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Research Article

Inhibition of Rhoa/Rho Kinase by Ibuprofen Exerts Cardioprotective Effect against Ischemic Reperfusion Injury in Rats

Gandhi T, Patel P*, Mehta A and Shah H
Pharmacology Department, Anand Pharmacy College, Gujarat, India

***Corresponding author:** Prexita Patel, Pharmacology Department, Anand Pharmacy College, Shri Ramkrishna Seva Mandal Opp. Town-Hall, Anand 388001, Gujarat, India

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Abstract

Ischemic Reperfusion Injury is the main cause of mortality globally, researchers are concentrating their efforts on heart protection. Ibuprofen inhibits rho-kinase, a downstream effector of a protein implicated in Ischemic Reperfusion Injury. The goal of this study is to deter Ischemic Reperfusion Injuryne whether ibuprofen has a cardioprotective effect in rats. In an experimental animal, Ischemic Reperfusion Injury was produced by undergoing coronary artery ligation operations. The rats were rando Ischemic Reperfusion Injuryzed into six groups: (I) Normal; (II) model; (III) Sham; (IV-VI) ISO þ ibuprofen (30, 60 and 90 mg/kg p.o). At the end of surgery Langendorff, cardiac marker enzymes, electrolytes, anti-oxidants and gene expression of ROCK-1 were investigated. Pre-treatment with ibuprofen (30, 60, 90 mg/kg, p.o.) for 21 days normalise Blood pressure and improved the ECG pattern, left ventricular function by avoiding ROCK-mediated damage, and prevented the rise in CK-MB, LDH, and Troponin-I and electrolyte level by maintaining cellular integrity. Further, ibuprofen downregulate the mRAN expression of ROCK-1 and preserved the cellular architecture of myocardial tissue. Ibuprofen provided cardioprotection in a model of myocardial infarction, by restoring most of the altered physical, physiological, bioche Ischemic Reperfusion Injurycal, haemodyna Ischemic Reperfusion Injuryc parameters, antioxidant status, and histological changes and by inhibiting ROCK-1 mRNA expression.

Keywords: Rho kinase; Ibuprofen; Coronary artery ligation; ROCK-1

Introduction

Cardiovascular Disease (CVD) is the leading cause of mortality globally, according to heart disease and stroke statistics from the American Heart Association, the National Institute of Health, and other government sources through 2021. Between 2015 and 2018, 126.9 Ischemic Reperfusion Injuryllion individuals in the United States had some type of cardiovascular disease. Although there are numerous cardiovascular consequences associated with CVD, myocardial infarction is the primary cause of all kinds of cardiovascular disease and the leading cause of morbidity and death globally [1]. In the United States, over 1.5 Ischemic Reperfusion Injuryllion instances of myocardial infarction (Ischemic Reperfusion Injury) occur each year, with a yearly incidence rate of about 600 cases per 100,000 persons [2]. Ische Ischemic Reperfusion Injurya causes the death of cardiac myocytes, resulting in myocardial infarction (Ischemic Reperfusion Injury) [3]. The Rho-kinase (Rho-associated coiled-coil protein kinase, ROCK) is a protein's downstream effector. RhoA is a key player in the development of myocardial infarction. With the aid of Rho GEF (Guanine Nucleotide Exchange Factor) and GTPase activating protein, Rho functions as a molecular switch, cycling between active GTP-bound and inactive GDP-bound states. Pre-treatment with particular Rho-kinase inhibitors such fasudil and Y-27632 improves hypertension, atherosclerosis, angina, heart failure, ische Ischemic Reperfusion Injurya reperfusion damage, stroke, coronary vasospasm, and thrombosis, according to previous research [4]. Rho-kinase has a detrimental function in the Ca2 independent route of eNOS production. Rho-kinase also increases the mRNA expression of pro-inflammatory mediators such as IL-6, IF-, thrombogenic molecules, and others. Various studies have shown that Rho kinase inhibitors prevent myocardial infarction by increasing arterial dilatation, haemodilution, preventing neutrophil accumulation, reducing infarct size, and improving neurological functioning in stroke patients. In ische Ischemic Reperfusion Injuryc myocardium, Rho-kinase inhibitors also reduce ROCK-mediated inflammatory responses [5]. This indicates that ROCK is important in myocardial infarction. In cultured neurons exposed to axon development inhibitors, ibuprofen, a non-steroidal anti-inflammatory medication, inhibits RhoA [6,7]. Because myocardial ROCK1 levels increased by 50% and ROCK2 levels increased by 40% in myocardial infarction, we may conclude that ROCK inhibitors have a protective effect against myocardial infarction [8]. In view of the foregoing, this study was conducted to deter Ischemic Reperfusion Injuryne the cardioprotective efficacy of ibuprofen against ische Ischemic Reperfusion Injuryc-reperfusion damage in rats induced by coronary artery ligation.

Materials and Methods

Drugs and preparation of solutions [9]

Ibuprofen was obtained as a free sample from pharmaceutical providers. Every day, ibuprofen was made fresh by suspending it in a

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0.5 percent Carboxymethyl Cellulose (CMC) solution.

Che ischemic reperfusion injurycals and kits

All the che Ischemic Reperfusion Injurycals used in this project were of analytical grade and were obtained from Astron che Ischemic Reperfusion Injurycals, Ahmedabad, and SD fine che Ischemic Reperfusion Injurycals, Mumbai, India. All the bioche Ischemic Reperfusion Injurycal tests were performed using the standard reagent kits purchased from i-chem. For gene expression study: RNAlater (Qiagen), TRI (Sigma), DNase I (Qiagen), First strand cDNA synthesis (Thermo scientific) and SYBR Green PCR kit (KAPA) were used. The primers were first designed by using NCBI BLAST primer tool and then commercially synthesized (Eurofins Geno Ischemic Reperfusion Injurycs).

Animals

The study utilised a healthy wistar (Male) weighing 250 10 g. The rats were housed in a set of 5 cages (1 group= 2 cages) under carefully regulated temperature (22 2°C), hu Ischemic Reperfusion Injurydity (55 5%), and light-dark cycle (12h/12h). The animals were given unrestricted access to a standard laboratory food (bought from Pranav Argo Pvt. Ltd) and unli Ischemic Reperfusion Injuryted water.

The protocol (APC/2017-IAEC/1716) of the experiment was approved by Institutional Animal Ethical Com Ischemic Reperfusion Injuryttee as per the guidance of the Com Ischemic Reperfusion Injuryttee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ischemic Reperfusion Injurynistry of Social Justice and Empowerment, Government of India.

Coronary artery ligation induced Myocardial Infarction [10]

The animals were divided into six groups, each having 10 individuals. Group I was a normal control, Group II was a model control, Group III was a Sham operated group, and Group IV-VI were Ibuprofen (30, 60 and 90 mg/kg) treatment controls, respectively.

Myocardial Infarction (Ischemic Reperfusion Injury) was induced by anaesthetizing rats by ad Ischemic Reperfusion Injurynistration of Ischemic Reperfusion Injuryxture of xylazine (25 mg/kg) and keta Ischemic Reperfusion Injuryne (70 mg/kg) intraperitoneal. By placing the animal on a heating pad, the rectal temperature was maintained at 37-38°C. The chest was opened at the fifth intercostal gap with an incision parallel to the ribs, and the ribs were spread to expose the heart. Sharp forceps were utilised to access the pericardium, and 6/0 polypropylene was used to ligate the coronary artery 2.0-3.0 mm below the anterior-inferior border of the heart's left atrium. Ische Ischemic Reperfusion Injurya was conducted for 30 Ischemic Reperfusion Injurynutes, followed by 45 Ischemic Reperfusion Injurynutes of reperfusion. Exa Ischemic Reperfusion Injurynation of the level of cardiac marker enzymes indicated the induction of Ischemic Reperfusion Injury (CK-MB, LDH) as shown in (Figure 1).

Collection of biological sample: blood and serum

Blood was obtained using a retro-orbital method under anaesthetic circumstances and kept at room temperature for 30 Ischemic Reperfusion Injurynutes. Serum was collected by centrifugation at 780g for 15 Ischemic Reperfusion Injurynutes, and it was then used to calculate cardiac marker enzymes (CK-MB, LDH, and Troponin-I), as well as electrolyte concentrations (NA+, K+, and Ca+2). At the same time, the heart was separated and washed with ice-cold physiologic saline before being utilised to estimate left ventricular function. For homogenate, histology, and RTq-PCR, a weighed heart was utilised. Antioxidant levels in cardiac homogenate were calculated.

Langendorff isolated perfused heart preparation [9]

An intraperitoneal (i.p.) injection of 500IU heparin and an intraperitoneal (i.p.) injection of 50-80 mg/kg pentobarbital were ad Ischemic Reperfusion Injurynistered to rats. After the rat went comatose and lost pedal reflex activity, the heart operation was commenced. To open its chest, a Ischemic Reperfusion Injuryd sternal thoracotomy was done, and the heart was quickly excised and immersed in oxygenated ice-cold modified Krebs-Henseleit buffer. The aorta was then cannulated and oxygenated modified Krebs-henseleit buffer was injected into the cannula. Finally, the cannula was swapped to connect with the Langendorff device. The apparatus had previously been adjusted at a constant flow rate of carbogen-saturated modified Krebs-Henseleit buffer (95 percent O_2 and 5 percent CO_2) and a temperature of 37°C. The latex balloon was carefully placed in the left ventricle of an isolated heart to evaluate the pharmacodyna Ischemic Reperfusion Injuryc reaction. The latex balloon was attached to the end of a polyethylene tube to estimate the hemodyna Ischemic Reperfusion Injuryc response, which was coupled with a pressure transducer. The balloon was swelled with 50% methanol to achieve a diastolic pressure of 5 to 6 mmHg. Left Ventricular End Diastolic Pressure (LVEDP), Heart Rate (HR), coronary flow rate, +dp/dtmax, and -dp/dtmax were the four hemodyna Ischemic Reperfusion Injuryc endpoints assessed.

Statistical analysis

The results were reported as mean Standard Error Of The Mean (SEM). One-Way Analysis Of Variance (ANOVA) was used in the statistical analysis, followed by Dunnett's post hoc test. P values of less than 0.05 were deemed significant.

Results

Effect of ibuprofen on electrocardiogram pattern in coronary artery ligation induced myocardial infarction

An electrocardiogram is a test that measures the heart's normal electrical activity. When compared to normal and sham operated animals, the coronary artery ligated animal (model control animal) displayed aberrant ECG and especially ST-Elevation. The ECG pattern improved after pre-treatment with ibuprofen (30, 60, 90 mg/kg p.o.) for 21 days as shown in (Figure 2).

Effect of ibuprofen on st-elevation in coronary artery ligation induced myocardial infarction

CAL treated rats showed significant ($p \leq 0.05$) rise in ST-Elevation as compared to normal control group and Sham opereated group. Pre-Treatment with Ibuprofen (30 and 60 mg/kg p.o.) shows significant decline in ST-elevation as compared to model control rats. However, 90 mg/kg p.o. presented significant rise in ST-level as shown in (Figure 3).

Effect of ibuprofen on lv function in coronary artery ligation induced myocardial infarction

When compared to the normal control group and the Sham

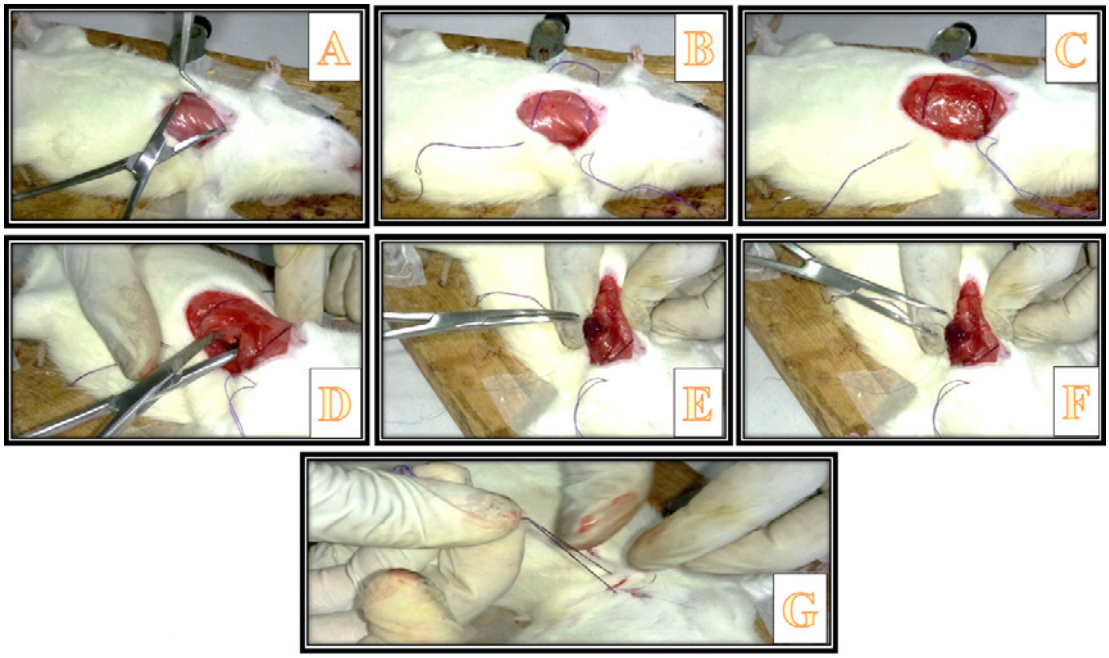

Figure 1: Coronary artery ligation induced Myocardial Infarction.

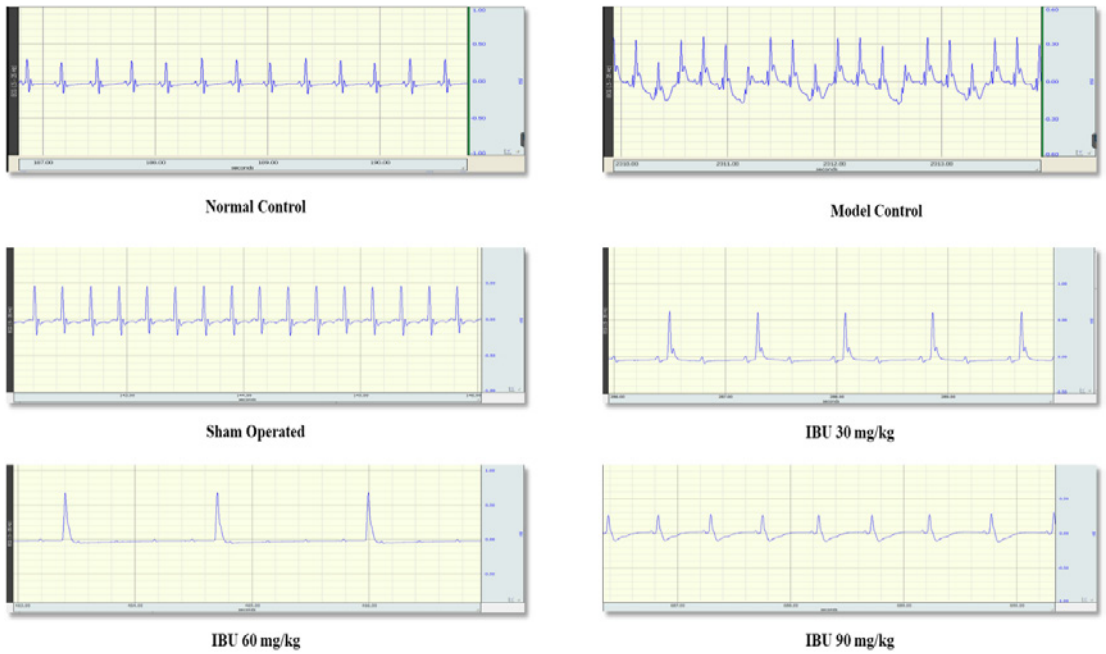

Figure 2: Effect of Ibuprofen on ECG Pattern in coronary artery ligation induced myocardial infarction.

operated group, CAL treated animals had a substantial (p0.05) increase in Heart Rate (HR), Blood Pressure (BP), and left Ventricular End-Diastolic Pressure (LVEDP), suggesting LV hemodyna Ischemic Reperfusion Injuryc load. In contrast to the usual control, it resulted in a substantial reduction in coronary flow rate and dp/dtmax. Ibuprofen pretreatment enhanced cardiac function, blood pressure, heart rate, coronary flow rate, dp/dtmax, and LVEDP as demonstrated in (Table 1).

Effect of ibuprofen on serum ck-mb and ldh level in coronary artery ligation induced myocardial infarction

Model control animals had substantially greater levels of CK-MB and LDH than normal control and Sham opereated animals (p 0.05). When compared to model control animals, pre-treatment with Ibuprofen (30, 60 and 90 mg/kg p.o.) substantially reduced the rise in CK-MB and LDH levels as shown in (Figure 4).

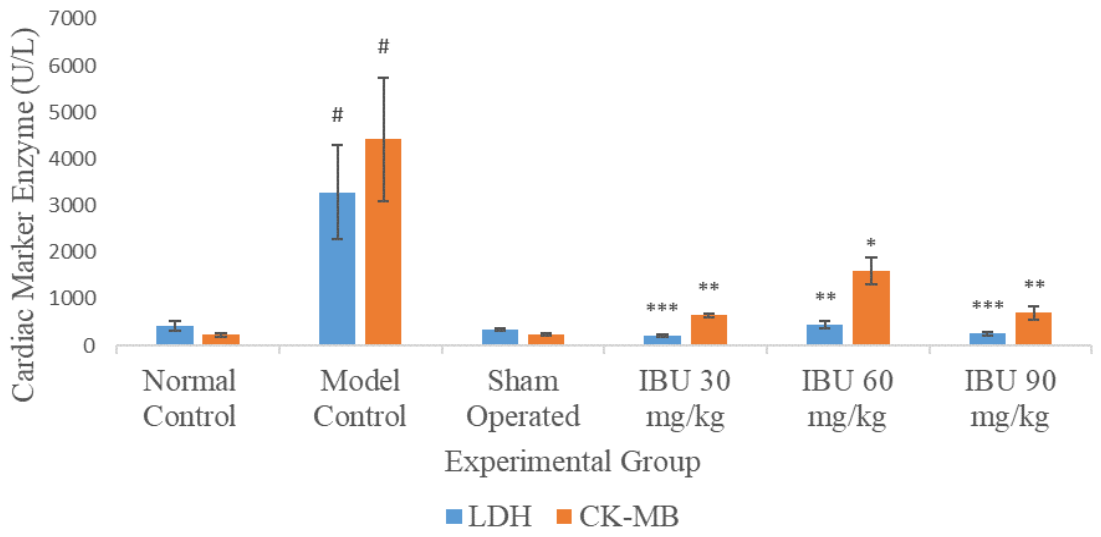

Figure 3: Effect of Ibuprofen on CK-MB and LDH activity in coronary artery ligation induced myocardial infarction.
The values expressed are as mean ± SEM (n=10). The statistical analysis was carried out by One-Way Analysis of Variance (ANOVA) followed by Dunnett's post-hoc test by using graph pad prism 9.2.0 whereas, #- significance difference from normal p≤0.05 and * - a significant difference from model p≤0.05. P values <0.0001 was set as a level of statistical significance respectively. # indicates significantly different from the normal control group and*, ** and *** Indicates significantly different from the model control group.

Table 1: Effect of Ibuprofen on left ventricular in coronary artery ligation induced myocardial infarction.

Groups	HR (bpm)	Coronary flow rate (ml/ Ischemic Reperfusion Injuryn)	Mean blood pressure	LVEDP	+dp/dt $_{max}$ (mmHg/s)	-dp/dt $_{max}$ (mmHg/s)
Normal control	335.97 ± 14.35	18.33 ± 0.98	98.87 ± 6.22	0.844 ± 0.51	801.6 ± 28.53	-721.4 ± 37.27
Model control	445.18 ± 1.87	11.50 ± 0.57 (#)	129.90 ± 2.11	2.481 ± 0.23	335.0 ± 8.03 (####)	-324.1 ± 8.23 (##)
Sham operated	337.20 ± 2.19	18.23 ± 0.30	116.30 ± 0.00	0.541 ± 0.27	565.1 ± 43.74	-687.3 ± 69.99
IBU (30 mg/kg)	406.52 ± 93.09	14.78 ± 2.25	89.86 ± 5.02	1.250 ± 0.06	549.9 ± 40.00 (***)	-12090 ± 105.70 (****)
IBU (60 mg/kg)	366.79 ± 69.85	17.07 ± 0.31 (*)	87.20 ± 4.97	0.857 ± 0.22	687.3 ± 39.24	861.5 ± 131.50
IBU (90 mg/kg)	328.18 ± 16.23	17.67 ± 0.31 (*)	91.62 ± 5.46	0.373 ± 0.09	343.7 ± 43.87	-303.4 ± 60.36

The values expressed are as mean ± SEM (n=10). The statistical analysis was carried out by One-Way Analysis of Variance (ANOVA) followed by Dunnett's post-hoc test by using graph pad prism 7.0. Whereas, # - significance difference from normal p≤0.05 and * - a significant difference from model p≤0.05. P values <0.0001 was set as a level of statistical significance respectively. #, ## and #### indicates significantly different from the normal control group and *, ***, and **** Indicates significantly different from the model control group.

Effect of ibuprofen infarction on cTnI level in coronary artery ligation induced myocardial infarction

When compared to normal control and sham operated animals, model control animals had a substantial (p 0.05) rise in cTnI levels. Pre-treatment with ibuprofen (30, 60 and 90 mg/kg p.o.) reduced the rise in cTnI levels substantially as shown in (Figure 5).

Effect of ibuprofen on serum electrolyte concentration level in coronary artery ligation induced myocardial infarction

CAL treated animal showed a significant($p \leq 0.05$) increase in sodium, calcium and decrease in potassium levels as compared to normal control group and Sham opereated group. Ibuprofen (30, 60 and 90mg/kg p.o. respectively) pre-treatment significantly prevented this damage as represented by data in (Table 2).

Effect of ibuprofen on antioxidant levels in coronary artery ligation induced myocardial infarction

In the CAL treated group, a significant (p 0.05) increase in MDA activity was detected, as well as a reduction in GSH, SOD, and catalase, as compared to the normal control group and the Sham operated group, which improved after treatment with Ibuprofen (30,60 and 90 mg/kg p.o.) as mentioned in (Table 3).

Effect of ibuprofen on rock-i gene expression in coronary artery ligation induced myocardial infarction

The effect of ibuprofen on cardiac ROCK-I mRNA expression was investigated in this study. When compared to the normal control group and the Sham operated group, the CAL treated group produced a substantial (p 0.05) upregulation of ROCK-I mRNA expression. Ibuprofen (30, 60 and 90 mg/kg) therapy, on the other hand, substantially reduced the CAL-induced up-regulation of ROCK-I mRNA expression as shown in (Figure 6).

Discussion

In comparison to other heart diseases, myocardial infarction has a high prevalence (Khan et al., 2020). ROCK activation is a new mechanism that plays a critical role in cardiovascular disease. It is

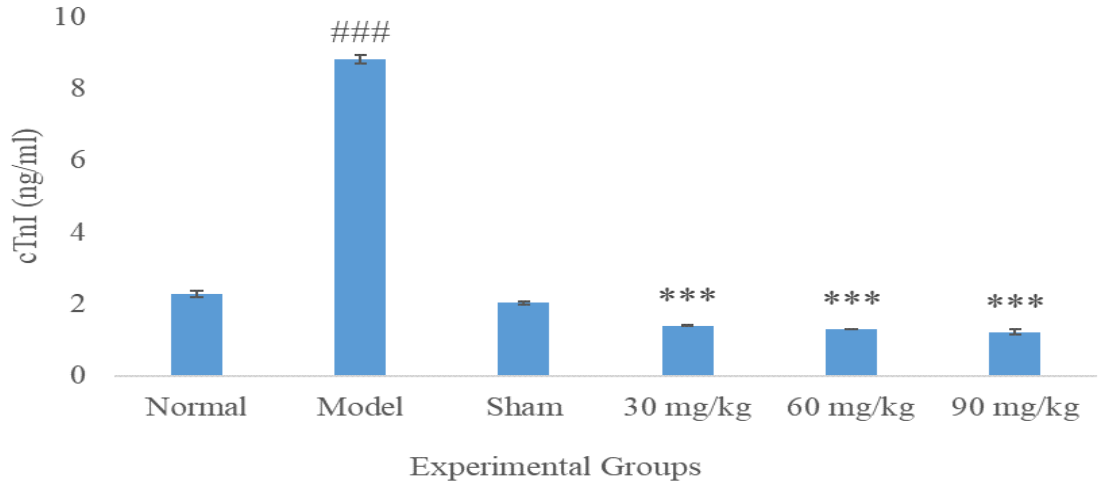

Figure 4: Effect of Ibuprofen on cTnI level in coronary artery ligation induced myocardial infarction.
The values expressed are as mean ± SEM (n=10). The statistical analysis was carried out by One-Way Analysis of Variance (ANOVA) followed by Dunnett's post-hoc test by using graph pad prism 9.2.0 whereas, #- significance difference from normal p≤0.05 and * - a significant difference from model p≤0.05. P values <0.0001 was set as a level of statistical significance respectively. ### indicates significantly different from the normal control group and *** Indicates significantly different from the model control group.

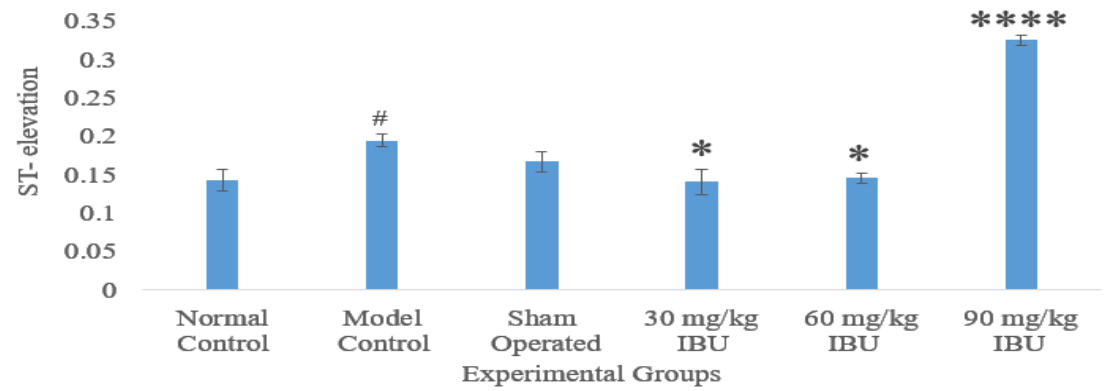

Figure 5: Effect of ibuprofen on ST-elevation in coronary artery ligation induced myocardial infarction.
The values expressed are as mean ± SEM (n=10). The statistical analysis was carried out by One-Way Analysis of Variance (ANOVA) followed by Dunnett's post-hoc test by using graph pad prism 9.2.0 whereas, #- significance difference from normal p≤0.05 and * - a significant difference from model p≤0.05. P values <0.0001 was set as a level of statistical significance respectively. # indicates significantly different from the normal control group and * and **** Indicates significantly different from the model control group.

Table 2: Effect of Ibuprofen on serum electrolyte concentration level in coronary artery ligation induced myocardial infarction.

Groups	Na^{+} conc. (mmol/l)	K^{+} conc. (mmol/l)	Ca^{+2} conc. (mg/dl)
Normal control	103.3±2.84	8.734±1.70	0.677±0.04
Model control	232.7±54.46 (#)	7.865±1.24	11.40±0.61
Sham operated	95.25±5.90	4.797±0.37	0.3917±0.09
IBU (30 mg/kg)	165.3±4.51	1.415±0.58 (**)	23.80±23.80
IBU (60 mg/kg)	150.9±3.06	3.261±0.29 (*)	31.53±20.69
IBU (90 mg/kg)	142.3±3.99	2.258±0.37 (**)	20.93±10.17

The values expressed are as mean ± SEM (n=10). The statistical analysis was carried out by One-Way Analysis of Variance (ANOVA) followed by Dunnett's post-hoc test by using graph pad prism 7.0. Whereas, # - significance difference from normal p≤0.05 and * - a significant difference from model p≤0.05. P values <0.0001 was set as a level of statistical significance respectively. # indicates significantly different from the normal control group and * and ** Indicates significantly different from the model control group.

linked with numerous cellular and molecular signalling pathways. ROCK inhibitors are effective in reducing Ischemic Reperfusion Injury associated with ROCK activation. The significance of ROCK inhibitors in causing positive effects in CAL-induced Ischemic Reperfusion Injury, on the other hand, is mainly unclear. Coronary Artery Ligation (CAL) creates high stress in the coronary artery, stimulating endothelin to bind to the G-Protein-Coupled Receptor (GPCR) and activating Rhokinase, which is important in myocardial infarction [11]. It causes myocardial necrosis, which results in cardiac dysfunction as well as a rise in lipid peroxidation, an increase in cardiac enzyme levels, and a reduction in antioxidant activity [12]. CAL's pathophysiological alterations are analogous to those seen in humans, making this model trustworthy and reliable (Halim1 et al., 2018). Cardiotoxicity is caused by CAL in the form of cytotoxic free radicals, increased intracellular Ca+2 overload, depletion of the high energy phosphate group, and oxidative stress, according to the

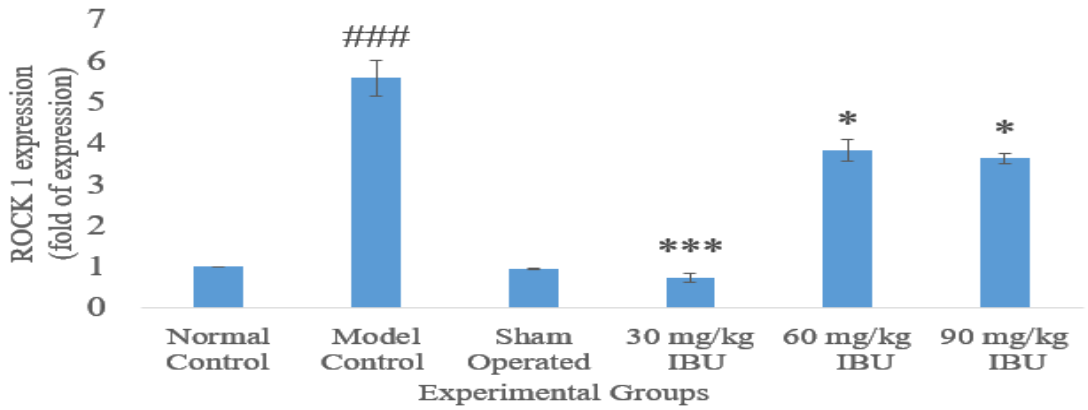

Figure 6: Effect of Ibuprofen on ROCK-1 expression in coronary artery ligation induced myocardial infarction.
The values expressed are as mean ± SEM (n=10). The statistical analysis was carried out by One-Way Analysis of Variance (ANOVA) followed by Dunnett's post-hoc test by using graph pad prism 9.2.0 whereas, #- significance difference from normal p≤0.05 and * - a significant difference from model p≤0.05. P values <0.0001 was set as a level of statistical significance respectively. ### indicates significantly different from the normal control group and * and *** Indicates significantly different from the model control group.

Table 3: Effect of Ibuprofen on antioxidant levels in coronary artery ligation induced myocardial infarction.

Groups	GSH(ug/ml)	SOD(ug/ml)	MDA(ug/ml)	Catalase(mmol/g of tissue)
Normal control	67.79±15.98	70.34±28.14	0.043±0.02	20.28±3.89
Model control	17.82±5.44	20.48±57.99	0.106±0.04	0.891±0.34
Sham operated	45.26±2.38	78.03± 5.08	0.053±0.02	15.15 ± 2.29
IBU (30 mg/kg)	4.825±1.49	518.8±660.9	0.079±0.04	0.249±0.10
IBU (60 mg/kg)	3.101±0.36	420.4±236.3	0.056±0.03	7.234±4.76
IBU (90 mg/kg)	6.259±1.38	412.9±165.3	0.039±0.01	0.073±0.05

The values expressed are as mean ± SEM (n=10). The statistical analysis was carried out by One-Way Analysis of Variance (ANOVA) followed by Dunnett's post-hoc test by using graph pad prism 7.0. Whereas, # - significance difference from normal p≤0.05 and * - a significant difference from model p≤0.05. P values <0.0001 was set as a level of statistical significance respectively.

research [13].

Ibuprofen inhibits RhoA activity via antagonising Peroxisome proliferative activated receptor gamma, according to several in-vivo and neural culture experiments (PPARgamma). Ibuprofen was tested in a CAL-induced model in rats in this study [14].

After CAL, ROCK activation causes myosin phosphatase enzyme inhibition, which causes hypercontraction, which leads to Ischemic Reperfusion Injury. Treatment with ibuprofen prevented all of these occurrences, indicating that it has a protective effect in Ischemic Reperfusion Injury. Following CAL surgery, ROCK activation causes suppression of the myosin phosphatase enzyme and downregulation of eNOS expression, resulting in hypercontraction and a rise in blood pressure. Ibuprofen pretreatment reduced the increase in blood pressure, indicating that it had a preventive effect against hypertension [15].

One of the greatest diagnostic markers for Ischemic Reperfusion Injury is an aberrant electrical impulse in the heart. CAL stimulates the Rho kinase, which affects the cell membrane permeability and electrical current, causing alterations in the ECG pattern, particularly ST segment elevation, which is indicative of infarction. ST-Elevation was observed in CAL-treated Ischemic Reperfusion Injuryce, and Ibuprofen pre-treatment significantly reduced CAL-induced ST-segment elevation, indicating that it has cell membrane maintaining action [9].

CAL induced Rho activation which is responsible for release of proinflammatory mediators and upregulate thrombogenic molecules, which li Ischemic Reperfusion Injuryts the regional blood flow and Parallel its downregulate the anti-apoptotic factor and upregulates the proapoptotic factor and cause cell necrosis, ultimately there is a change in the cytoskeleton, which alters the Left Ventricular (LV) function, which is the major predictor of causing death (Kalogeris and , Christopher P. Baines, Maike Krenz, 2014). When compared to ISO group animals, moderate left ventricular impairment was seen in CAL-treated rats, but ibuprofen pre-treated groups showed improvement in LV function.

Troponin-I is an essential diagnostic marker for cardiac illness, with its level rising in pathological heart conditions, particularly in Ischemic Reperfusion Injury, and CK-MB, a cardiac-specific enzyme, rises in ventricular septal defects. LDH is widely expressed in heart muscle and produced after tissue injury, and Troponin-I is an essential diagnostic marker for cardiac illness, with its level rising in pathological heart conditions, particularly in Ischemic Reperfusion Injury. Pretreatment with ibuprofen decreased the CAL-induced rise in blood levels of cardiac marker enzymes. It's possible that Ibuprofen, by protecting membrane integrity, Ischemic Reperfusion Injuryght prevent these enzymes from leaking [9].

Electrolytes are necessary for maintaining body function, especially in the heart, where they aid in the conduction system's

maintenance. CAL-induced Rho activation alters membrane permeability, affecting electrolyte levels [17]. When compared to normal control Ischemic Reperfusion Injuryce, CAL treated animals had a substantial rise in sodium, a negligible decrease in potassium, and a negligible increase in calcium. When compared to the model control group, pre-treatment with ibuprofen reversed the CAL-induced change in ion concentrations such as Na+, Ca+2, and K+.

In experimental animals, CAL surgery has been shown to cause significant oxidative stress and lipid peroxidation in the heart. The proposed mechanism for oxidative stress is that it indirectly regulates the GTPase pathway and inactivates the phosphatase, which propagates kinase signalling and activates Rho, causing ische Ischemic Reperfusion Injuryc-reperfusion injury, which causes severe impairment in cellular functions and necrotic lesions in the rat myocardium [18]. However, natural antioxidants (GSH, SOD, Catalase) have played a role in preventing oxidative damage; however, following CAL surgery, these enzymes' mechanisms are no longer sufficient to deal with the avoidance of excessive ROS production, and their protective impact has halted [19]. The activity of antioxidant enzymes was shown to be significantly reduced in the current investigation. Induction of Ischemic Reperfusion Injury by CAL resulted in an increase in MDA and a decrease in GSH, SOD, and Catalase in model control Ischemic Reperfusion Injurie as compared to normal control animals and a sham operated group, indicating that oxidative stress was present in the CAL group. While pretreatment with ibuprofen for 21 days results in a non-significant increase in glutathione reductase, superoxide dismutase, and catalase activities, as well as a decrease in MDA levels in CAL-treated rats.

Rho-kinase is one of RhoA's downstream effectors, cycling between a GDP-bound inactive state and a GTP-bound active state, and its expression rises with Ischemic Reperfusion Injury [20,21]. When compared to the normal control group and the sham operated group, the CAL-treated group had higher ROCK-I mRNA expression in the cardiac tissues, which was reduced by ibuprofen therapy. These data support the hypothesis that the RhoA/ROCK pathway is implicated in CAL-induced Ischemic Reperfusion Injury and that ibuprofen therapy can reduce RhoA and ROCK-I expression. Ibuprofen appears to have a cardioprotective impact based on improvements in LV dysfunction, ECG pattern, ST-segment elevation, serum cardiacmarkers, electrolyte levels, antioxidant enzymes, and ROCK-I down-regulation.

Conclusion

Our findings show that pre-treatment with ibuprofen for 21 days protects against ische Ischemic Reperfusion Injuryc-reperfusion injury by restoring normal ECG patterns and ST-segment elevation, improving most of the altered physiological, bioche Ischemic Reperfusion Injurycal, and haemodyna Ischemic Reperfusion Injuryc parameters, maintaining antioxidant status, preventing cellular damage, and suppressing ROCK-1 mRNA expression.

Authors' Contributions

Tejal Gandhi: Evaluation of estimated parameter and writing manuscript; Prexita Patel: Generation of hypothesis, estimation of parameter and writing manuscript; Arpit Mehta: Estimation of parameter; Hittal Shah: Gene exprssion study.

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