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Cardiac damage following renal ischemia reperfusion injury increased with excessive consumption of high fat diet but enhanced the cardiac resistance to reperfusion stress in rat☆

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ABSTRACT

Renal ischemia-reperfusion (IR) injury inflicts remote cardiac dysfunction. Studies on rats fed with a high-fat diet (HD) showed contradictory results: some demonstrated increased sensitivity of the heart and kidney to IR injury, while others reported resistance. In this study, we examined cardiac dysfunction and compromised cardiac tolerance associated with renal IR in HD and standard diet (SD) fed rats. Male Wistar rats fed with HD or SD diet for 16 weeks were subjected to either renal sham or IR protocol (bilateral clamping for 45 min and reperfusion for 24 h). The hearts isolated from these rats were further subjected to normal perfusion or IR procedure to study cardiac response. Renal IR surgery negatively affected cardiac function with substantial changes in the cardiac tissues, like mitochondrial dysfunction, elevated oxidative stress, and inflammation. HD-fed rat hearts exhibited hypertrophy at the end of 16 weeks, and the consequential impact on the heart was higher in the animals underwent renal IR surgery than with sham surgery. However, the IR induction in the isolated heart from renal sham or renal IR operation showed significant tissue injury resistance and better physiological recovery in HD-fed rats. However, in SD-fed rats, only hearts from renal IR-operated rats showed resistance to cardiac IR, whereas hearts from renal sham-operated rats were more susceptible to IR damage. The augmented IR resistance in the heart with prior renal surgery was due to preserved mitochondrial bioenergetics function, reduced oxidative stress, and activation of the PI3K/AKT signaling axis.

1. Introduction

Renal ischemic injuries are developed by the consequential impact of diminished or absence of blood flow during transplantation surgeries, vascular surgeries, or renal stenosis [1]. Although immediate reperfusion of the ischemic area is the essential step in reducing the ischemic damage, the clinical interventions adopted for reperfusion itself lead to unavoidable damage, named ischemia-reperfusion (IR) injury [2]. Incidentally, pathological changes in the kidney due to IR not only induce renal damage but also can instigate the release of a cascade of mediators that promote injury in the other organ or deteriorate its function [3].

Distant organ injury associated with ischemic acute kidney injury (AKI) represents dysregulation in the complex interplay of

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various mechanisms and pathways that coordinate the overall function of different organ systems [4]. The liver, lungs, heart, brain, and kidney form interconnected networks, and thus, the disturbance or dysfunction in one organ can affect the performance of another organ [5]. Understanding the molecular and cellular interactions involved in distant organ injury is essential for developing novel therapeutic strategies to mitigate its clinical implications [5,6]. Cardiac dysfunction/failure, one of the most frequent and serious consequences associated with ischemic AKI, is reported to be the major cause of the rehospitalization of renal patients [7]. Several key pathways contribute to cardiac injury associated with ischemic AKI, including systemic inflammation, oxidative stress, endothelial dysfunction, immune dysregulation, and microvascular dysfunction [8]. These pathological events interact in a complex manner, leading to cellular dysfunction, structural alterations, and impaired cardiac function [5,6,8].

According to Shiao et al. (2015), the impact of distant organ injury in ischemic AKI becomes even more severe in the presence of comorbidities [9]. The presence of co-morbidities can independently make alterations in the cardiovascular system, which often contribute to the severity and complexity of the disease [9]. Obesity, one such condition with accompanying metabolic abnormalities, worsens the outcomes of ischemic heart disease [10,11]. In fact, the beneficial effects of obesity in the ischemic heart were also reported [12]. However, the majority of pre-clinical studies showed the negative effect of obesity on ischemic heart [13–15]. The animal studies have demonstrated that obesity combined with insulin insensitivity reduces myocardial resistance to ischemia-reperfusion (IR) injury [16]. Early studies have shown that nutritional status can be a strong predictor of post-operative outcome [17,18]. Few studies have shown that short-term high-fat diet administration before surgery is detrimental to the body's response to surgical stress [19,20]. On the other hand, reduction of nutrients or energy transiently before surgery is beneficial [20–22]. Similarly, few early studies have documented the risk for surgery with long-term over-nutrition [20].

The present study investigated the effect of renal reperfusion on the heart to withstand IRI in a rat model administered with a high-fat diet, a condition known to exacerbate myocardial vulnerability to IRI. Understanding the potential cardioprotective or deleterious effects of renal reperfusion in the setting of a high-fat diet could have significant clinical implications for patients undergoing cardiovascular procedures.

2. Methodology

2.1. Animal model and surgical procedures

Male Wistar rats weighing 120–150 g were procured from the Central Animal Facility, SASTRA deemed to be University. The animals were acclimated for at least one week prior to the experiments under standardized conditions, including a temperature of 22 $\pm$ 1 °C, humidity of 55 $\pm$ 5 %, and a 12-h light/dark cycle. All animal procedures complied with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (565/SASTRA/IAEC/RPP).

2.2. In Vivo renal ischemia-reperfusion

The rats were assigned to two major groups (n = 24 per group), namely normal diet-fed (ND) and high-fat diet-fed (HD). The HD rats were maintained in a high-fat diet for 16 weeks, whereas the ND group rats were maintained in a normal diet. The high-fat diet was prepared by adding 40 % fat (beef tallow) to the powdered chow pellets and then re-pelleted using a hand pellet presser. The total energy of the standard diet for normal animals was 395 kcal/100 g (65 % carbohydrates, 24 % proteins and 11 % fat), and the high-fat diet had a total energy of 540 kcal/100g (40 % carbohydrates, 20 % proteins and 40 % fat). Plasma levels of total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides and glucose were estimated at the end of treatment period using kit method (Agappe, India).

At the end of 16 weeks, each group was subdivided into 2 subgroups (n = 12) - 1) Sham- In this group, the right and left renal pedicles of rats were surgically exposed without clamping, and the incision was sutured after 45 min, and the animal was recovered for 24 h 2) Ischemia-reperfusion (IR) - The renal peduncle of the rats were exposed, and ligated using non-traumatic vascular clamps to induce ischemia for 45 min and removed the ligation to initiate the reperfusion for 24 h.

At the end of reperfusion, plasma levels of creatinine and blood urea nitrogen (BUN) were measured using a kit method as per the manufacturer's instructions (Agappe, India). From each group, kidneys (n = 3) were stored in formalin for histopathological analysis using hematoxylin/eosin. The pathomorphological lesions were examined under the microscope based on the following pathologies according to EGT (Endothelium, glomeruli, tubular) scoring system- Congestion/haemorrhage, glomerular congestion, tubular dilatation, tubular degeneration, tubular necrosis and presence of eosinophilic casts. The changes were scored on a 4-point scale depending on the severity (0- nil/absent, 1- mild, 2- moderate, 3- marked, 4- severe), and the average score is presented [23].

2.3. Ex Vivo isolated rat heart perfusion

Following the sham or renal ischemia-reperfusion surgery, rat hearts from all the groups were excised after anesthetizing the animals with sodium thiopentone (60 mg/kg of body weight). The excised hearts were mounted onto a Langendorff apparatus and continuously perfused with Krebs-Hensleit (KH) buffer of pH 7.4 according to the assigned groups (n = 6), as described below. Ischemia was induced by stopping the buffer flow to the heart for 30 min. Throughout the experiment, heart rate (HR), left ventricular developed pressure (LVDP), Left ventricular end-diastolic pressure (LVEDP), and rate pressure product (RPP) were recorded and calculated using LabChart physiological data analysis software (ADInstruments Inc, Sydney, Australia).

Group 1: Normal Perfusion (NP): Rat hearts perfused with KH buffer for 120 min.

Group 2: Heart Ischemia-Reperfusion (HIR): After 30 min of stabilization with KH buffer, ischemia was induced for 30 min, followed by 60 min of perfusion with KH buffer.

The enzymatic activity of the injury marker enzymes lactate dehydrogenase (LDH) and creatine kinase (CK) was utilized to evaluate the cardiac injury. The enzymatic activity of these enzymes was measured in perfusate and tissue samples from the various experimental groups, as previously described. LDH activity was evaluated by monitoring the absorbance of NADH at 340 nm while measuring the conversion of lactate to pyruvate. The concentration of inorganic phosphate, which is liberated during ATP conversion, was detected using a reagent and computed at 660 nm to determine CK activity [24].

2.4. Histopathology

The hearts were thoroughly cleaned, weighed, and rinsed with ice-cold normal saline. A section of the heart was fixed in 10 % formalin (v/v), embedded in paraffin, cut into 5 μm sections, and stained with hematoxylin and eosin for histopathological examination under a light microscope. Three heart tissue samples per group were analyzed for histological changes [24].

2.5. TUNEL staining

TUNEL assay was used to assess myocardial DNA strand breaks. Fluorescence detection kit (Takara Bio Inc., Japan) and fluorescence microscopy were employed for this purpose.

2.6. Isolation of mitochondrial subpopulations

The subsarcolemmal (SSM) and interfibrillar (IFM) mitochondria from rat hearts were isolated following the procedure described by Palmer et al., in 1985. The differential centrifugation technique was utilized for the isolation process [25].

2.7. Antioxidant enzymes and Lipid Peroxidation

Thiobarbituric acid reactive substances (TBARS) and glutathione (GSH) concentrations, as well as the activities of glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD), and catalase enzymes, were measured in cardiac tissue samples using previously described methods [26].

2.8. Mitochondrial analysis

i) Activity of Electron Transport Chain (ETC) Enzymes: The activities of various electron transport chain enzymes, including rotenone-sensitive NADH-oxidoreductase (NQR), succinate decylubiquinone DCPIP reductase (SQR), ubiquinol cytochrome-c reductase (QCR), and cytochrome *c* oxidase (COX), were analyzed spectrophotometrically following established procedures [27].
ii) ATP Level and ATP Producing Capacity: ATP levels in isolated mitochondria were measured using the ATPlite system (Perkin Elmer). The ATP-producing capacity was evaluated in the presence of specific energy substrates (glutamate/malate and succinate) and compared with non-energized conditions [28].

2.8.1. Inflammation

Tissue levels of TNF-α (KB3145, Krishgen BioSystems, India) and IL-6 (KB3068, Krishgen BioSystems, India) were measured in heart homogenate using ELISA kits according to the manufacturer's instructions.

2.8.2. Gene expression

qPCR (ABI7500, Thermo Scientific, USA) was performed to analyse the expression of nuclear-encoded β-actin, TNF-α, IL-6, PI3K, and Akt in rat heart samples. mRNA extraction was performed using TRIzol reagent, and cDNA was synthesized using a kit method as per the manufacturer's instructions. Gene expression was quantified using Sybr green chemistry, and the expression of genes was normalized with β-actin. The expression of genes was calculated as per the procedure of Livak and Schmittgen [29]. The primer sequence of the genes is presented in Supplementary Table 1.

3. Protein expression

The tissue samples from the left ventricle of the myocardium were homogenized in an ice-cold RIPA lysis buffer. Protein concentrations were determined using Lowry's technique, and proteins were then denatured for 15 min at 80 °C in SDS lysis buffer. Following separation in a 5 % stacking and 10 % resolved SDS-PAGE gel, the protein samples were transferred onto 0.45-m PVDF membranes. p-PI3K (Tyr 458/Tyr 199) (CST #4228), *p*-AKT (Ser 473) (CST #4060), Total Akt (CST #C67E7), Total PI3K (CST #4257), and β-actin (CST # 13E5) were used as primary antibodies to probe the membranes. The membranes were blocked with 5 % Bovine Serum Albumin in Tris-buffered Saline with 0.1 % Tween (TBST) for 1h. Following three TBST washes, the membranes were incubated with anti-rabbit secondary antibody (CST #7074) in TBST for 1 h at room temperature. After three washings, the blot membranes were

imaged using a Chemi-Doc XRS (BioRad, USA) employing a chemiluminescent detection system (ECL, BioRad, USA). Quantity-One (BioRad, USA) image analysis software was used to assess the relative band intensities.

3.1. Statistical analysis

The data were presented as mean ± standard deviation of the mean (SD). Intergroup comparisons were performed using one-way analysis of variance, followed by post hoc Dunnet's test to determine differences among the groups. A significance level of $p < 0.05$ was considered statistically significant.

4. Results

4.1. Cardiac changes associated with diet

The changes associated with standard and high-fat diets were assessed in the heart isolated from SD and HD-fed rats. Heart weight to body weight ratio was found to be elevated in HD rats compared to SD rats, indicating possible hypertrophy in the myocardium. This was further analyzed at the molecular level using the mRNA expression of Atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) genes, which were normalized with the housekeeping gene βactin. The results suggested a significant ($p < 0.05$) up-regulation of BNP and downregulation of ANP genes that collectively indicate the presence of cardiac hypertrophy in HD myocardium compared to the SD heart (Fig. 1A–C).

Diagnostic criteria for metabolic syndrome include high abdominal adiposity, high triglycerides, low high-density lipoprotein (HDL) cholesterol, and high fasting glucose [30]. Hence, the presence of metabolic syndrome evaluated in the HD animals by the abdominal fat content, triglycerides, cholesterol and glucose levels in the study animals (both SD and HD). According to the results shown in Supplementary Fig. 1, after 16-weeks treatment with high-fat diet, plasma triglycerides (HD-189.2 ± 22.7, SD-120.4 ± 19.3), total cholesterol (HD-234.3 ± 12, SD-147.5 ± 8.2), LDL-C (HD-95.8 ± 7.1, SD-47.4 ± 4.1) and HDL-C (HD-106.5 ± 4.2, SD-67.1 ± 6.4) concentrations of HD rats were significantly ($p < 0.05$) increased. The glucose level showed no difference between HD and SD rats (Supplementary Fig. 1). The abdominal fat content was significantly high in HD rats compared to SD which taken together confirm the metabolic alterations in HD but unable to ascertain the metabolic syndrome.

4.2. The effect of bilateral renal artery ligation on biochemical parameters and damage in the kidney

In order to study the distant effect of renal surgery, we adopted renal sham operation and IR surgery and evaluated the surgical effect on renal tissue and its physiology and the results are given in Fig. 2. Unlike sham-operated rat kidneys, IR surgery induces extensive tubular damage and cast formation, with a significantly higher histological tissue injury score and increased urinary kim-1 level indicating renal tissue damage. Further, we observed decreased Na–K ATPase activity in the renal tissue of IR rats. Injury markers like creatinine and BUN were elevated in the plasma with IR surgery in both SD and HD compared to its own control. Incidentally, the pattern of changes in these parameters was similar in both SD and HD fed rat, where the negative effect was predominant in HD-fed rats (renal injury score: SD-IR-11 ± 2, HD-IR- 18 ± 2; Na–K ATPase activity: SD-IR-5.4 ± 0.68, HD-IR- 2.71 ± 0.52; urinary Kim-1: SD-IR-4.2 ± 0.3, HD-IR- 6.7 ± 0.4) (Fig. 2A–D)

4.3. Cardiac hemodynamic alterations with renal injury

Table 1 shows the impact of renal surgery on cardiac physiological changes. Sham renal surgery did not impart any significant change in the hemodynamics of the heart, where the parameters like LVDP, RPP, and LVEDP were insignificantly changed between the heart obtained from SD and HD fed rats (LVDP: SD_RSHS- 89 ± 4, HD_RSHS-84 ± 3; RPP: SD_RSHS- 31 ± 4, HD_RSHS- 28 ± 2; LVEDP: SD_RSHS-8± 1, HD_RSHS-11 ± 3). But IR surgery in the kidney inflicts significant change in cardiac hemodynamics from the renal

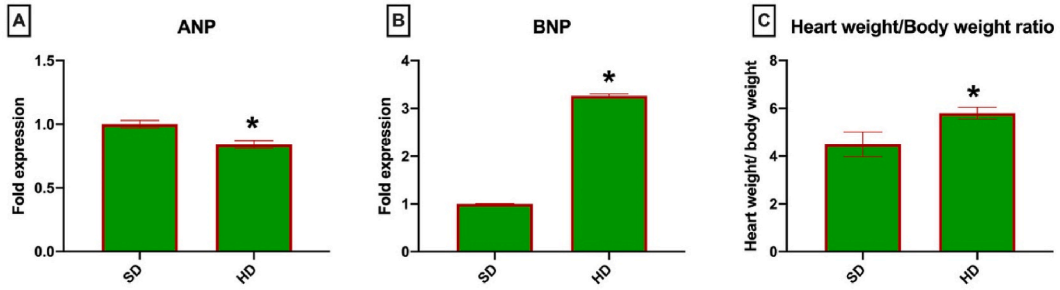

Fig. 1. Cardiac changes associated with SD and HD feeding in rats. Gene expression of A) ANP, B) BNP and C) Heart weight/body weight ratio *$p <$ 0.05 vs SD. The data are presented as mean ± SD (n = 6/group). One-way ANOVA, followed by post-Dunnet's test was used to analyse the data. SD-Standard diet, HD-High fat diet.

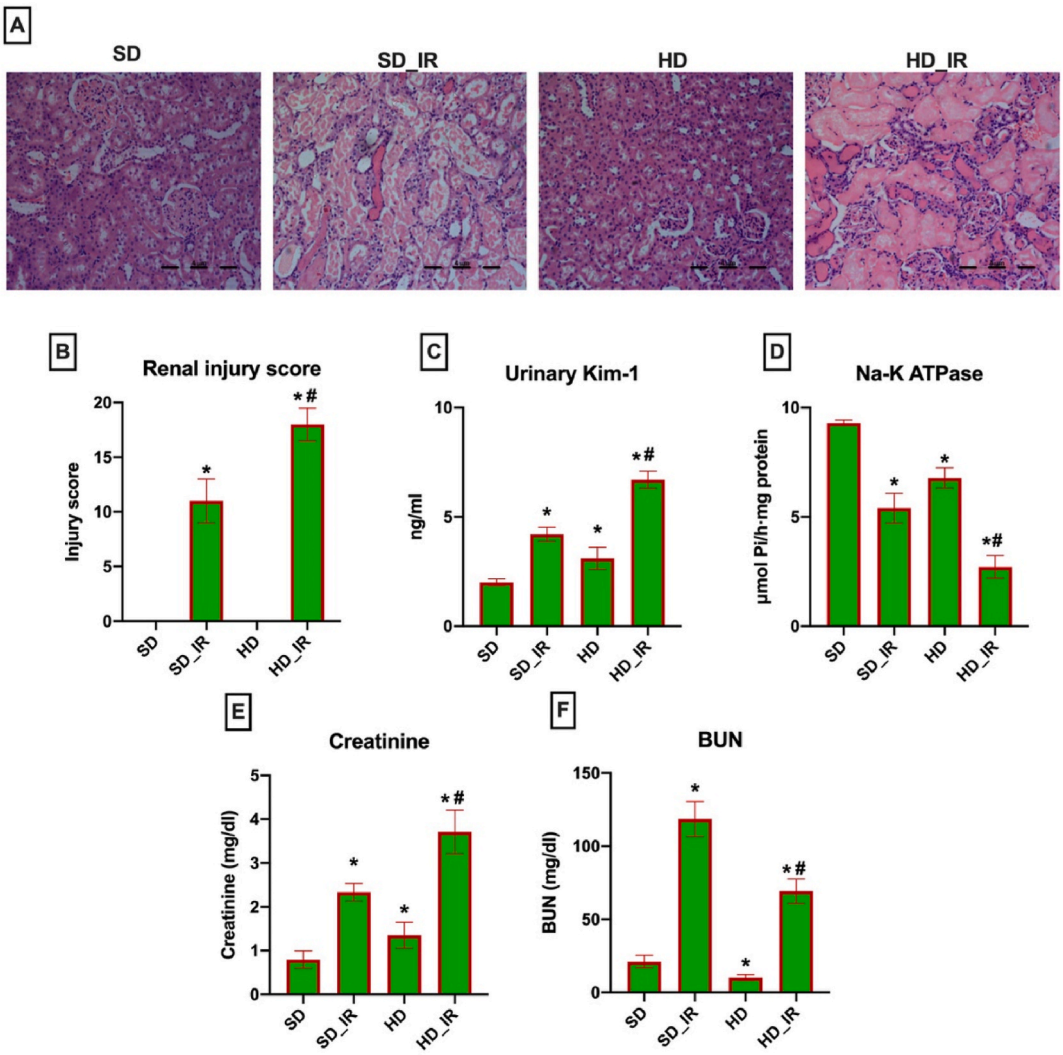

Fig. 2. Renal Injury in SD and HD rats subjected to IR. A) Representative H and E stained images (n = 3) (The images are presented at 10x magnification) B) Renal injury score C) Urinary levels of Kim-1, D) Na–K ATPase activity, E) Plasma creatinine and F) Blood urea nitrogen. *$p < 0.05$ vs SD, #$p < 0.05$ vs HD. The data are presented as mean ± SD (n = 6/group). One-way ANOVA, followed by post-Dunnet's test was used to analyse the data. SD- Standard diet, HD-High fat diet.

Table 1
Cardiac hemodynamic function and Injury.

	SD_RSHS	SD_RRHS	SD_RSHR	SD_RRHR	HD_RSHS	HD_RRHS	HD_RSHR	HD_RRHR
LVDP	89 ± 4	83 ± 5	36 ± 5$^{\#\$}$	64 ± 6$^{\#\$}$	84 ± 3	72 ± 2*$^{\#}$	77 ± 4*$^{\#}$	85 ± 4*$^{\$}$
LVEDP	8 ± 1	10 ± 2	48 ± 3$^{\#\$}$	17 ± 5$^{\#}$	11 ± 3	15 ± 3$^{\#}$	13 ± 2*$^{\#}$	9 ± 1*$^{\$}$
RPP	31 ± 4	28 ± 5	12 ± 1$^{\#\$}$	22 ± 3$^{\#}$	28 ± 2	22 ± 5$^{\#}$	24 ± 4*$^{\#}$	25 ± 2
Injury score	0 ± 0.00	1.33 ± 0.5	8.3 ± 0.5$^{\#\$}$	5 ± 0.5$^{\#}$	2 ± 0.2	3 ± 0.1	4 ± 0.2*	3 ± 0.1

*$p < 0.05$ HD group vs respective SD group, #$p < 0.05$ vs SD_RSHS, \$ $p < 0.05$ vs SD_RRHS and & $p < 0.05$ vs HD_RRHS. The data are presented as mean ± SD (n = 6/group). One-way ANOVA, followed by post-Dunnet's test was used to analyse the hemodynamic data. Histopathological injury score analyzed using Kruskal–Wallis test. SD- Standard diet, HD-High fat diet, HS- Heart Sham, HR- Heart ischemia reperfusion, RS- Renal sham, RR- Renal ischemia reperfusion.

sham surgery and also between SD (SD_RRHS: LVDP- 83 ± 5, LVEDP-10 ± 2, RPP-28 ± 5) and HD (HD_RRHS: LVDP- 72 ± 2, LVEDP-15 ± 3, RPP-22 ± 5) fed rat. Hemodynamic decline was more prominent in HD-fed rats (Table 1).

To check the responsive ability of the heart towards ischemia-reperfusion injury, an IR procedure was induced in an isolated rat heart. Heart from renal sham-operated rats showed a striking decline in hemodynamic parameters (LVDP, LVEDP, and RPP) from

control after cardiac IR induction. These observations were noted only in rats fed with SD (LVDP- 36 ± 5, LVEDP- 48 ± 3, RPP- 12 ± 1). On the contrary, HD-fed rats showed intact hemodynamic parameters even after cardiac IR was induced (HD_RSHR: LVDP- 77 ± 4, LVEDP-13 ± 2, RPP-24 ± 4).

Incidentally, the same pattern of changes was retained in HD-fed rats even when the hearts from renal IR-operated rats were subjected to cardiac IR stress (HD_RRHR: LVDP- 64 ± 6, LVEDP-17 ± 5, RPP-22 ± 3).

Interestingly, compared with renal sham-operated animals, hemodynamic parameters were significantly improved in IR hearts from SD-fed rats that had priorly undergone renal IR.

4.4. Cardiac alterations in histopathology after renal surgery

Fig. 3A–H gives the histopathological alterations and its injury score, where the heart from the renal sham-operated rat exhibits mild pathology only in those animals fed with HD (SD_RSHS-0±0.00, HD_RSHS-2±0.2). However, the histological alterations in hearts were elevated in both SD and HD-fed rats when analyzed in the hearts obtained from the animals that underwent renal IR surgery (SD_RRHS-1.33 ± 0.5, HD_RRHS 3 ± 0.1). Effectively, the changes noted were similar between SD and HD.

However, cardiac histological changes increased when the isolated hearts were subjected to IR stress to identify their responsive ability. According to the results in Fig. 3, hearts obtained from renal sham surgery showed a significant elevation in the pathological alterations in the SD-fed rats (SD_RSHR-8.3 ± 0.5, SD_RSHS- 0 ± 0.00). Still, this change was mild and insignificant from its sham heart in HD-fed rats (HD_RSHR-4±0.2, HD_RSHS-2±0.2).

Incidentally, the pathological alterations in IR heart decreased in both SD (SD_RRHR-5±0.5) and HD-fed rats when they underwent prior IR renal surgery (HD_RRHR- 3 ± 0.1).

4.5. Cardiac injury assessment after renal surgery

Cardiac injury was evaluated by measuring the TUNEL-positive cells and the activities of CK and LDH in both perfusate and cardiac tissue, and the results are given in Fig. 4. TUNEL-positive cells were relatively absent in renal sham-operated rat hearts in HD and SD-fed rats. A mild increase in the level of TUNEL-positive cells was noted in cardiac tissue after renal IR surgery (Fig. 4A). The changes were similar between HD and SD-fed rats.

When hearts were subjected to further IR challenge to understand their tolerance, the number of TUNEL-positive cells excessively increased in SD-fed rats than in HD-fed rats, where the animals had priorly undergone renal sham operation. However, TUNEL-positive cells were low in IR rat hearts from both SD and HD-fed animals when they previously underwent renal IR surgery (Fig. 4A).

Fig. 4 depicts the activities of CK and LDH in both coronary perfusate and tissue. According to the results, sham renal surgery in SD and HD rats exhibited a mild increase in LDH (SD_RSHS-1.53 ± 0.09, HD_RSHS-1.79 ± 0.08) and CK (SD_RSHS- 0.12 ± 0.01, HD_RSHS-0.21 ± 0.01) activities in the heart perfusate in HD rat heart from SD rat. The corresponding decline in the tissue was noted only in LDH activity (Fig. 4B–E).

Renal IR surgery induced significant elevation of LDH and CK in HD-fed rat perfusate (HD_RRHS: LDH- 2.4 ± 0.12, CK- 0.28 ± 0.04), where the corresponding decline in its tissue level (HD_RRHS: LDH- 2.13 ± 0.17, CK- 13.53 ± 0.89) was noted. This significant change was absent in SD-fed rats (Fig. 4B–E).

Subjecting IR procedure in isolated rat heart after renal sham surgery elicits a significant increase in LDH (SD_RSHR-3.4 ± 0.18, HD_RSHR-1.85 ± 0.05) and CK (SD_RSHR-0.57 ± 0.01, HD_RSHR-0.26 ± 0.03) in the perfusate in SD than HD fed rat compared with

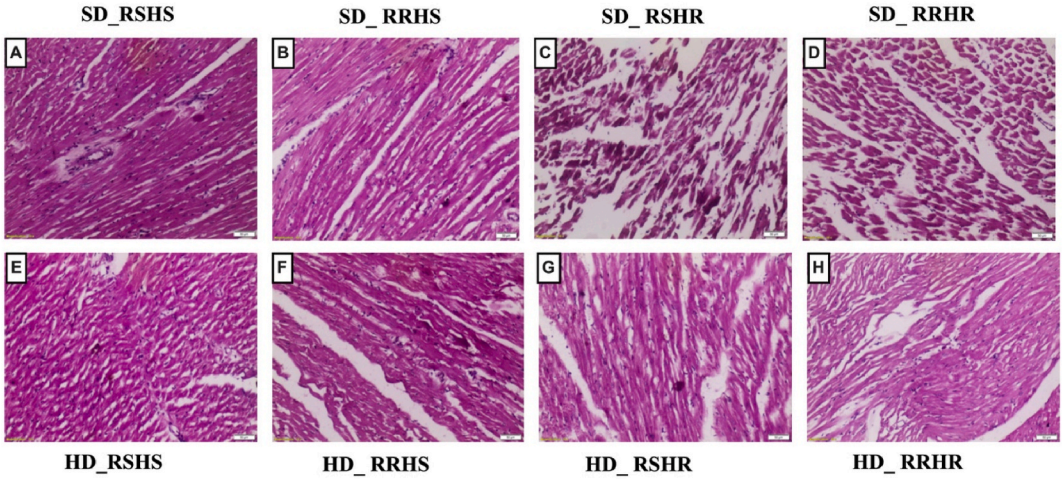

Fig. 3. A) Representative H and E images of different experimental groups (n = 3) (The images are presented at 10x magnification). SD-Standard diet, HD-High fat diet, HS- Heart Sham, HR- Heart ischemia reperfusion, RS- Renal sham, RR- Renal ischemia reperfusion.

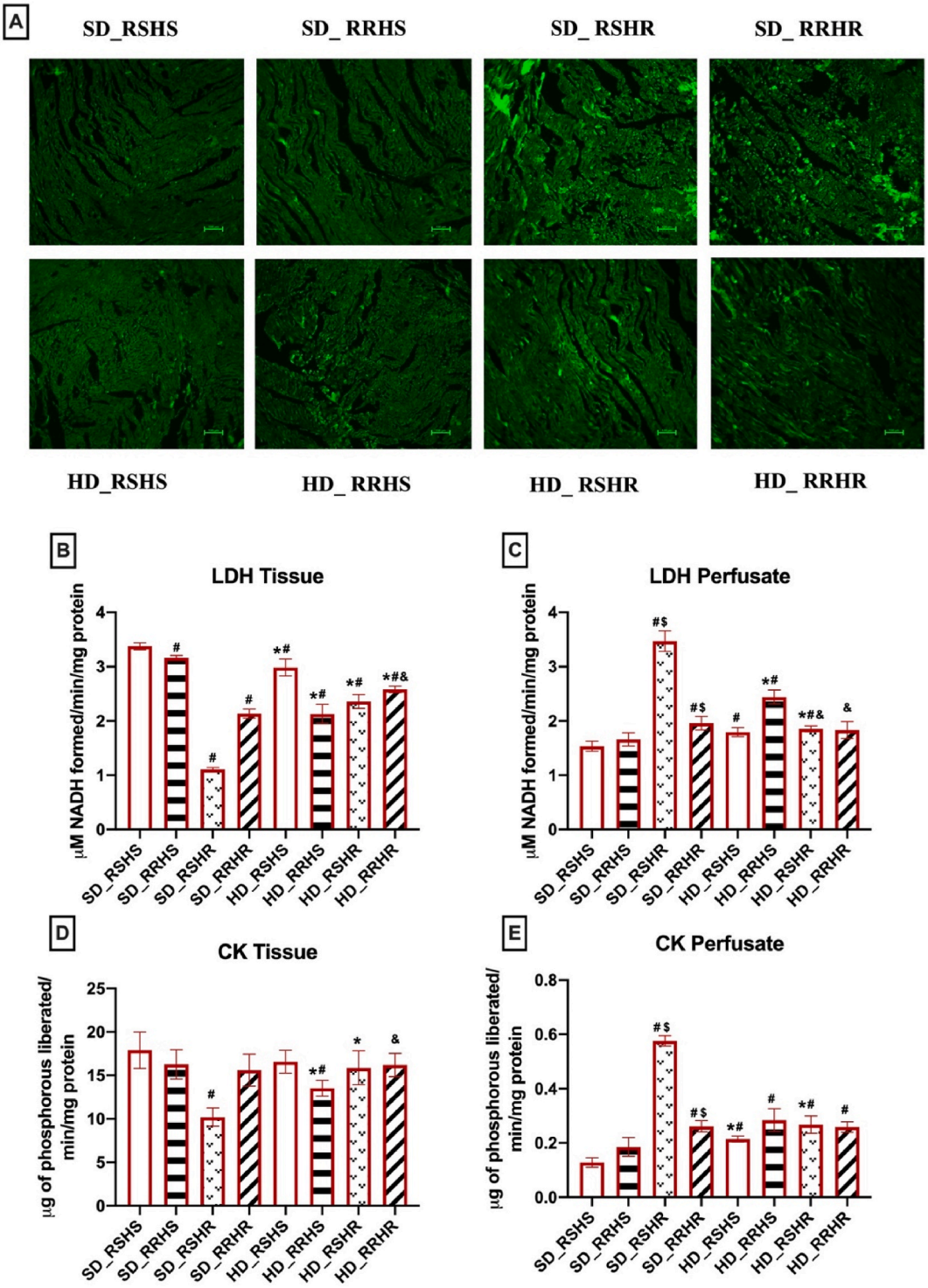

Fig. 4. A) Representative TUNNEL images of different experimental groups (n = 3) (The images are presented at 10x magnification). Biochemical analysis of B) LDH activity in the tissue C) LDH activity in the perfusate D) CK activity in the tissue and E) CK activity in the perfusate. *p < 0.05 HD group vs respective SD group, #p < 0.05 vs SD_RSHS, $ p < 0.05 vs SD_RRHS and & p < 0.05 vs HD_RRHS. The data are presented as mean ± SD (n = 6/group). One-way ANOVA, followed by post-Dunnet's test was used to analyse the data. SD-Standard diet, HD-High fat diet, HS- Heart Sham, HR- Heart ischemia reperfusion, RS- Renal sham, RR- Renal ischemia reperfusion.

its own controls. A corresponding decrease was found in the tissue level of these enzymes in these groups (Fig. 4B–E). Similarly, IR-challenged isolated rat hearts from the animals that had already undergone renal IR surgery showed relatively less cardiac injury in both SD and HD-fed animals from their respective controls.

4.6. Myocardial oxidative stress assessment after renal surgery

Myocardial oxidative stress was evaluated by measuring TBARS, GSH/GSSG, and activities of catalase and SOD. The results are given in Fig. 5. The TBARS and GSH/GSSG levels were non-significantly changed between SD and HD-fed rats in the myocardial tissue from the rat subjected to the renal sham-operated animal. This observation was identified in the SSM fraction of mitochondria. Still, in the IFM fraction, a significant change was noted between SD and HD rat hearts concerning TBARS and GSH/GSSG. When the animals were subjected to renal IR surgery, the decline in GSH/GSSG and increased TBARS were observed in the myocardial tissue from the control. However, no significant change was found between SD and HD-fed rats. In fact, these changes were prominent in the IFM fraction of mitochondria. Heart from the animal subjected to renal sham surgery when challenged to IR increased TBARS in both SSM and IFM fractions of SD and HD-fed animals. Similarly, GSH/GSSG levels were significantly decreased in both IFM and SSM of SD and HD-fed animals (Fig. 5A and B).

SOD and catalase activity were insignificantly changed between SD and HD-fed animals that underwent renal sham surgery. Renal IR surgery slightly decreased the activities of SOD and catalase in the heart. The pattern of changes was similar in both SD and HD-fed animals and between IFM and SSM. However, heart IR from the animal already underwent renal sham surgery significantly declined both SOD and catalase activities in cardiac tissues, which was strikingly present in SD-fed animal SSM and IFM fraction of HD-fed animals. However, preserved antioxidant activities were observed in the IR myocardium isolated from the SD and HD animals that had already undergone renal IR surgery (Fig. 5C and D).

DCFH-DA fluorescent probe was used to detect ROS, and according to Fig. 5E, HD-fed myocardial tissue exhibited significantly higher DCFH-DA levels than SD-fed sample rats that underwent renal sham operation (SD_RSHS-0.98 ± 0.02, HD_RSHS-1.3 ± 0.01). Subsequent renal IR surgery cardiac sample showed significant elevation in both SD and HD-fed rat hearts of almost similar magnitude (SD-RRHS-1.65 ± 0.12, HD_ RRHS – 1.58 ± 0.04). But IR challenge of isolated hearts maintained a higher level of DCFH-DA level only in SD-fed rat hearts (SD-RSHR-1.68 ± 0.14, HD_RSHR- 1.22 ± 0.12). Contrary to this observation, the IR heart from the animal already underwent renal IR showed higher DCFH-DA in the HD-fed heart than the SD-fed heart.

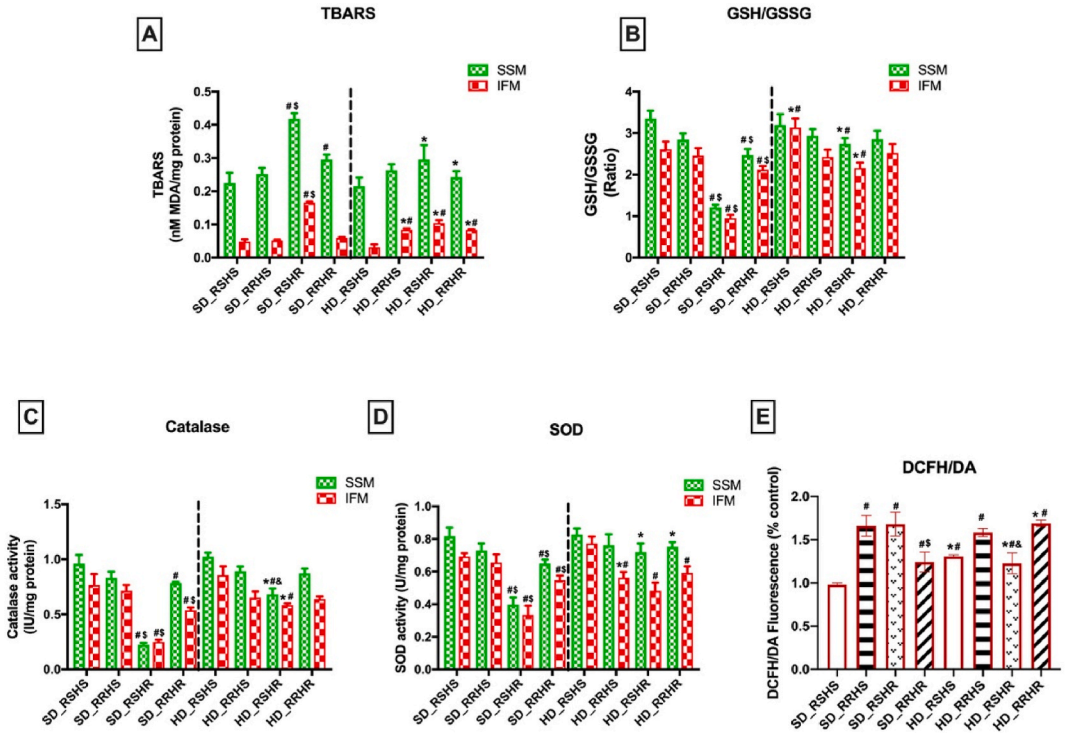

Fig. 5. Spectrophotometric analysis of oxidative stress in tissue homogenate and mitochondria. A) TBARS B) GSH: GSSG ratio, C) catalase activity, D) SOD activity in the respective groups and E) DCFH/DA analysis in the tissue. *p < 0.05 HD group vs respective SD group, #p < 0.05 vs SD_RSHS, $ p < 0.05 vs SD_RRHS and & p < 0.05 vs HD_RRHS. The data are presented as mean ± SD (n = 6/group). One-way ANOVA, followed by post-Dunnet's test was used to analyse the data. SD-Standard diet, HD-High fat diet, HS- Heart Sham, HR- Heart ischemia reperfusion, RS- Renal sham, RR- Renal ischemia reperfusion.

4.7. Evaluating the mitochondrial dysfunction in the heart after renal surgery

Renal sham surgery did not impart any significant change in the proximal ETC enzymes like NQR and SQR between SD and HD-fed animals or between IFM and SSM (Fig. 6A–D). However, the distal ETC enzymes like QCR and COX did show significant variation between SD and HD rat heart mitochondria. This was more predominantly seen in the IFM fraction of mitochondria. Moreover, the total ATP level in the IFM fraction of mitochondria was also found to be decreased in HD-fed cardiac mitochondria from SD rat heart mitochondria (ATP level in IFM: SD_RSHS- 332 ± 18, HD_RSHS- 268 ± 21) (Fig. 6E). Compared to its own sham, the ATP levels and ETC enzyme activities insignificantly differed in SD and HD rats that underwent renal IR surgery, except for the distal ETC enzymes, where HD rats showed a significant decline. Heart challenged to IR showed distinct effects in SD and HD-fed rats. In IR hearts from SD, ETC enzymes and ATP significantly declined when the rats priorly underwent renal sham surgery. However, in a similar experimental setup, HD-fed IR rat hearts exhibited comparatively better ETC activities. These observations were supported by improved ATP levels in HD_RSHR rat hearts (SSM- 199 ± 9.2, IFM-214 ± 9.6).

IR heart from the animal that already underwent renal IR showed an improved ATP level and corresponding ETC enzyme activities in HD, similar to that of its sham control (HD_RRHS), indicating protection (Fig. 6E). But for SD-fed rat hearts, even though a similar protocol improved ATP level and ETC enzyme activities, the protection was not near to its sham control (SD_RRHS).

4.8. Expression of TNF-α, IL-6, PI3K, and AKT in the myocardial tissue after renal surgery

Fig. 7 provides the gene expression and protein levels of TNF-α and IL-6 in the cardiac tissue. Expressions of the above proteins at the gene level were insignificantly changed between SD and HD rat hearts from animals that underwent renal sham surgery. However, significant upregulation of IL-6 and TNF-α was noted in the cardiac tissue of rats that underwent renal IR surgery (Fig. 7A and B). In fact, this upregulation was even higher in HD-fed rat hearts. IR challenge of the heart from animals subjected to renal sham surgery further increased the expression of TNF-α and IL-6 in both SD and HD-fed rats, except IL-6 in HD rats. In alignment with the gene expression, the protein levels of TNF-α and IL-6 measured via ELISA were also changed (Fig. 7C and D). Incidentally, IR induction in the heart after renal IR surgery lessens the expression of TNF-α and IL-6, and their protein levels from the RSHR group in SD-fed rat hearts.

To further investigate the modulation of PI3K and AKT signaling molecules (the major components of the cardioprotective RISK pathway) during renal surgery in rats with different diet regimen, we analyzed the mRNA gene expression of PI3K and Akt genes in the myocardium. The results showed that the gene expression of PI3K and Akt did not show much difference between the SD and HD groups (Fig. 7E and F). However, gene expression patterns of PI3K and AKT exhibited a significant decline in SD-fed IR rat hearts but

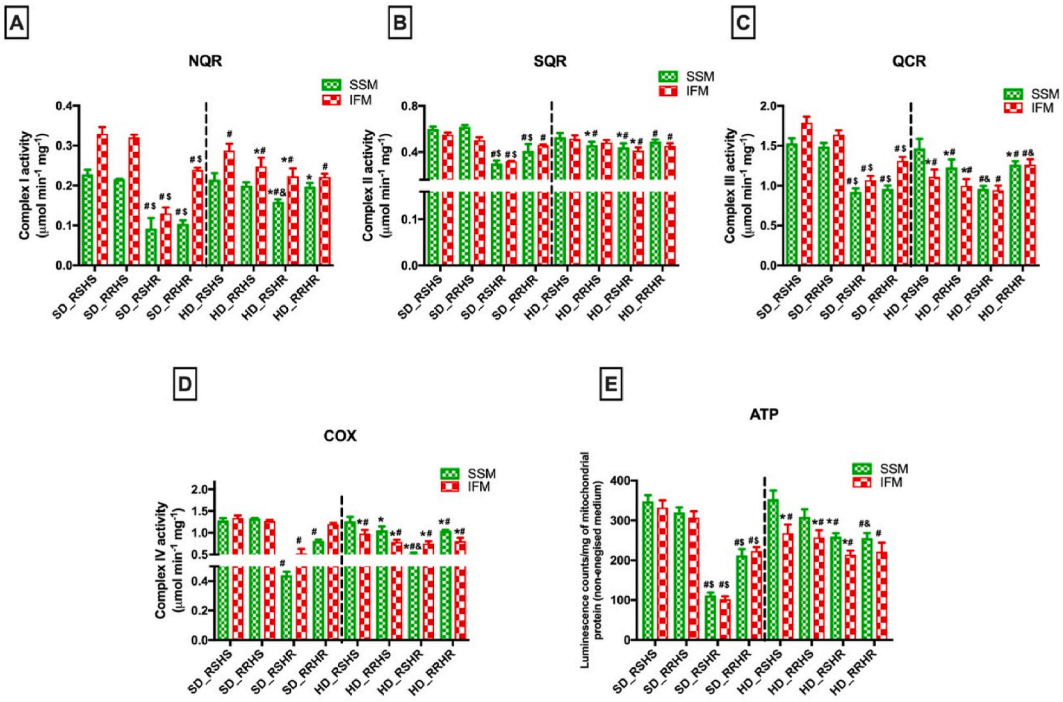

Fig. 6. Mitochondrial electron transport chain enzyme activities in cardiac mitochondrial subpopulations using spectrophotometry-based analysis A) NQR (Complex I), B) SQR (Complex II), C) QCR (Complex III), D) COX (Complex IV) activities and E) ATP level in the mitochondrial samples isolated from heart tissues. *p < 0.05 HD group vs respective SD group, #p < 0.05 vs SD_RSHS, $ p < 0.05 vs SD_RRHS and & p < 0.05 vs HD_RRHS. The data are presented as mean ± SD (n = 6/group). One-way ANOVA, followed by post-Dunnet's test was used to analyse the data. SD-Standard diet, HD-High fat diet, HS- Heart Sham, HR- Heart ischemia reperfusion, RS- Renal sham, RR- Renal ischemia reperfusion.

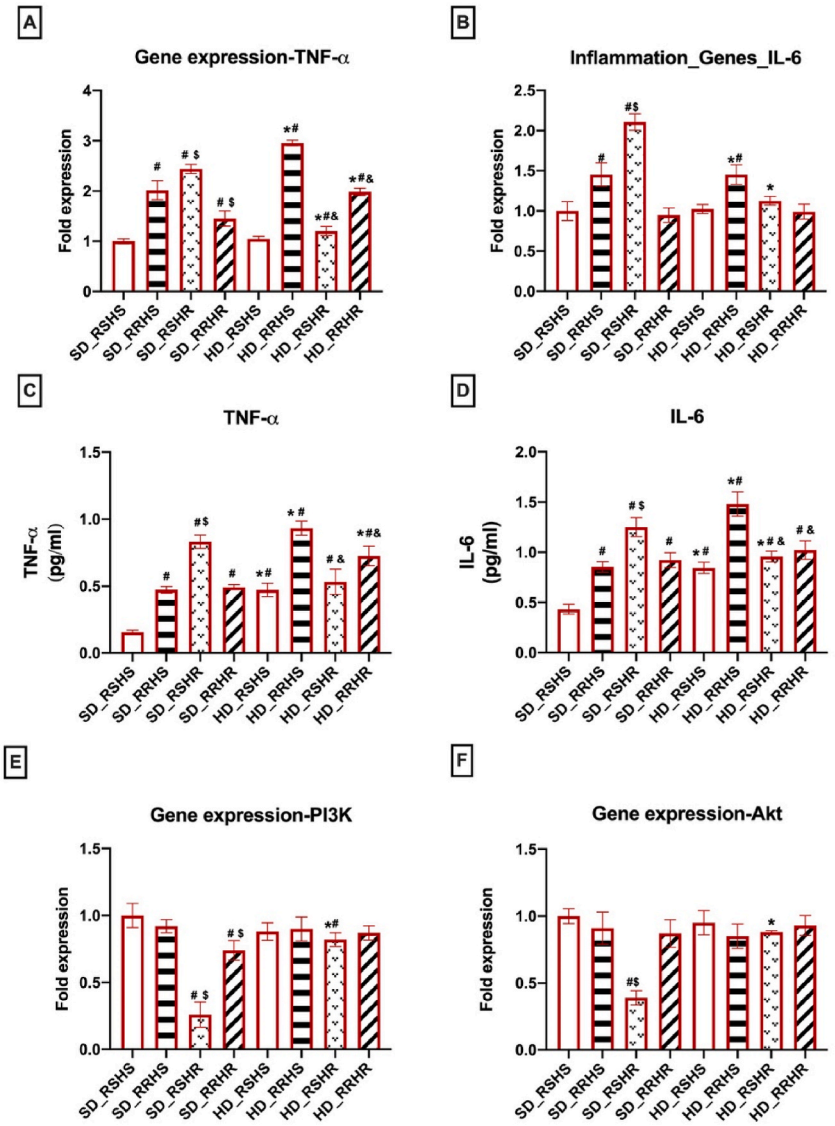

Fig. 7. Gene expression analysis of A) TNF-α and B) IL-6 in the cardiac tissue, protein levels of C) TNF- α and D) IL-6 in the cardiac tissue. PI3k/Akt signaling pathway determined by qRTPCR technique. A) mRNA expression changes of PI3k B) mRNA expression changes of Akt. *p < 0.05 HD group vs respective SD group, #p < 0.05 vs SD_RSHS, $ p < 0.05 vs SD_RRHS and & p < 0.05 vs HD_RRHS. The data are presented as mean ± SD (n = 6/group). One-way ANOVA, followed by post-Dunnet's test was used to analyse the data. SD-Standard diet, HD-High fat diet, HS- Heart Sham, HR-Heart ischemia reperfusion, RS- Renal sham, RR- Renal ischemia reperfusion.

not in HD-fed IR rat hearts after renal sham surgery. This was supported by the expression levels of the phosphorylated forms of these proteins (Fig. 8A–C). Renal IR surgery resisted this reduction in the gene and protein levels of PI3K and AKT in both SD and HD rat hearts when subjected to further IR insult.

5. Discussion

Many reports in the literature show the negative effect of cardiac surgery on kidney function that eventually leads to the development of AKI [31,32]. However, very few literature is available in support of the negative impact of renal surgery on cardiac function [33]. This effect of surgery on the distant organs also depends on co-existing morbidities and risk factors [34]. Excessive high-fat diet consumption is considered a risk factor for many non-communicable diseases, post-operative outcomes, and rehabilitation efficiency [35]. However, its role in imparting distant cardiac abnormality linked to renal IR surgery is unknown and is addressed in this study. The negative effect of renal surgery on the heart was evaluated with respect to the basal cardiac functional alterations and its ability to respond to additional IR stress in the heart. The major findings of the present study are as follows.

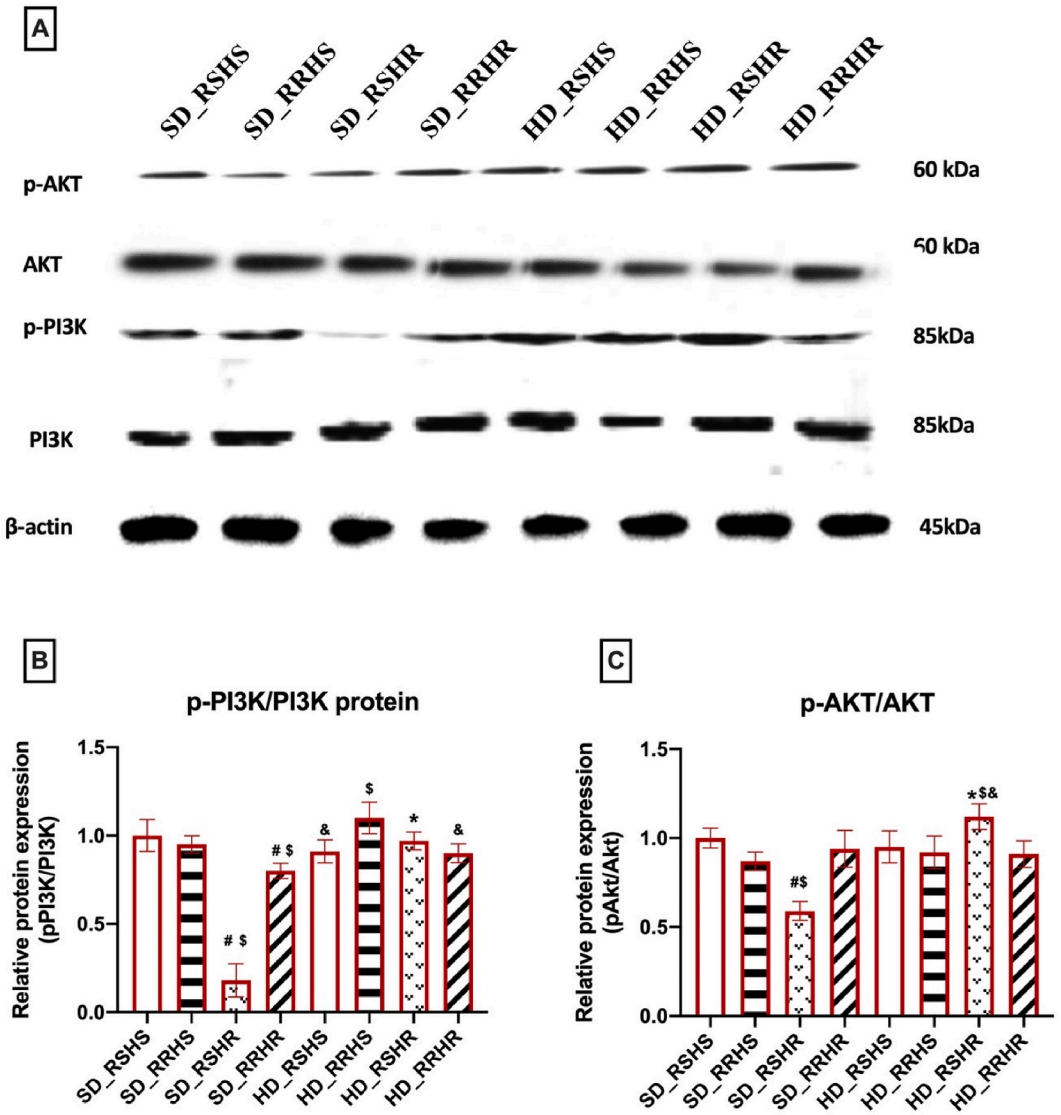

Fig. 8. Expression analysis of PI3K/AKT pathway A) Representative blot image of p-PI3k, Total PI3K, *p*-AKT (Ser 473), Total AKT and β actin proteins, B) Relative protein expression of p-PI3k/PI3K, C) Relative protein expression of *p*-AKT/AKT in the cardiac tissue *p < 0.05 HD group vs respective SD group, #p < 0.05 vs SD_RSHS, $ p < 0.05 vs SD_RRHS and & p < 0.05 vs HD_RRHS. The data are presented as mean ± SD (n = 3/group). One-way ANOVA, followed by post-Dunnet's test was used to analyse the data. SD-Standard diet, HD-High fat diet, HS- Heart Sham, HR-Heart ischemia reperfusion, RS- Renal sham, RR- Renal ischemia reperfusion. The uncropped blot images are included in the supplementary file.

i) The impact of renal sham surgery on the heart was similar in both rats fed with SD and HD diets, where the cardiac hemodynamics and the cardiac injury measured by histology were insignificantly changed in HD rat hearts from SD. Except for DCFH-DA and protein levels of TNF-α and IL-6, all other cellular measurements that include ETC enzyme activity, gene expression of PI3K, AKT, TNF-α, IL-6, and antioxidant enzymes activities were insignificantly changed between SD and HD rat heart.

ii) Renal IR surgery induced insignificant cardiac physiological deterioration with mild tissue injury in SD-fed rats. However, these changes were significant in HD-fed rat hearts, with a substantial increase in TUNEL-positive cells, oxidative stress, declined mitochondrial ETC enzyme activities, and elevated inflammation. The majority of these parameters significantly exhibited the adverse effect in HD-fed rat hearts from SD-fed rat hearts.

iii) Cardiac IR of rats already underwent renal sham surgery exhibited severe cardiac injury and declined hemodynamics in SD-fed rat hearts. But HD-fed rat hearts with similar experimental protocol resist the IR injury effectively where the cardiac physiological function and tissue injury were mildly affected. Cardiac oxidative stress, mitochondrial function, and inflammation measured support the above findings. Effectively, the pro-survival signaling pathways that provide the myocardium with the ability to resist IR, such as PI3K/AKT signaling, were insignificantly lower in the SD-fed rat heart compared to the HD-fed rat heart.

iv) When we induce IR to the heart from an animal that has already undergone renal IR surgery, combat the cardiac IR effectively in both SD and HD-fed rats. However, the overall resistance of the heart to withstand IR injury was higher in HD-fed rats. The cellular mediators like oxidative stress, mitochondrial function, inflammatory markers, and prosurvival signaling pathway well supported these observations.

A Plethora of evidence in the literature substantiates the development of AKI in cardiac patients undergoing open heart surgery, where the subjects commonly experienced ischemia-reperfusion-associated abnormalities. This enhanced the risk of mortality by 5-fold for these patients [4,36–38]. Similarly, many studies have documented the cardiovascular risk in patients undergoing dialysis and those who developed AKI [39]. Additionally, studies have reported cardiac dysfunction, injury, damage, and fibrosis associated with AKI [40]. Renal transplantation recipients have a 40 % risk of developing cardiovascular-related events within 36 months after transplantation, where the latter procedure is known to be associated with renal ischemia-reperfusion injury [41]. Even though the mortality rate of kidney transplant recipients is considerably reduced, the proportion of cardiovascular-related deaths remains the major known reason for death in transplant patients [42,43]. Identifying, treating, and preventing the risk factors may decrease mortality, as per the observations made by many clinicians [44].

The significant risk factors associated with CVD-linked post-kidney transplant are obesity, hyperlipidemia, diabetes mellitus, hypertension, smoking, and gender [45]. However, the underlying mechanism of these risk factors that influence the development of CVD in renal patients who underwent surgery with ischemia-reperfusion impact is not well explored. In the present study, we focused on the influence of a high-fat diet on renal surgery linked to cardiac dysfunction in the rat experiment model. Accordingly, we found that renal sham surgery of 45 min did not cause any significant deterioration in the cardiac physiological function (measured via LVDP, LVEDP, and RPP) or inflict any significant tissue injury (measured via histopathology, TUNEL, and cardiac injury markers). In general, cardio renal mediators that initiate the cross-talk between the heart and kidney can cause organ dysfunction [46]. One of these mediators is metabolites' altered products that may intensify with stress factors like surgery [5]. Hence, in the HD-fed rats, even though insignificant cardiac function and injury were noticed from SD-fed rats, the pattern of changes in HD-fed rats was towards pathology in renal sham-operated rats.

However, when we subjected the kidney to IR injury (unavoidable abnormalities normally associated with any significant organ surgery), the sign of cardiac dysfunction (deteriorated hemodynamic indices) and development of tissue injury was substantial. IR injury is known to generate excessive ROS production, release inflammatory responses, and induce mitochondrial dysfunction [47]. In a combined heart and kidney failure, the critical mechanism involved in the balance between oxidative stress and antioxidant defense, mitochondrial integrity, and pro and anti-inflammation [22,48,49]. In the present study, we noted a significant alteration in these parameters, distinct between the SD and HD-fed rats. Unlike the SD-fed heart, the HD-fed rat heart exhibited slightly higher injury and dysfunction. This was mainly due to the significant elevation in the inflammation in the cardiac tissue and the associated mild decrease in mitochondrial function. Many studies demonstrated the direct relationship between inflammation and mitochondrial dysfunction in developing cardiovascular abnormalities [50–52]. There exist many reports that link inflammation between a high-fat diet and diseases [53,54]. Regardless of the cause, inflammation is a cellular adaptation event to restore homeostasis [55]. Hence, in response to this trigger, the mitochondrial energy supply declined, especially in the IFM fraction, which eventually influenced the decline in cardiac physiology.

Further, when we inflict an IR injury in an isolated heart from an HD-fed rat that underwent sham or IR operation, the physiological function of the heart was intact with relatively mild tissue injury. Further, we found that ETC enzyme activities of mitochondria, including the IFM fraction, were intact, substantiating the effective adaptive response of mitochondria in response to renal surgery. This was further evident when we compared the SD-fed rat heart from the animal subjected to renal IR with the HD-fed animal with a similar experiment protocol. However, the present study did not throw light on when the maladaptive response of mitochondria may develop in the presence of risk factors like a high-fat diet. The use of male rodents in this research is also a significant limitation, as it limits the applicability of the findings to female rats. The study's emphasis on young male Wistar rats is an additional limitation. Age plays a crucial role in physiological responses and results in Ref. [56]. Young animal results may not necessarily apply to older animals [57]. Further research may be necessary in these directions.

Declarations

5.1. Ethics statement

The animal experiments were conducted according to the guidelines of the Committee for the purpose of conduct and supervision of experiments on animals, India, with prior approval from the Institutional Animal Ethical Committee (565/SASTRA/IAEC/RPP).

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Data availability statement

The data associated with the study has not been submitted to any data repository. The datasets generated and analyzed during the

current study will be made available by the corresponding author upon reasonable request.

Supplementary Table 1
Primer details

PRIMER	SEQUENCE (5′ to 3′)
TNF- α –F	CGCTCTTCTGTCTACTGAAC
TNF- α –R	TTCTCCAGCTGGAAGACTCC
IL-6 –F	CACTTCACAAGTCGGAGGCT
IL-6 –R	AGCACACTAGGTTTGCCGAG
PI3K–F	GCATCAGTGGCTCAAGGACAAG
PI3K–R	CAAGATAAAGGTTGCCACGCAGT
Akt-F	AGGAGGAGGAGGAGATGGA
Akt-R	GGTCGTGGGTCTGGAAAG
β-actin-F	GTGTGGTCAGCCCTGTAGTT
β-actin-R	CCTAGAAGCATTTGCGGTGC

CRediT authorship contribution statement

Priyanka N. Prem: Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Gino A. Kurian:** Writing – original draft, Supervision, Resources, Project administration, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e22273.

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