exercise preconditioning boost the strength and consolidate the cardiac muscles to work under stress. Despite the presence of traditional knowledge about health benefits of aerobic exercise, it is yet to be brought into the clinical arena. In spite of few impending challenges subjected to additional investigations, aerobic exercise preconditioning shows a high degree of promise.

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by perpetual hyperglycaemia due to lack of insulin or insulin resistance. Prevalence of type 2 diabetes mellitus is certainly high across the world as well as it majorly responsible for micro and macro vascular abnormalities [1]. The current statistics of IDF report reveals the increased ubiquity of diabetic population (about 326.5 million and about 429 million are at risk) across the globe, which is projected to rise up to 693 million by the year 2045. The global mortality rate of diabetes is about 10.7% which is significantly much higher than other diseases. The mortality rate with cardiac disease in diabetic patients is nearly 1.7 times higher than non-diabetic patients [2]. As the incidence of DM has gradually raised from last two decades, it has become a thrust area to revisit the therapeutic managements. Some traditional holistic approaches have been considered as potential strategy but deficit scientific evidences or unexplored mechanism limits their therapeutic application. Aerobic exercise is a conventional and integrated therapeutic management to regulate the metabolic function. Clinical study reports that physical exercise can develop myocardial tolerance against ischemic injury [3]. Whereas, sedentary life style has been reported as the major culprit of various metabolic disorders and associated vascular diseases [4]. As suggested by WHO, about 23% of adults (men 20% and women 27%) have sedentary life style across the globe, whereas in

☆ This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.
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https://doi.org/10.1016/j.lfs.2019.03.077
Received 8 March 2019; Received in revised form 27 March 2019; Accepted 30 March 2019
Available online 01 April 2019
0024-3205/

developed countries, this ratio is higher (men 26% and women 35%) than developing (men 12% and women 24%) countries [5]. Indeed, the vital health benefits of aerobic exercise have been described from ancient time by Susruta (an Indian physician in 600 BC240), Hippocrates (460–377 BCE) and Plato (427–347 BCE) [6,7]. Moreover, some recent evidence also suggests the significant mitigation of metabolic dysfunction and coronary heart diseases [8]. Increased physical activity can also improve the demand of blood and oxygen to the skeletal muscle and removal of metabolites, carbon dioxide and other toxic substance due to increased circulation [9]. In-addition, some other detrimental factors including fatty diet, regular binge drinking, active/passive smoking, lethargic physical state, dyslipidaemia, atherosclerosis and hypertension epithet for the cardiac complications. Whereas, the recent studies explored the promising therapeutic potential of physical exercise against these prominent root cause of cardiovascular diseases [10]. Moreover, the aerobic exercise including yoga also provide the health benefits including strengthening of cardiovascular muscles, upsurge the ability of contraction/dilation, angiogenesis, regulation of blood pressure and autonomic tone [11]. Although the scientific reports conformed the cardiac health benefits of aerobic exercise and reduce the rate of premature death. But the therapeutic impact of aerobic exercise on temporal dynamics of pre and post myocardial infarction with mechanistic changes in diabetic state is still unexplored which grounds the present investigation.

2. Material and method

2.1. Animals

Experimental animals for present study was approved by the Institutional Animal Ethics Committee as per the guidelines of 'Committee for the Purpose of Control and Supervision of Experiments on Animals' (CPCSEA) New Delhi, India (CPCSEA\AIP\2013\004). Albino wistar rats weighing (180-200 g) were used in the present investigation. Animals were first acclimatized in experimental room of animal house for one week prior to start the experimentation. All animals were maintained at free access to food and water *ad libitum* with normal day and night cycles (ambient humidity (60% ± 10%) and temperature (20 ± 2 °C)).

2.2. Chemicals

Streptozotocin (STZ) was purchased from HiMedia Laboratories, Mumbai, India. Enzymatic kits including cardiac troponin I and CK-MB, LDH were procured form Logotech India Pvt. Ltd. (Delhi, India) and Transasia Bio-Medicals Ltd., India respectively. Moreover, enzymatic kits for total cholesterol, TNF-α, IL-6 and IL-10 assay kits were procured form Ray Biotech India. All other chemicals used in the present study were of analytical grade.

2.3. Preparation of high fat diet (HFD)

HFD was prepared by mixing of 45.5% standard chow, 22.7% lard, 22.7% vegetable shortening and 9% sucrose, whereas the standard diet (for control group) consisted 100% chow. One-time prepared HFD was stored for 4 days (temperature, 7 °C, ± 2). The standard diet of rat chow provided 3.97 Kcal g^{-1} whereas, the HFD provided 6.25 Kcal g^{-1} [12].

2.4. Induction of experimental DM

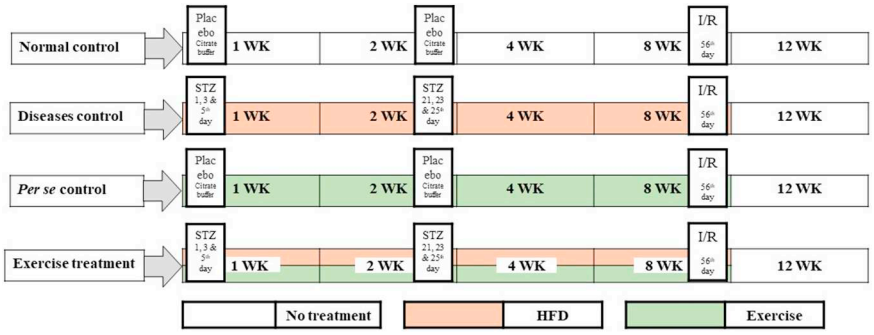
DM was induced by free access feeding of HFD and administration of double cycle repetitive dose (DCRD) of freshly prepared STZ solution (by citrate buffer at pH 4.5) as reported in our previous report [12]. A low dose of STZ (20 mg/kg/day) were given to each animal on 1st, 3rd, and 5th day in the first cycle and the similar treatment was repeated on 21st, 23rd and 25th day (after 15 days of first cycle) in the second cycle to induce sustained and chronic DM [12]. The rats with blood glucose levels over 300 mg/dl were considered as diabetic.

2.5. Induction of experimental ischemia/reperfusion (I/R) injury

Rats were anaesthetized by intraperitoneal injection of 80 mg/kg ketamine and 10 mg/kg xylazine. A small incision was made on neck to expose trachea and was cannulated by a polyethylene tube connected to a rodent respirator with a tidal volume of 1.0 mL/100 mg body weight (60 breaths/min). Then left thoracotomy was performed and pericardial tissue was removed under a microscope. The coronary artery was ligated (silk suture (6–0) for 30 min) followed by reperfusion as reported in our previous report [4].

2.6. Experimental protocol

Sixty experimental animals were randomly divided into four groups (normal control, diseases control, *per se* control and treatment group respectively) where each group comprise of fifteen animals.



Group 1 (normal control): Animals were fed with standard chow diet and DCRD of placebo (citrate buffer) were administered on 1st, 3rd, 5th day (first cycle) and 21st, 23rd, 25th day (second cycle). After eight weeks animals were exposed with I/R injury.

Group 2 (diseases control): Animals of diseased group were fed with HFD and DCRD of STZ (20 mg/kg/*i.p*/day) was administered by intraperitoneal injection [12]. At the end of eight weeks, all animals of this group were exposed with I/R injury.

Group 3 (*per se* control): Animals of this group were fed with standard diet and exposed with swimming training (90 min/day) up to eight weeks [4]. I/R injury was induced after eight weeks.

Group 4 (Treatment group): Animals were fed with HFD and DCRD of STZ (20 mg/kg/*i.p*/day). In-addition, animals of this group were simultaneously exposed with swimming training (90 min/day). At the end of eight weeks all animals of this groups were also exposed with I/R injury.

2.7. Biochemical estimation

All biochemical investigations were carried at different time interval (1st, 2nd, 4th, 8th and 12th week) to access the temporal dynamics of pre and post MI in diabetic rats (all biochemical estimations at 8th week were carried after 24 h of I/R induction). Blood sample of each animals were collected and was isolated by centrifugation method (7000*g* for 10 min at 2–4 °C). The serum was used to estimate various cardiac biomarkers (CK-MB, LDH, cTnI), inflammatory biomarkers (TNF-α, IL-6 and IL-10) and renal biomarkers (BUN, serum creatinine and micro-proteinuria) as per the manual of assay kits respectively. Furthermore, serum nitrite/nitrate concentration was measured to estimate the bioavailability of NO as described in our previous study [4]. Whereas, the oxidative stress level of myocardial tissue was estimated by TBARS and superoxide anion generation to measure the instantaneous changes in myocardial oxidative stress in correspondence to swimming induce aerobic exercise [4].

2.8. Histological assessment of cardio-renal tissue

Isolated heart and kidney were stored in 10% formalin solution. The tissues were further embedded in paraffin wax, then the thin slices were cut and this section was fixed on slide. The sections were stained with hematoxylin and eosin and were observed under inverted microscopy.

2.9. Statistical analysis

All the data is represented as mean ± SD. Results are analysed by using one-way ANOVA followed by Tukey's multiple comparison tests. A '*p*' value < 0.01 was considered as statistically significant.

3. Results

3.1. Effect of aerobic exercise on blood glucose

A persistent blood glucose level was observed in normal control group up to 12 weeks (in range of 97.32 to 98.87 mg/dl). Similarly, the *per se* control group has also shown comparable observation (in range of 97.69 to 98.47 mg/dl) with normal control group at every time point. Whereas, the disease control group demonstrated a significant increase in blood glucose level (about three times more) as compared to normal control at each point of observation which confirmed the presence of DM. Moreover, the exercise group has shown a substantial reduction in glucose level at different time point (pre and post MI) including 1st, 2nd, 4th, 8th, 12th week by 30%, 34%, 48%, 61% and 67% respectively as compared to disease group (Table 1).

3.2. Effect of aerobic exercise on lipid profile

A marked increase in serum total cholesterol (TC), low density lipoprotein (LDL) and triglycerides (TG) were observed in diabetic animals, whereas the level of HDL was significantly reduced in disease control animals as compared to normal rats. The normal concentration of TC was ranging in 126.56 to 127.56 mg/dl in normal control animals which was comparable to *per se* control animals (in range of 122.45 to 124.54 mg/dl) at each point of measurement (1st, 2nd, 4th, 8th and 12th week). In-addition, a partial augmentation in TC level was observed at 1st and 2nd week, whereas, a significant surge of TC was measured at 4th, 8th and 12th week as compared to normal control. Conversely, a significant reduction of TC level (about 22%, 27% and 33%) was observed in treatment group at 4th, 8th and 12th week respectively (Table 1). Additionally, the serum level of LDL and TG was measured in range of 44.45 to 45.65 mg/dl and 140.48 to 142.64 mg/dl respectively in normal control animals. The *per se* group also demonstrated similar range for LDL and TG (42.87 to 44.76 mg/dl and 141.56 to 143.43 mg/dl respectively) measured at different time points. Whereas, the disease control animals were shown a considerable increase of LDL and TG (about 2 folds and 1.4 folds at each point of measurement) as compared to normal control group. A significant diminution of LDL and TG were observed in treatment group animals. The percentage reduction of both biomarkers in exercise treated animals were found as 39%, 39%, 43%, 45%, 53% and 15%, 15%, 16%, 17%, 19% respectively at 1st, 2nd, 4th, 8th, 12th week (Table 1). Furthermore, the serum level of HDL in normal control group (in range of

Table 1
Table shows the effect of exercise training on glucose and various lipid parameters, TC indicates total cholesterol; HDL indicates high density lipoprotein; LDL indicates low density lipoprotein; TG indicates triglycerides. All data are expressed as Mean ± SD, $^{###}p < 0.001$ as compared with normal, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ as compared to diseases control.

Treatment	Week	Normal control	Diseases control	Exercise *per se*	Exercise + diabetic I/R
Serum glucose (mg/dl)	1st	98.87 ± 7.37	302.14 ± 12.23$^{###}$	97.69 ± 11.23	212.62 ± 11.36***
	2nd	97.43 ± 7.33	308.23 ± 12.34$^{###}$	98.47 ± 9.96	202.13 ± 12.58***
	4th	98.68 ± 6.51	313.34 ± 11.65$^{###}$	98.19 ± 12.65	162.15 ± 9.26***
	8th	97.32 ± 7.15	318.76 ± 9.45$^{###}$	98.25 ± 11.45	122.42 ± 11.57***
	12th	97.53 ± 5.34	314.65 ± 12.67$^{###}$	98.11 ± 12.44	102.22 ± 10.57***
TC (mg/dl)	1st	127.23 ± 11.4	131.12 ± 9.34	122.45 ± 9.67	128.56 ± 8.23*
	2nd	126.56 ± 12.6	148.47 ± 10.45	123.67 ± 9.49	129.67 ± 8.34*
	4th	127.22 ± 11.7	167.8 ± 5.4$^{###}$	123.23 ± 8.9	129.34 ± 7.45***
	8th	126.56 ± 12.5	176.34 ± 8.7$^{###}$	124.54 ± 9.5	128.14 ± 8.25***
	12th	127.45 ± 11.4	198.73 ± 9.6$^{###}$	124.32 ± 11.5	132.67 ± 7.45***
HDL (mg/dl)	1st	17.64 ± 0.62	15.68 ± 0.5$^{###}$	17.57 ± 0.8	20.24 ± 0.75***
	2nd	17.84 ± 0.84	15.45 ± 0.6$^{###}$	17.68 ± 0.6	21.38 ± 0.45***
	4th	17.56 ± 0.67	14.76 ± 0.4$^{###}$	17.34 ± 0.8	21.68 ± 0.65***
	8th	17.78 ± 0.56	14.53 ± 0.5$^{###}$	17.47 ± 0.7	22.58 ± 0.45***
	12th	17.45 ± 0.78	13.84 ± 0.6$^{###}$	17.57 ± 0.8	23.85 ± 0.74***
LDL (mg/dl)	1st	45.57 ± 4.78	96.46 ± 7.45$^{###}$	42.87 ± 6.45	59.15 ± 5.45***
	2nd	45.56 ± 4.54	95.38 ± 5.34$^{###}$	44.76 ± 7.67	58.49 ± 6.36***
	4th	45.43 ± 3.56	96.34 ± 7.36$^{###}$	43.15 ± 6.24	54.36 ± 4.15***
	8th	44.45 ± 4.45	96.32 ± 5.45$^{###}$	44.34 ± 5.14	52.44 ± 6.53***
	12th	45.65 ± 3.33	96.36 ± 6.26$^{###}$	44.13 ± 6.56	45.09 ± 5.73***
TG (mg/dl)	1st	141.35 ± 7.53	184.34 ± 9.66$^{###}$	142.24 ± 7.33	156.43 ± 7.23***
	2nd	140.48 ± 6.77	184.56 ± 7.56$^{###}$	142.45 ± 7.41	157.36 ± 6.76***
	4th	141.26 ± 8.53	185.47 ± 9.87$^{###}$	141.76 ± 8.78	155.56 ± 7.46***
	8th	142.64 ± 6.36	186.76 ± 8.43$^{###}$	141.56 ± 8.56	154.23 ± 7.82***
	12th	141.46 ± 7.57	188.57 ± 8.75$^{###}$	143.43 ± 9.65	153.45 ± 7.47***

17.45 to 17.84 mg/dl) was found comparable to *per se* group where the observed level of HDL was in range of 17.34 to 17.68 mg/dl. Moreover, the disease group revealed a significant fall in HDL level that was measured as 11%, 13%, 16%, 18% and 21% at 1st, 2nd, 4th, 8th and 12th week respectively. Conversely, a substantial upsurge of HDL level was perceived in aerobic exercise treated group by 15%, 17%, 19%, 21% and 27% at 1st, 2nd, 4th, 8th and 12th week respectively as shown in Table 1.

3.3. Effect of aerobic exercise on cardiac biomarkers

At the initial phase (1st and 2nd week) of exercise an insignificant variation of CKMB level was observed among all groups. Whereas, during middle phase (2nd and 4th week) of experimental protocol, a significant increase of CKMB level was observed in disease group as compared to normal control. Furthermore, a slight increase of CKMB level was observed in *per se* group and treatment group of aerobic exercise as compared to normal and disease group which revealed the temporary hypoxia (during strenuous exercise) -induced cardiac damage. After induction of experimental I/R at 8th week a significant increase in CKMB level was observed in all group as compared to initial and middle phase. The increased CKMB level in disease group was significantly higher than normal control group at 8th week. The treatment group showed a considerable diminished level of CKMB as compared to disease group, whereas comparable to *per se* treatment group. At post I/R phase (12th week), the level of CKMB of treatment group was comparable to the level at I/R induction (8th week), whereas the *per se* group exhibited a significant reduction which clearly revealed the impact of aerobic exercise against I/R damage.

The similar observation was found for LDH and cTnI level, where the initial phase (1st and 2nd week) was not significantly impacted whereas the middle phase (4th week) experienced comparatively increase level of these cardiac biomarker than initial phase. Moreover, the level of LDH and cTnI at I/R phase (8th phase) were significantly raised in all groups respectively as compared to initial and middle phase. Additionally, the detailed observation of the finding at 8th week revealed the considerable increase of LDH and cTnI level in disease group respectively. Whereas the increasing of these cardiac biomarkers was significantly hindered in aerobic exercised groups (both pes se and treatment group). On the later phase, even the presence of LDH and cTnI level were higher as compared to initial and middle phase but it was considerable low as compared to I/R phase (8th week). The results show interesting consequences in exercised group where the level of LDH and cTnI were comparatively less altered at initial, middle and later phase.

Serum level were further analysed for nitrite/nitrate concentration to speculate the bioavailability of endothelium-derived relaxing factor (NO). At initial phase (1st and 2nd week), a slight reduction of serum nitrite/nitrate concentration was observed in disease group as compared to normal control, whereas, no significant changes were observed in *per se* group. During the middle phase, a significant reduction of serum nitrite/nitrate level was found in disease group which was significantly blocked in aerobic exercised groups (both in *per se* and treatment group) as compared to normal control (Fig. 1). The similar observation was found after I/R stage (8th week) and post I/R phase (12th week). These observations clearly reveal the ability of aerobic exercise to maintain the level of serum nitrite/nitrate concentration.

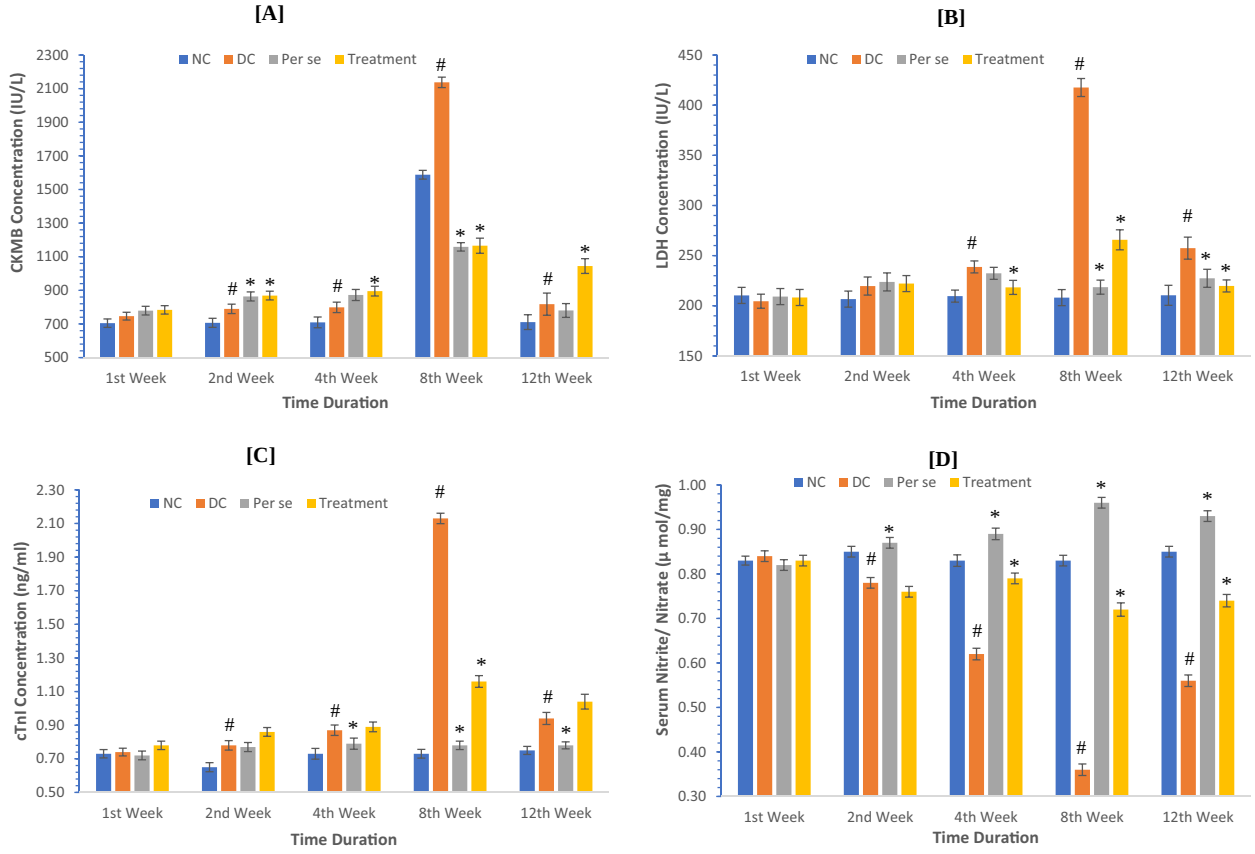


Fig. 1. Temporal estimation of various cardiac biomarkers including CKMB (A), LDH (B), cTnI (C), serum nitrite/nitrate level (D), in different groups. All data are expressed as Mean ± SD, value of significance has represented by ($^{\#}$) $p < 0.01$ as compared with normal and (*) represents $p < 0.01$ as compared with diseases group at respective time point.

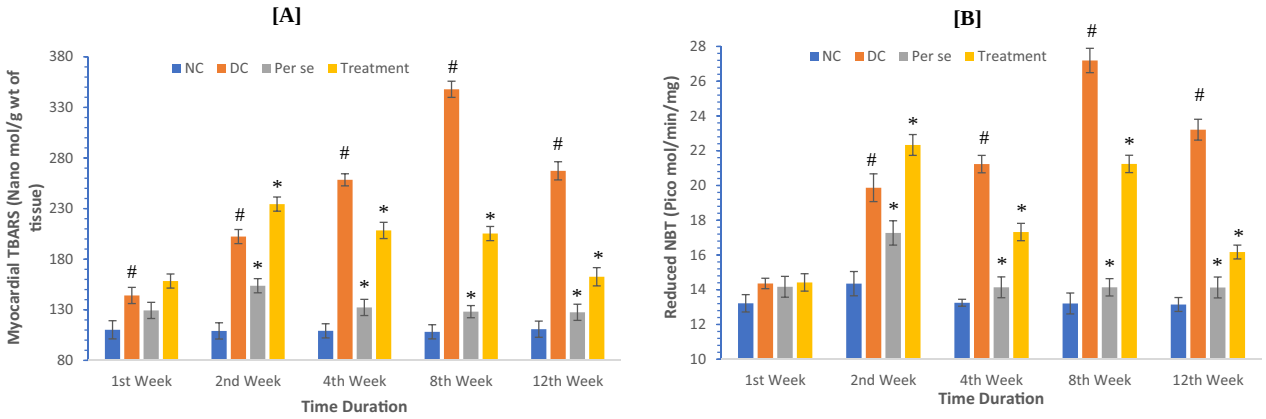


Fig. 2. Temporal assessment of various oxidative stress biomarkers including myocardial TBARS (A), myocardial reduce NBT level (B) in different groups. All data are expressed as Mean ± SD, value of significance has represented by (#) $p < 0.01$ as compared with normal and (*) represents $p < 0.01$ as compared with diseases group at respective time point.

3.4. Effect of aerobic exercise on oxidative stress

Oxidative stress of myocardial tissue was measured by serum TBARS and reduced NBT level. Oxidative stress was significantly surged in disease control group as well as in aerobic exercise group (both in *per se* and treatment group) as compared to normal control animals at initial and middle phase (1st, 2nd week and 4th week respectively). Whereas at middle phase, the findings revealed an interesting observation which confirms the reversal of increased oxidative stress level in aerobic exercise groups (either in *per se* group or in treatment group). After induction of I/R lesion (8th week), a considerable elevation of myocardial TBARS and NBT-reduction in disease group as compared to normal control group, whereas no significant increase was observed in *per se* and treatment group (Fig. 2). The post I/R phase (12th week) also exhibited the similar observation where the level of oxidative stress in exercised group was comparable with initial and middle phase (pre-I/R stage).

3.5. Effect of aerobic exercise on inflammatory cytokines

Both pro- and anti-inflammatory cytokines were measured at different time point (pre and post I/R phase). At initial phase (1st week), there was no significant difference observed in IL-6 for all group whereas at 2nd week, disease control and aerobic exercised groups (both *per se* and treatment) shown significant higher concentration of IL-6 as compared to normal control group. Similar consequences were observed at middle phase as in 2nd week. Moreover, post-operative animals (at 8th week) were shown a higher release of IL-6 in all treated groups (disease control group and exercised group), but the increase of IL-6 level in exercised groups were comparatively lower than disease control group which showed that aerobic exercise obstruct the overall upsurge of IL-6 level. During the post-operative phase (12th week) diabetic group showed a significant higher concentration of IL-6 as compared to normal control, whereas the *per se* group was shown the comparable concentration of IL-6 level with normal control group. Treatment group revealed a slight interruption of IL-6 release as compared to disease control group.

Serum TNF-α and IL-10 were also observed in a similar pattern of release in different groups and at different time of observation. The concentration of TNF-α and IL-10 were significantly increased in disease group as compared to normal control at each time point (1st, 2nd, 4th, 8th and 12th week) of measurement. Whereas, no statistically significant difference was observed in normal control and *per se* group for both TNF-α and IL-10 respectively at each point of measurement. Furthermore, the treatment group significantly reduced the serum TNF-α and IL-10 level at every time point of measurement as shown in Fig. 3.

3.6. Effect of aerobic exercise on renal biomarkers

Assessment of renal biomarkers were done by measuring BUN, serum creatinine and microproteinuria at all different time point. The concentration of all renal biomarkers was significantly higher in disease group as compared to normal control, whereas normal control and *per se* group retained equivalent level of all biomarkers respectively. The treatment of aerobic exercise significantly reduced the level of all renal biomarkers and the observation confirmed the therapeutic impact of aerobic exercise in renal protection against diabetes and associated complications. Moreover, the consequences also revealed that the pre-

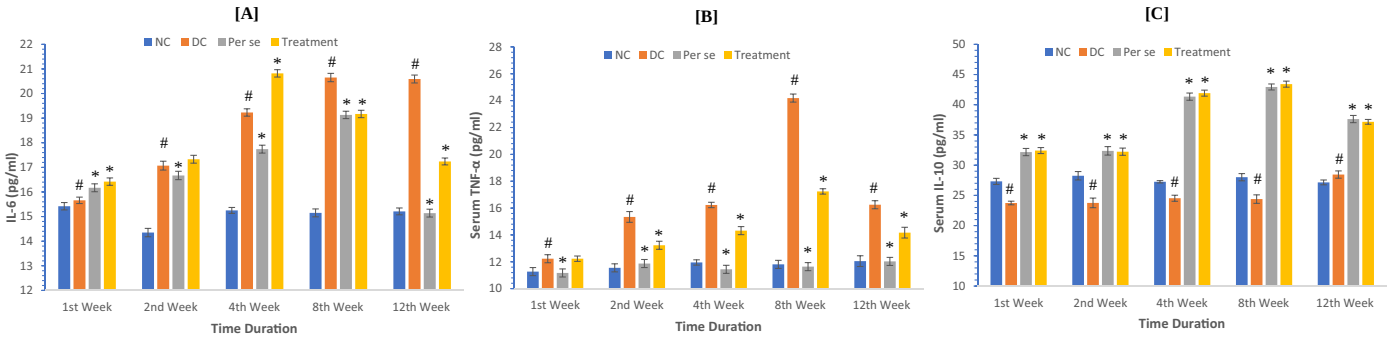


Fig. 3. Temporal assessment of various inflammatory cytokines release in serum including IL-6 (A), TNF-α (B) and IL-10 level (C) in different groups. All data are expressed as Mean ± SD, value of significance has represented by (#) $p < 0.01$ as compared with normal and (*) represents $p < 0.01$ as compared with diseases group at respective time point.

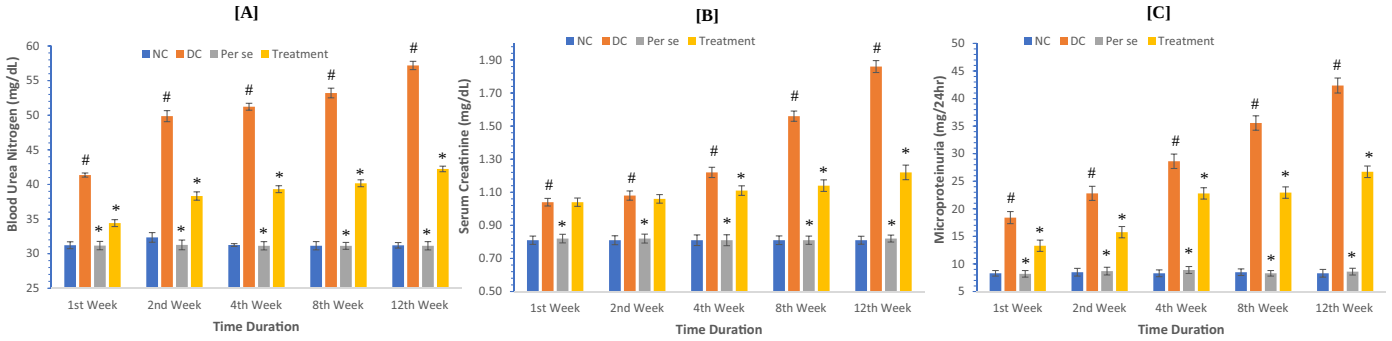


Fig. 4. Temporal assessment of various renal biomarkers including BUN (A), serum creatinine (B) and microproteinuria level (C) in different groups. All data are expressed as Mean ± SD, value of significance has represented by ($^{\#}$) $p < 0.01$ as compared with normal and (*) represents $p < 0.01$ as compared with diseases group at respective time point.

phase of myocardial I/R was completely un-altered as the observations were comparable at 1st and 4th week. Moreover, the measurement of BUN after 24 h of I/R (at 8th week) was also found almost similar as at 4th week. Indeed, the serum creatinine and microproteinuria level were significantly raised at 8th week for disease group as compared to pre-I/R phase. Whereas, the treatment group significantly prevented the damage which was observed by reduced level of serum creatinine and microproteinuria at 8th week (Fig. 4). Furthermore, at post-I/R phase (12th week), the level of serum BUN, serum creatinine and microproteinuria were comparatively higher than pre-I/R phase and 8th week in disease group which revealed the subsequent pathological impact of myocardial I/R on renal functions. Treatment group had consistently reduced level of all biomarker respectively at post-I/R phase (12th week) also.

3.7. Effect of aerobic exercise on structural abnormalities of myocardial tissue

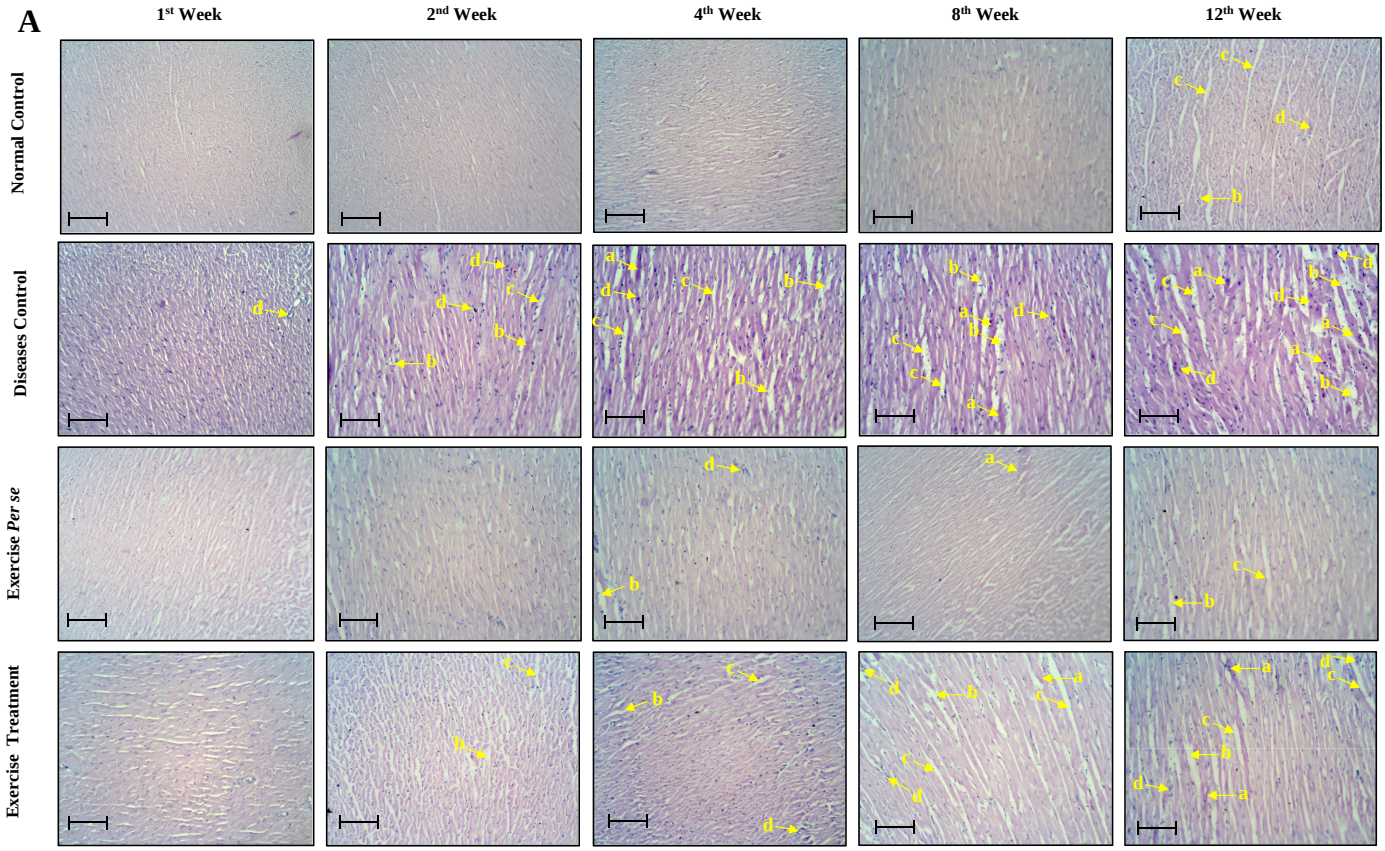
Histological assessment of cardiac tissue was done at all different time point (pre- and post- I/R injury) as shown in Fig. 5(A). The histological photomicrograph of normal control animals showed no structural alteration at any point (1st, 2nd and 4th week) before inducing experiment I/R injury. Whereas, the histology of post-I/R phase of normal control rats indicated the presence of perivascular cuffing, increased intercalated space and nuclei karyolysis which revealed the structural alteration due to I/R insult. Disease control group showed major structural lesions during pre- I/R phase also due to presence of chronic hyperglycaemia and other lipid profile which are the root cause of cardiovascular abnormalities. Moreover, the detailed observation of disease control group revealed the greater existence of leukocyte aggregation in perivascular space, increased space between intercalated discs and karyolysis in pre- I/R phase at each time points (1st, 2nd and 4th week) as compared to cardiac tissue of other groups. Additionally, the post I/R phase of disease control photomicrograph comprised the presence of contraction band of myocardium which represents the necrosis of cardiac tissue. The photomicrograph of *per se* group indicated the comparable observation to normal control. However, mild presence of perivascular cuffing and karyolysis was observed at pre- I/R phase (4th week) which may develop due to temporary hypoxia to the myocardial tissue induced by vigorous exercise. Whereas, post- I/R phase shows some structural alteration as observed in normal control group but the total impact of damage was lower as compared to normal control group. Histological micrograph of aerobic exercised diabetic animal showed minor structural alteration during both pre and post I/R phase as compared to disease control animals. The presence of perivascular space and increased intercalated space during initial and middle phase (2nd and 4th week) showed myocardial damage. Additionally, the band of necrosis and karyolysis at post- I/R phase revealed the potential of I/R lesions in diabetic as well as regular exercised animals. Although, the structural alteration was also observed in pre- and post I/R phase in treatment group but the impact of damage was significantly lowered as compared to disease group.

3.8. Effect of aerobic exercise on structural abnormalities of renal tissue

The histological evaluation of renal tissue of normal control animals showed the presence of normal glomerular and tubules at different time point (pre- and post- I/R phase). Whereas, the post- I/R phase (12th week) of normal control group confirmed the presence of mild blood vessels congestion. The photomicrograph of disease group demonstrated the absence of normal tubule at both pre- and post- I/R phase (1st, 2nd, 4th, 8th and 12th week). Conversely, vacuolation of endothelial lining glomerular tufts and necrosis of endothelial lining of renal tubules was observed at pre- I/R phase (1st, 2nd and 4th week). Moreover, the post- I/R phase (8th and 12th week) of disease group contained vacuolation, necrosis of endothelial lining and congestion of renal blood vessels that revealed the diabetes associated damage in renal tissue. Exercise *per se* group showed the comparable observations with normal control. However, small damage of renal tissue was confirmed by presence of vacuolation and necrotic lining at initial and middle phase (1st, 2nd and 4th week). Post- I/R phase of exercise groups also represented the small impact of vacuolation. Even the normal glomerular and renal tubules were observed at all stages of measurement, but the minor renal injury may occur due to strenuous exercise and myocardial abnormalities. Furthermore, the treatment group significantly diminished the structural alteration at initial and middle phase (pre- I/R phase; 1st, 2nd and 4th week) which was confirmed by abolished necrosis and renal blood vessel congestion as shown in Fig. 5(B). Moreover, the post- I/R phase showed the lower impact of injury as compared to disease control group that was observed by the presence of normal glomerular and renal tubule.

4. Discussion

The protecting temperament of aerobic exercise against metabolic disorders and MI has been an area of interest in the scientific community for several decades. Many studies pointed either pre or post treatment of various therapeutic managements against the root cause of pathological event [3,4]. High fat intake, DM and sedentary life mimic the current scenario of human lifestyle which ultimately endorse the increased prevalence and mortality of microvascular diseases. Sedentary lifestyle leads to various health disorders including obesity, dyslipidemia, diabetes and heart diseases. Several studies reported that regular physical exercise ameliorate the risk of DM and associated micro/macro vascular diseases [3,4,6–9,13]. Lack of pharmacological evidences to endorse their associated mechanism limits the clinical preference. Present investigation targeted the concurrent fluctuation in structural and functional parameters pertaining to different conditions and treatments given to animals.



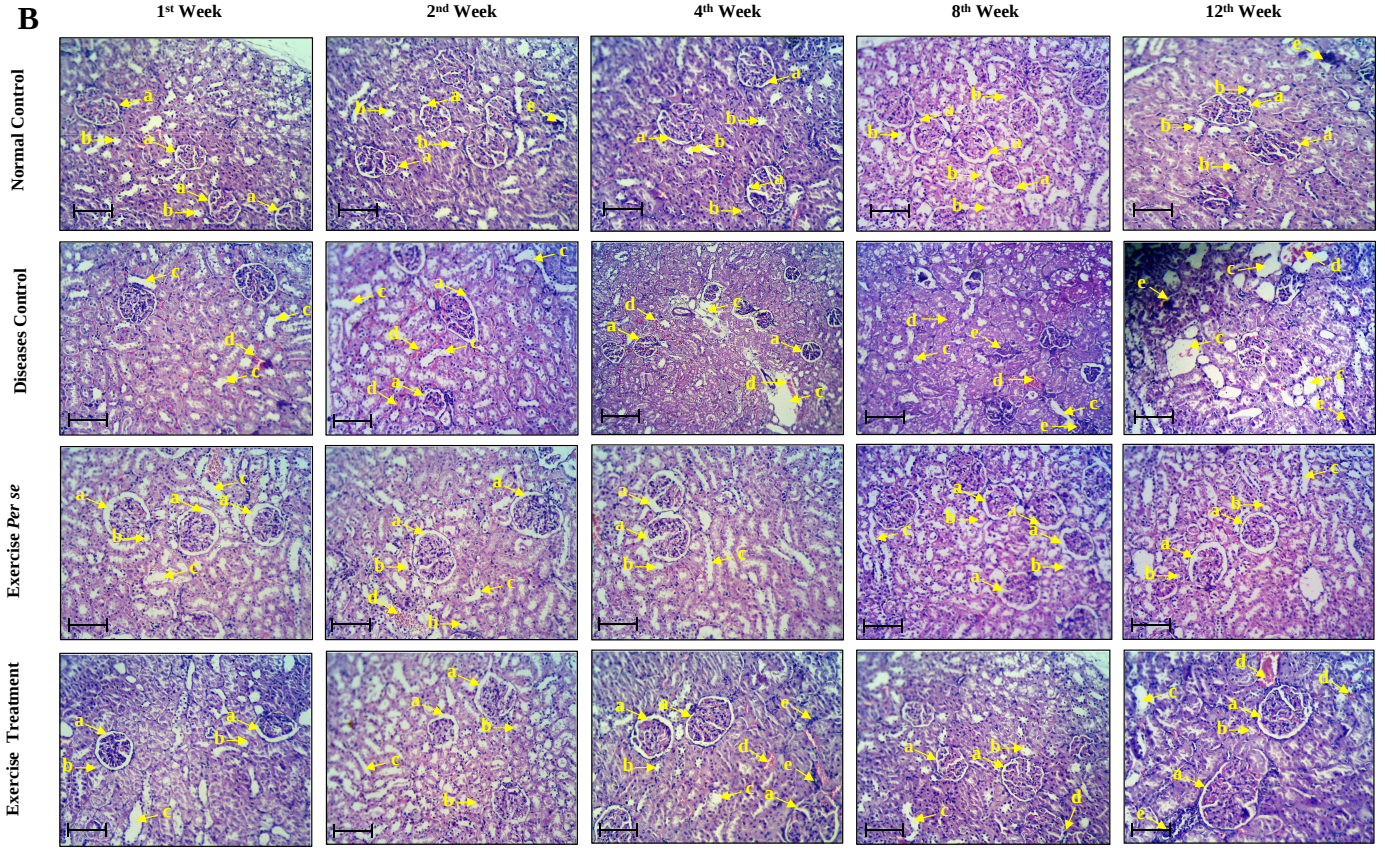


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Fig. 5. The histological section of cardiac tissue (A) is highlighted as: 'a' represents contraction band necrosis, 'b' represents perivascular cuffing, 'c' represents increased intercalated space (widened intracellular spaces), 'd' represents nuclei karyolysis marked in different photomicrographs of different groups of animals. Moreover, the renal histological section (B) is highlighted as: 'a' represents normal glomerulus, 'b' represents normal renal tubules, 'c' represents vacuolation of endothelial lining glomerular tufts, 'd' represents necrosis of epithelial lining renal tubules, 'e' represents congestion of renal blood vessels. The histological examination was performed using Motic Microscope BA310 (Motic, USA) at 20× (scale bar = 100 μm).

Rats fed with HFD and STZ administration results to insulin resistance, β-cell destruction and high level of glycated haemoglobin as reported in our previous study which cause significant hyperglycemia [12]. DM eventually produce dyslipidemia, oxidative stress, release of proinflammatory cytokines and heart diseases [14]. The similar observations (three times higher fold of blood glucose was measure in disease group) were obtained in the present investigation which confirm the occurrence of DM and associated disorders. Whereas, marked increase in serum TC, LDL, TG and reduced HDL concentration further confirmed the existence of dyslipidemia. Aerobic exercised treated animals significantly reduced the blood glucose level, TC, LDL, TG and upsurge the HDL level during pre and post myocardial I/R lesion. The consequence precisely confirmed the protective impact of aerobic exercised against DM and dyslipidemia which are the major root of CVD. Moreover, the slight increase of CKMB, LDH, cTnI were observed at initial and middle phase in exercise group compared to disease group that revealed the minor cardiac damage induced due to temporary hypoxia during strenuous exercise [15]. The intensification of these cardiac biomarkers was significantly higher after 24 h of I/R induction (8th week) which endorse the impact of cardiac damage. But at post-I/R phase, this damage was reduced in all groups but significant reduction was measured in exercise animals because of preconditioning of cardiac tissue during heavy exercise. The preconditioning of myocardial tissue also upsurges the level of serum nitrite/nitrate (NO) level as reported in previous study [4]. Our study confirms the upsurge of NO regulation in aerobic exercised group and also revealed the protection against I/R injury in diabetic animals during pre- and post-MI phase. Additionally, several recent studies revealed the presence of high oxidative stress due to DM which has corroborated by the consequence of present investigation [12,15]. Even, the oxidative stress in aerobic exercise animals were also higher than normal control which further explained the notion of induction of ROS during heavy exercise consistently. During the induction of temporary hypoxia in myocardial tissue, oxidative stress has been generated due to anerobic metabolism. The synthesis of ROS during pre-MI phase was slightly more which found to be constant at later (middle and post- I/R phase) stage in aerobic exercise groups against diabetic group. The consequences confirmed the regulatory potential of aerobic exercise against ROS synthesis during adverse condition. Myocardial I/R injury is also an inflammatory condition where the overproduction of inflammatory cytokine (IL-6 and TNF-α) mediator results to severe proinflammatory reactions and provoking inflammation [16,17]. We have investigated the relation between aerobic exercise preconditioning and -associated cardioprotective effect through its anti-inflammatory potential. Pro-inflammatory cytokines (TNF-α and IL-6) levels were prominently provoked by I/R and temporary hypoxia produced during strenuous exercise as compared to normal control group at early phase (1st, 2nd and 4th week). Whereas, during experimental exposure of I/R injury, treatment of aerobic exercise significantly suppresses the increased level of TNF-α and IL-6. The TNF-α and IL-6 levels at post-I/R phase (12th week) were not altered in exercise group as compared to pre-I/R phase (1st, 2nd and 4th week) even the animals had chronic hyperglycemia. The outcomes revealed that exercise induced preconditioning reduce the concentrations of IL-6 and TNF-α as compared to diabetic I/R models which may be the possible mechanism to its cardioprotective effects after reperfusion. Moreover, the anti-inflammatory cytokines (IL-10) were slightly increased in disease group at all pre- and post-I/R phase. Whereas, the exercise preconditioning significantly upsurge the level of IL-10 at pre- and post-I/R phase which produce anti-inflammatory response against I/R injury. The consequences endorse the regulation of pro-inflammatory and anti-inflammatory cytokines by aerobic exercise as uncontrolled release of inflammatory mediators exacerbates protein wasting and organ dysfunction [18–20].

Notably, other assessments including BUN, microproteinuria and serum creatinine were carried to understand the effect of exercise preconditioning on renal tissue which showed a significant drop in all biomarkers at all stage (pre- and post-I/R phase) as compared to disease group. These findings suggest that the main driver of the drop in renal biomarkers is aerobic exercise -induced preconditioning, anti-oxidative environment, anti-inflammatory potential and anti-diabetic action before and after I/R phase as suggested by previous reports [21,22]. Furthermore, pre- and post exercise preconditioning also exerts some influence on structural permanency during and after MI as we previously conceded by the observation of histological assessment of cardio-renal tissue which were slightly altered in exercise group as compared to diabetic I/R animals [4]. Consequently, imaging of pre- and post-MI tissue characterized to contraction band necrosis, perivascular cuffing, increased intercalated space and nuclei karyolysis for myocardial tissue, whereas, vacuolation of endothelial lining glomerular tufts, necrosis of epithelial lining renal tubules and congestion of renal blood vessels are accountable for renal tissue. Pre- and post-aerobic exercise preconditioning quantifying diminished structural mutation as compared to disease control group.

By the conclusion of all assessments it has been observed that aerobic exercise causes a temporary hypoxia to myocardial tissue at early phase. However, the persistent exposure of hypoxic condition for longer duration boost the strength of cardiac muscles and consolidate the cells to work under stress [15,23,24]. These constructive insurgencies instigate preconditioning in cardiac tissue which protect the heart muscle during actual pathological condition or MI. Although we have shown the temporal expression pattern for aerobic exercise preconditioning, but a little is known about the signalling cascades that overturn the pathology of diabetic I/R injury. Our results show that preconditioning (a potent cardioprotective strategy) and short ischemia duration have a major impact on the intensity and dynamics of post-MI.

Acknowledgment

The authors are grateful to Dr. Ashok K. Chauhan, Founder President, Ritnand Balved Education Foundation (RBEF) and Amity Group of Institutions for providing the infrastructure and support.

Conflict of interest

The authors declare that there is no conflict of interest.

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