RESEARCH PAPER

Protective effect of Nrf2 modulator - Quercetin in myocardial infarction

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ABSTRACT

Despite abundant therapies of Myocardial Infarction (MI), there always remains an opportunity (perhaps need) to identify novel targets. One such emerging target is NAD(P)H:quinone oxidoreductase 1 (NQO-1), which acts downstream in Nrf2 cascade. This study was planned to investigate the effect of Nrf2 activator quercetin on NQO-1 modulation in Sprague Dawley (SD) rats. The MI was induced by left anterior descending (LAD) coronary artery was ligated for 30 minutes, followed by two hours of reperfusion, to create an animal model of myocardial ischemia/reperfusion (I/R). The animals were randomized into six groups receiving either vehicle (control), LAD surgery (model) or treatments (Telmisartan (10 mg/kg) and quercetin (10, 25, 50 mg/kg) for 45 days. Various haemodynamic parameters, left ventricular functions, biological markers, lipid and protein peroxidative marker, antioxidant enzymes activity as well as histology (heart) were carried out. The expression of mRNA of NQO-1 gene in heart homogenate was estimated. Treatment with quercetin significantly prevented the rise in infarction, organ weight, improved left ventricular function, cardiac markers profile, restored antioxidant enzymes along with decrease in lipid and protein peroxidation. The normal architecture of heart was preserved in histopathological analysis by quercetin treatment. The upregulation of NQO-1 gene in heart by quercetin resulted in normal myocardial cells. Altogether, quercetin prevented infarction, improved cardiac function by augmenting the innate cell defence system in LAD surgery. Such promising effects are attributed to novel cellular level target, NQO-1, which have multiple effects like regularisation of oxidative stress, eNOS activation and inhibition of ACE shedding.

Keywords: Quercetin; Nrf2 activator; Keap1 inhibitor; Apoptosis; Cardiovascular

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1. INTRODUCTION

Cardiovascular diseases (CVDs) have surpassed all other causes of death in recent years as the leading cause of death worldwide. The majority of the pathogenic effects causing abnormalities in the heart and blood vessels are caused by CVDs.[1] An array of conditions that includes rheumatic heart disease, cerebrovascular illness, and coronary heart disease.[2] The WHO estimated that 17.9 million deaths, or 32% of all fatalities globally, would be attributable to CVDs in 2019. The majority of these deaths were due to heart attacks and strokes. Atherosclerosis, coronary artery disease, myocardial infarction and arterial hypertension are the primary underlying causes of CVDs.[1,3]

Myocardial infarction (MI), the most prevalent kind of CHD, can be brought on by either acute or chronic myocardial ischemia. Heart attacks can also be referred to as MIs. A blockage or near-blockage of a coronary artery brought on by the growth of plaque and thrombus, which is a hallmark of MI.[4] This degenerative situation, wherein the amount of glycogen in the cells diminishes and the amount of oxygen given to the cells decreases, causes reduced blood flow and consequent tissue damage or death (infarction) of the afflicted area.[4,5] The fundamental features of MI include diastolic and systolic dysfunction, coronary artery embolism, cocaine-induced ischemia, coronary dissection, and coronary vasospasm, as well as ST-segment deviation, which might culminate in a heart attack.[6]

Myocardial infarction (MI) is more common in Asian countries like India, Pakistan, Bangladesh, Sri Lanka, and Nepal than it is in industrialised countries.[7]Around 126 million people worldwide, or 1.72% of the world's population, are affected by myocardial infarction. Nine million fatalities each year are brought on by this illness worldwide.[8,9] AHA estimates that 805,000 Americans reported having a MI, whereas 81.8% of Indians were impacted by myocardial infarction.[7]

By clearing the blockage in the blood artery, the oxygen flow must be restored, called reperfusion, in order to balance the abnormality's need for and supply of oxygen, which efficiently reduces infarct size and reduces mortality. Beside this, reperfusion following thrombosis causes the production of free radicals or reactive oxygen species (ROS), which can potentially cause damage to cardiac tissues.[10] As a result, cardiac ischemia and reperfusion (I/R) injury plays a significant role in myocardial infarction, and inhibiting ROS is an crucial therapeutic approach for mitigating I/R injury. In order to lessen the ROS damage brought on by

ischemia and reperfusion at, a number of antioxidants are said to be effective.[11]

Despite the fact that there are several medications available for MI treatment, they all have a number of negative effects.[12] Additionally, only 24% of individuals with MI receive treatment in the real world, and none of the drugs that are currently on the market target the genetic causes of MI at their root. Due to their safety, effectiveness, cultural acceptance, and fewer side effects, plant-based medications are becoming more and more popular worldwide.[13] Plant-based medicine can significantly help to attain the objective of universal health coverage by being incorporated in a people-centered health system that combines curative treatments with preventative care, stated in "WHO global report on traditional and complementary medicine 2019".[14] Given that plants offer an abundance of bioactive components with therapeutic value, the reasoning for using plant-based medicine is hardly unexpected. Plants may be used to target the various molecular aspects of the multiple-cause pathophysiology of MI, such as the destruction of plaque and thrombus formation, reduction of inflammatory markers, modification of different biochemical levels, genetic modulation, scavenging of reactive oxygen species (ROS), and augmentation of cellular antioxidants.[13] The well-known polyphenolic flavonoid antioxidant quercetin is our target chemical. It is generally known that oxidative stress plays a part in conditions like MI. A group of vital proteins known as the phase II metabolic enzymes detoxify xenobiotics and have recently been identified as a potential target for lowering oxidative stress. NAD(P)H:quinone oxidoreductase (NQO1), one of the main phase II detoxification mechanisms, is the subject of thiNADs investigation. Due to its capacity to catalyse the conversion of poisonous and reactive quinones and quinone imines to their less reactive and toxic hydroquinones forms, NQO1 is one of the most notable enzymes in cellular defence.[15] Quercetin and its equivalents have been shown in several in-vitro experiments to increase the activity of the NRF2-ARE system, which stands for nuclear factor erythroid 2-related factor-antioxidant response element system.[16,17] Cells consistently and constitutively produce Nrf2, guaranteeing that it responds quickly to oxidative, inflammatory, and metabolic stressors. The downstream cascade of antioxidant genes, comprises of heme oxygenase-1 (HO-1), NQO1, and glutamatecysteine ligase modifier subunits, is likewise increased by the activation of NRF-2.[16,18] The in-vitro research has demonstrated quercetin's ability to activate NQO-1. In the MCF-7 human breast cancer cell line, Valerio et al. demonstrated enhanced NQO1 transcription in response to quercetin.[19] According to an in-vivo investigation done on adult mice, quercetin boosted the protein and gene expression of the NQO-1 gene in the liver.[20] In light of this, the objective of the functional investigation was to investigate the possibility that the NRF2 activator quercetin may influence the downstream NQO-1 gene and lower the infract size.

2. MATERIALS & METHODS

Materials

2.1 Procurement of Drug

From Sigma Aldrich Pvt. Ltd., quercetin was purchased. The Torrent Research Centre in Ahmedabad provided the telmisartan for purchase.

2.2 Chemicals and Kits

Epinephrine, thiobarbituric acid (TBA), trichloroacetic acid (TCA), tris buffer, 5-5'-dithiobis [2-nitrobenzoic acid] (DTNB, Ellman's Reagent), 5-thionitrobenzoic acid (TNB), TRIazol, used in the study were of analytical grade and procured from Sigma-aldrich Pvt. Ltd. Ethylenediaminetetraacetic acid (EDTA), carboxy methyl cellulose (CMC), sodium citrate, citric acid, potassium dihydrogen phosphate (KH2PO4), sodium bicarbonate (NaHCO3), potassium chloride (KCl), and glucose were of analytical grade and were obtained from Merck Pvt. Ltd, Hyderabad. Calcium chloride (CaCl2), magnesium sulphate (MgSO4), sodium chloride (NaCl), and sodium pyruvate were of analytical grade and were obtained from Chemdyes Cop., Rajkot. All the biochemical assay was performed using standard kits (creatine kinasemuscle/Brain (CK-MB), lactate dehydrogenase (LDH), electrolytes (Na+ and K+ and Ca+2), creatinine, blood urea nitrogen (BUN), and uric acid), which were procured from i-chem Jeev Diagnostic Pvt. Ltd, Chennai. Primers were purchased from Eurofins Genomics, Bangalore. qRT-PCR Kit was purchased from applied biosystems, Thermoscientific Inc., Ahmedabad.

2.3 Experimental Procedures

2.4 Selection of Dose

The dose of Telmisartan (10 mg/kg) and three doses of quercetin (10 mg/kg, 25 mg/kg and 50 mg/kg) were selected from literature.[39]

2.5 Induction of myocardial infarction

For the investigation, male 12-week-old Sprague Dawley (SD) rats were purchased from the Zydus Research Centre in Ahmedabad. Six rats were kept in each cage in a controlled setting that conformed

with CPCSEA guidelines. Rats were fed a conventional laboratory chow diet that was purchased from Pranav Agro Pvt. Ltd., Vadodara, and were given free access to RO water. The experimental protocol APC/2018-IAEC/1808 was authorized by Anand Pharmacy College's Institutional Animal Ethical Committee (IAEC).

The left anterior descending (LAD) coronary artery was ligated for 30 minutes, followed by two hours of reperfusion, to create an animal model of myocardial ischemia/reperfusion (I/R). Ketamine (90 mg/kg) and xylazine (10 mg/kg) were delivered intraperitoneally (i.p.) to anaesthetize the animals. Heparin (500 IU) was also injected to avoid blood clotting. A respirator with a tidal volume of 6-8 ml/kg and a frequency of 80 breaths/min was used to provide positive pressure breathing with 95% O2 and 5% CO2. The LAD was tied 2-3 mm from its origin for 30 minutes to imitate I/R, and a left thoracotomy was done to expose the heart. After two hours after reperfusion, blood samples were taken, the animals were killed, and the heart tissues were removed and stored at -80°C for future investigation.

The rats were divided into six groups of ten each, based on their body weight, using a random allocation method.

Group I - Normal control group; Administered distilled water.

Group II - CAL Model; Rats were subjected to a 30 min Left Anterior Descending LAD coronary artery ligation followed by a 2 h reperfusion

Group III- Standard control; Telmisartan 10 mg/kg (Tel 10 mg/kg); Std. drug Tel (10 mg/kg, p.o.) Prior to 45 days of LAD coronary artery ligation + LAD coronary artery ligation

Group IV-VI: Quercetin 10 mg/kg (Que 10 mg/kg), Quercetin 25 mg/kg (Que 25 mg/kg) , Quercetin 50 mg/kg (Que 50 mg/kg) respectively; Que (10 mg/kg, 25 mg/kg and 50 mg/kg p.o.) Prior to 45 days of LAD coronary artery ligation + LAD coronary artery ligation.

During the study protocol, the weight, blood pressure, and ECG of the rats were recorded. Following 45 days of pre-treatment, the rat's blood pressure and ECG were measured once again using Biopac Student Lab (MP-36 Biopac Systems, Inc).[42,43] After the completion of the 45-day pre-treatment period, blood samples were collected from the rat's retro-orbital plexuses under Ketamine (50 mg/kg) + Xylazine (10 mg/kg) anesthesia. The serum was separated from the blood and analyzed for various biochemical parameters such as Ca+2[44,45,46,47] levels, creatine kinase-muscle/brain (CK-MB)[48], lactate dehydrogenase (LDH)[49], and troponin-I.[40] Following this, the rats were humanely euthanized by administering an overdose of ketamine (90 mg/kg) and xylazine (15 mg/kg), and their hearts were isolated, cleaned, and weighed.

At the end, the langendorff study was performed on isolated heart for determining various LV functions like dp/dt max, dp/dt min as well as left ventricular end diastolic pressure (LVEDP).[50]

A portion of the heart was homogenized with 10 ml of ice-cold Tris-hydrochloride buffer (0.1M, pH 7.4) and then centrifuged to prepare homogenates for the determination of stress marker and antioxidant parameters (MDA, SOD, GSH, Catalase)[51,52,53]. At the conclusion of the experiment, the animals were humanely euthanized, and their hearts were removed, cleaned, weighed, and rinsed with phosphate buffer. The hearts were then stored in 10% formalin for preservation. A portion of the heart was set aside for hematoxylin-eosin staining to assess myocardial degeneration, hypertrophy, and infiltration of inflammatory cells. The area of the myocardial infarct was also measured using the TTC staining technique.[41]

A fraction of the heart tissue was reserved for gene expression analysis using real-time polymerase chain reaction (RT-PCR) to study the mRNA levels of Bax, Bcl2, Caspase-3, Nrf2, HO-1, IL-6, TNF-alpha, and Nf-kb.

2.6 Statistical analysis

A values are represented as mean ± SEM. For statistical analysis, one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test utilizing GraphPad prism v6.01. Significant change was set at $p<0.05$.

3. RESULTS

3.1. Estimation of anthropometric parameters

2.1.1 Effect of quercetin on Body weight

The body weight was insignificantly decreased in CAL induced myocardial infarct animals (260 ± 7.83) as compared to normal control group animals (276 ± 5.84). Telmisartan 10 mg/kg and quercetin 50 mg/kg were found to exhibit significant ($p<0.001$) rise in body weight (304 ± 8.31, 309 ± 7.42 respectively) as compared to model control group.

Quercetin 10 mg/kg and 25 mg/kg treated animals showed insignificant change in body weight (256.9±5.31, 251.9±4.525 respectively) comparatively model control group.

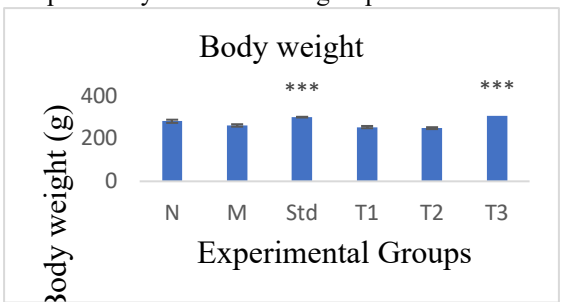


Figure 1 Effect of quercetin on Body weight

Results were presented as mean ± SEM, with n=10 animals in each group.
*Where ***p<0.01 vs Model control group.*

3.1.2 Effect of quercetin on Heart weight and Heart / Body weight ratio

The Model control group animals displayed significant (p<0.001) decline in heart weight (0.78 ± 0.021) as compared to normal animals (1.1 ± 0.043). Heart / Body weight ratio was also significantly (p<0.01) reduced in CAL induced MI rats when compared to normal animals. The treatment with quercetin and telmisartan exhibited significant increase in heart weight and heart/body weight ratio as compared to model control group animals.

Table 1 Effect of quercetin on heart weight and Heart / Body weight ratio

Experimental groups	Heart weight (g)	Heart / Body weight ratio
Normal control	1.1025 ± 0.0044	0.0038 ± 0.0002
DOCA model	0.7887 ± 0.0028 (##)	0.0029 ± 0.0001 (##)
Tel 10mg/kg	1.0000 ± 0.0037 (**)	0.0032 ± 0.0001(**)
Que 10mg/kg	0.9212 ± 0.0040 (*)	0.0035 ± 0.0006 (*)
Que 25 mg/kg	0.9310 ± 0.0039 (*)	0.0037 ± 0.0001 (**)
Que 50mg/kg	1.18 ± 0.0034 (**)	0.0037 ± 0.0001 (**)

*The results were reported as mean ± SEM, with a total of 8 animals in each group. Where ##p<0.01 vs Normal control group. Where *p<0.05, **p<0.01 vs Model control group.*

3.2 Assessment of hemodynamic changes

2.2.1 Effect of quercetin on SBP

The infarcted rats indicated a significant (p<0.01) increase in systolic blood pressure at the end of the study protocol (158 ± 6.265) as compared to normal control group (117.8 ± 1.109). The significant prevention observed in all doses of quercetin treated rats (132.4 ± 4.78, 130.7 ± 4.372, 127.7 ± 7.518 respectively) as shown in below figure and also telmisartan 10 mg/kg treated rats (119.3 ± 2.692) has shown significant incline in SBP as compared to model control group rats.

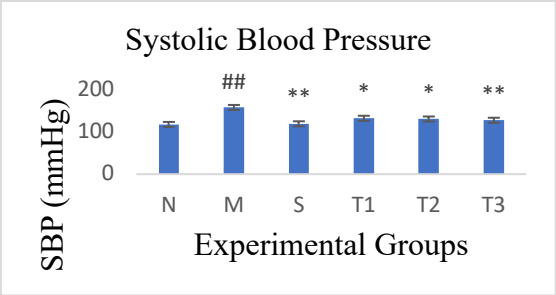


Figure 2 Effect of quercetin on Systolic Blood Pressure (SBP)

Results were presented as mean ± SEM, with n=10 animals in each group.
*Where ##p<0.01 vs Normal control group. Where *p<0.05, **p<0.01 vs Model control group.*

3.2.2 Effect of quercetin on DBP

The diastolic blood pressure was significantly (p<0.01) elevated in CAL induced myocardial infarct animals (113.7 ± 6.232) as compared to Normal control group (82.11 ± 2.659). Telmisartan 10 mg/kg and quercetin 50 mg/kg treated rats showed significant (p<0.05, p<0.01 respectively) decrease in diastolic blood pressure (97.1 ± 1.244, 65.05 ±10.73 respectively) as compared to CAL induced myocardial infarct animals.

There is no significant prevention observed in quercetin 10 mg/kg and 25 mg/kg (97.1 ± 3.366, 90.34 ± 2.584 respectively).

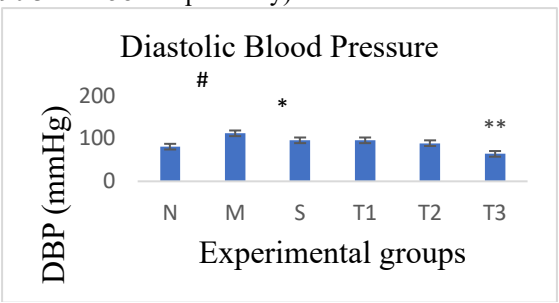


Figure 3 Effect of quercetin on Diastolic Blood Pressure (DBP)

Results were presented as mean ± SEM, with n=10 animals in each group.
*Where, ##p<0.01 vs Normal control group. Where *p<0.05, **p<0.01 vs Model control group.*

3.2.3 Effect of quercetin on MAP

The significant (p<0.01) prevention in MAP achieved by the treatment with telmisartan and quercetin 25 mg/kg and 50 mg/kg treated animals (105 ± 1.339, 103.8 ± 2.919, 103.1 ± 2.162 respectively) as compared to infarcted animals while insignificant change was experiential in quercetin 10 mg/kg treated animals (108.5 ± 2.658).

The MI animals presented significant (p<0.01) rise in mean arterial pressure (128.5 ± 5.833) as compared to normal group animals (94.02 ± 2.09).

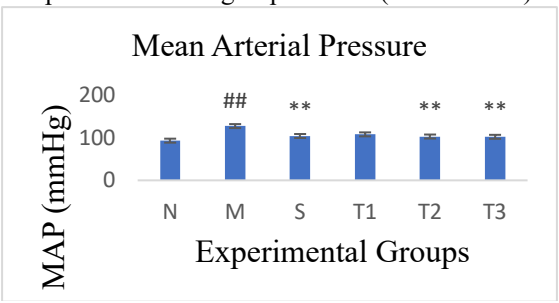


Figure 4 Effect of quercetin on Mean Arterial Pressure (MAP)

Results were presented as mean ± SEM, with n=10 animals in each group.
*Where ##p<0.01 vs Normal control group. Where **p<0.01 vs Model control group.*

3.3. Estimation of biochemical parameters in serum

2.3.1 Effect of quercetin on Ca^{2+} ion level

The marked ($p<0.01$) increase in Ca^{+2} level was seen in CAL induced myocardial infarct animals (14.68 ± 1.557) as compared to normal control group (9.05 ± 0.4481). Telmisartan 10 mg/kg and quercetin 50 mg/kg treated animals presented significant ($p<0.01$) decrease in Ca^{+2} level (8.057 ± 0.9524, 8.611 ± 1.298 respectively) when compared to model control. An insignificant decrease in Ca^{2+} level was observed in quercetin (10 mg/kg, 25 mg/kg) treated animals (11.57 ± 1.994, 10.97 ± 0.4878 respectively) as compared to model control group.

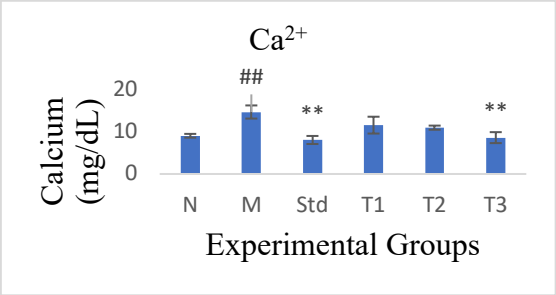


Figure 5 Effect of quercetin on Ca^{2+} ion level

Results were presented as mean ± SEM, with n=10 animals in each group.

*Where ##p<0.01 vs Normal control group. Where **p<0.01 vs Model control group.*

3.3.2 Effect of quercetin on Creatine kinase (CK-MB) level

A substantial ($p<0.001$) elevation in cardiac marker CK-MB was noticed in CAL induced myocardial infarcted animals (1131 ± 243.5) as compared to normal control group (331.6±36.93). The animals treated with telmisartan and all doses of quercetin exhibited significant ($p<0.01$, $p<0.001$ respectively) decline in elevation of CK-MB level (547.1 ± 120.8, 483.3 ± 77.73, 456 ± 131.8, 403.7 ± 72.93 respectively) as compared to model control group.

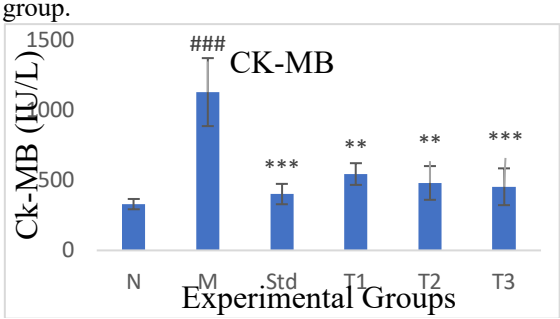


Figure 6 Effect of quercetin on Creatine kinase (CK-MB) level

*Results were presented as mean ± SEM, with n=10 animals in each group. Where ###p<0.01 vs Normal control group. Where **p<0.05, ***p<0.01 vs Model control group.*

3.3.3 Effect of quercetin on Lactate dehydrogenase (LDH) level

The animals of model control group indicated significant ($p<0.001$) elevation in LDH level in CAL induced myocardial infarction when compared to normal animals. The significant ($p<0.001$) prevention in elevation of LDH level was achieved by treatment with telmisartan and quercetin as compared to model control group.

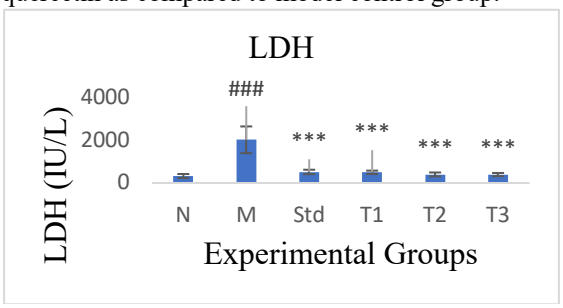


Figure 7 Effect of quercetin on Lactate dehydrogenase (LDH) level

Results were presented as mean ± SEM, with n=10 animals in each group.

*Where ###p<0.01 vs Normal control group. Where ***p<0.01 vs Model control group.*

3.3.4 Effect of quercetin on Troponin-I level

An insignificant elevation in troponin-I level was observed in CAL induced myocardial infarct animals (0.8167 ± 0.003645) as compared to normal control group (0.731 ± 0.01332). On the other side, an insignificant decrease in elevated troponin-I level was seen in telmisartan and quercetin treated groups as compared to model control group.

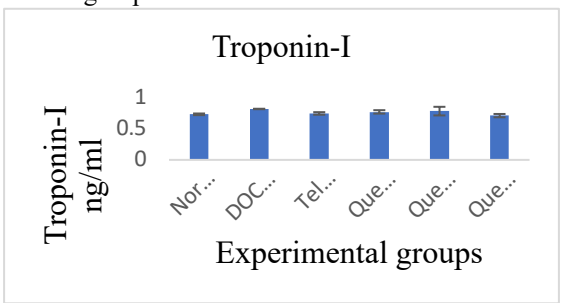


Figure 8 Effect of quercetin on Troponin-I level

Results were presented as mean ± SEM, with n=10 animals in each group.

3.4. Effect of quercetin on Left ventricular function

At the end of 45 days of study protocol, LV function was measured by performing langendorff assay. CAL induced myocardial infarct animals noticed with significant ($p<0.001$) rise in LVEDP as compared to normal control group, indicating LV haemodynamic overload. While, quercetin 10 mg/kg, 25 mg/kg and 50 mg/kg and telmisartan 10 mg/kg significantly ($p<0.05$, $p<0.01$, $p<0.001$ respectively) prevented this raise in LVEDP (1.322 ± 0.2423, 0.9402 ± 0.1527, 0.9463 ± 0.1207, 1.003 ± 0.08215 respectively) and improved the normal heart function.

Moreover, model control animals observed with significant ($p<0.001$) decrease in coronary flow rate (10.625 ± 1.18). However, treatment with quercetin 25 mg/kg, 50 mg/kg and telmisartan 10 mg/kg showed significant ($p<0.05$, $p<0.001$ respectively) increase in coronary flow rate (13.44 ± 1.79, 15.778 ± 1.20, 18.22 ± 1.38, 19.125 ± 0.991 respectively) as compared to model control group. Besides, quercetin 10 mg/kg treated rats showed insignificant elevation in coronary flow rate (15.778 ± 1.20) as compared to model control group.

In contrast, CAL induced myocardial infarction produced a noteworthy ($p<0.05$) decrease in $+dp/dt_{max}$ and $-dp/dt_{min}$ in model control animals. The decrease in $+dp/dt_{max}$ and $-dp/dt_{min}$ significantly ($p<0.05$, $p<0.01$ respectively) declined by telmisartan 10 mg/kg and quercetin 50 mg/kg as compared to model control group. While, this reduction in $+dp/dt_{max}$ and $-dp/dt_{min}$ insignificantly prevented by quercetin (10 mg/kg and 25 mg/kg) as compared to model control group.

Table 2 Effect of quercetin on Left ventricular function

Experimental groups	LVEDP (mmHg)	CFR (ml/sec)	+dp/dt max (mmHg/s)	-dp/dt min (mmHg/s)
Normal control	1.125 ± 0.422	19.75 ± 0.5	10.67 ± 0.644	-18.53 ± 1.016
DOCA model	2.649 ± 0.159 (###)	10.625 ± 1.18 (###)	3.568 ± 0.505 (#)	-3.617 ± 0.760 (###)
Tel 10mg/kg	1.003 ± 0.082 (***)	19.125 ± 0.991 (***)	9.571 ± 0.787 (*)	-9.011 ± 1.542
Que 10 mg/kg	1.322 ± 0.242 (*)	13.44 ± 1.79 (*)	7.586 ± 1.916	-7.104 ± 1.481
Que 25 mg/kg	0.940 ± 0.152 (**)	15.778 ± 1.20 (*)	6.608 ± 1.928	-7.269 ± 1.853
Que 50mg/kg	0.946 ± 0.120 (***)	18.22 ± 1.38 (***)	10.82 ± 1.317 (**)	-10.94 ± 1.756 (**)

Results were presented as mean ± SEM, with n=10 animals in each group.
*Where, ###p<0.01 vs Normal control group. Where, *p<0.01, **p<0.0, ***p<0.01 vs Model control group.*

3.5. Estimation of stress marker and endogenous antioxidant enzymes in heart homogenate

2.5.1 Effect of quercetin on Malondialdehyde level

The MDA level was evaluated in heart homogenate towards the end of 45 days study protocol. The MDA concentration was elevated significantly ($p<0.001$) in model control (0.3647 ± 0.076) when compared with normal control (0.1369 ± 0.049). The significant ($p<0.05$, $p<0.001$ respectively) drop was observed in quercetin 25 mg/kg, 50 mg/kg and telmisartan 10 mg/kg treated animal groups (0.1735 ± 0.062, 0.0831 ± 0.0058, 0.06543 ± 0.011) as compared to model control group. Also, quercetin (10 mg/kg) exhibited decrease in MDA level (0.1924 ± 0.039) as compared to model control group, but change was insignificant.

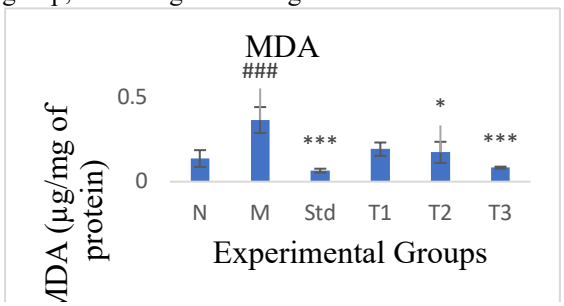


Figure 9 Effect of quercetin on MDA level

Results were presented as mean ± SEM, with n=10 animals in each group.
*Where ###p<0.01 vs Normal control group. Where *p<0.05, ***p<0.01 vs Model control group.*

3.5.2 Effect of quercetin on Superoxide dismutase level

After 45 days of protocol, the significant ($p<0.01$) decrease in the SOD level was observed in MI (70.42 ± 21.45) animals as compared to normal control group (105.9 ± 20.98). The treatment with quercetin 25 mg/kg, 50 mg/kg and telmisartan 10 mg/kg significantly ($p<0.05$, $p<0.01$ respectively) restored this decreased SOD level (123.3 ± 31.38, 126.6 ±13.69, 126.1 ± 14.24 respectively) as compared to model control group. While, quercetin (10mg/kg) insignificantly restored the SOD level (88.01 ± 4.431) as compared to model control group.

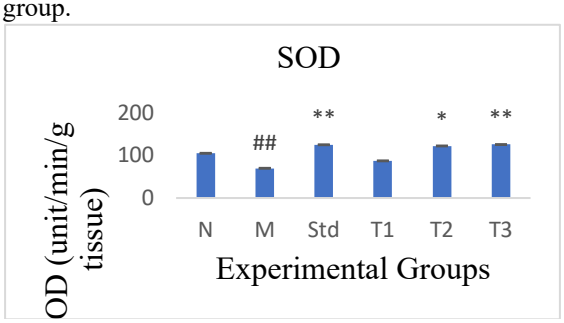


Figure 10 Effect of quercetin on SOD level

Results were presented as mean ± SEM, with n=10 animals in each group.
*Where ##p<0.01 vs Normal control group. Where *p<0.05, **p<0.01 vs Model control group.*

3.5.3 Effect of quercetin on Glutathione level

The concentration of GSH of the model control was considerably ($p<0.05$) lowered when compared with normal control group (0.3941 ± 0.107). The drug treatment with telmisartan markedly ($p<0.05$) prevented this decreased GSH level (0.3421 ± 0.09591). All doses of quercetin insignificantly prevented this decreased level of GSH as compared to model control group.

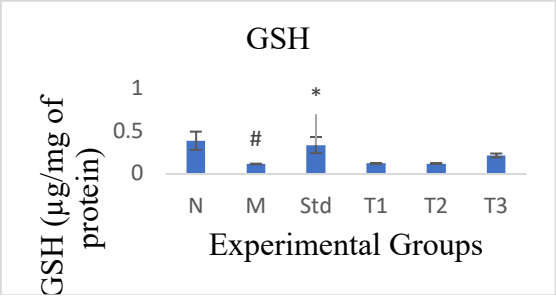


Figure 11 Effect of quercetin on GSH level

Results were presented as mean ± SEM, with n=10 animals in each group.

*Where #p<0.05 vs Normal control group. Where *p<0.05 vs Model control group.*

3.5.4 Effect of quercetin on Catalase level

A significant ($p<0.01$) dropped in the CAT level was seen in CAL induced myocardial infarct animals (0.4153 ± 0.1162) when compared to the normal control (0.9916 ± 0.05163). Telmisartan and higher dose of quercetin treated rats presented significant ($p<0.01$, $p<0.05$ respectively) restoration in catalase level (1.05 ± 0.1222, 0.734 ± 0.1311 respectively) as compared to model control group. An insignificant decline in CAT level was experiential in quercetin (25 mg/kg, 50 mg/kg) (0.4584 ± 0.1801, 0.6502 ± 0.2385) as compared to model control group.

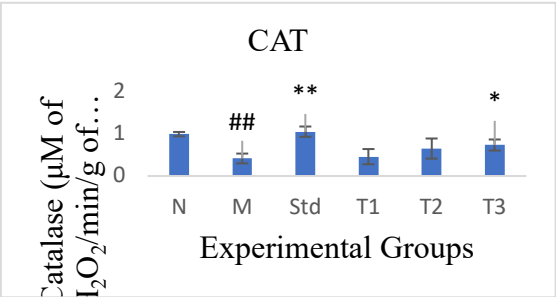


Figure 12 Effect of quercetin on CAT level

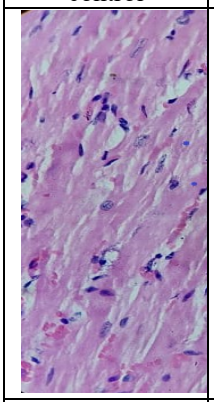
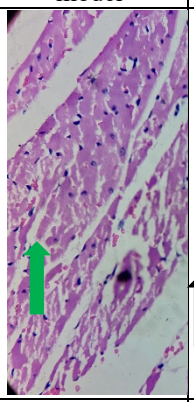
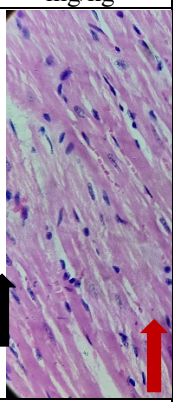
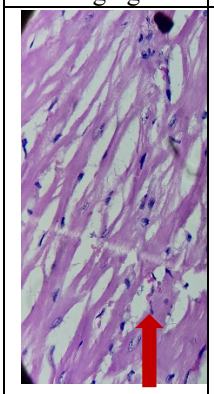
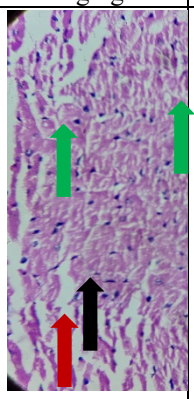
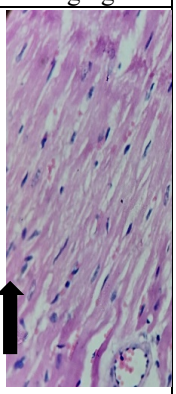
Results were presented as mean ± SEM, with n=10 animals in each group.

*Where ##p<0.01 vs Normal control group. Where *p<0.05, **p<0.01 vs Model control group.*

3.6. Effect of quercetin on histopathology of Heart

Normal control group animals shown normal tissue histology with normal shape of myocardial cells having single, oval shaped nuclei present in to the centre of cardiomyocytes and normal muscle fibres with all normal structure. However, CAL induced myocardial infarct animals showed myocardial cells infiltration and necrosis. The irregular spread of cardiac myofibers and edema in myocardium was observed in the model control group as compared to normal control group. The treatment with telmisartan (10 mg/kg) and quercetin (10, 25 and 50 mg/kg) displayed improvement in histopathological changes as compared with model control group.

Table 3 Histopathological assessment of rat's heart tissue

1. Normal control	2. DOCA model	3.Tel 10 mg/kg
		
4. Que 10 mg/kg	5. Que 25 mg/kg	6. Que 50 mg/kg
		

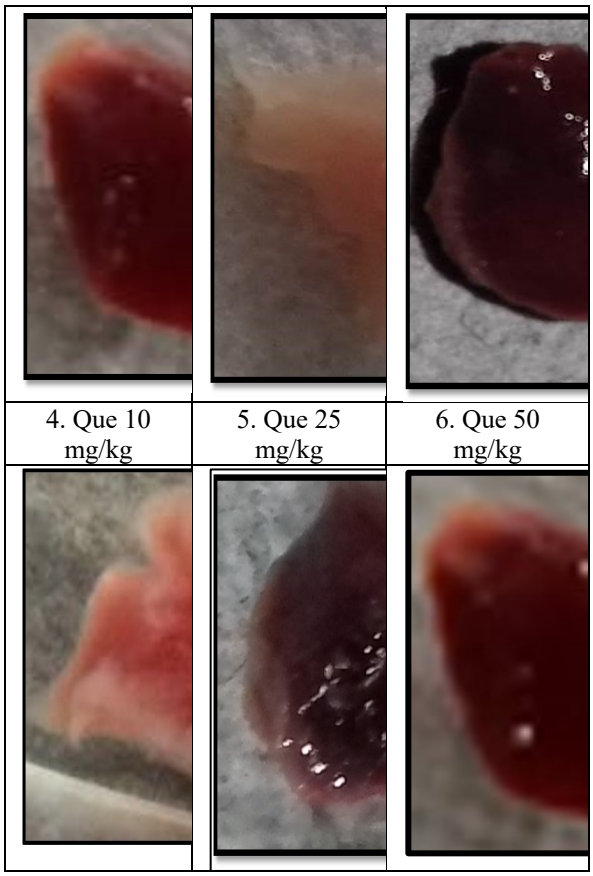
Indications: inflammatory infiltration in myocardial cells (red arrow), ischemic necrosis (green arrow) and tissue edema (black arrow).

3.7 Effect of quercetin on myocardial infarct area

In comparison with normal group, larger infarct area was observed in heart of model control group as evident by pale colour. Treatment with telmisartan 10 mg/kg and quercetin 50 mg/kg indicated noteworthy decrease in infarct area. While, a few infarcted areas was noticed in quercetin 10 mg/kg and 25 mg/kg therapy.

Table 4 Effect of quercetin on myocardial infarct area

1. Normal control group	2. DOCA model	3.Tel 10 mg/kg



1). Normal control group animals indicated no infarction. 2). Model control group animals showed significant large infarct area. 3). Std. drug Telmisartan 10 mg/kg receiving animals showed reduction in area of infarction. 4). Quercetin 10 mg/kg group showed infarction. 5). Quercetin 25 mg/kg group rats presented slight decrease in infarction. 6). Quercetin 50 mg/kg receiving rats demonstrated noticeable reduction in infarction.

2.8 Effect of quercetin on mRNA expression

In contrast to the normal, decrease in Nrf2, HO-1 and Bcl2 mRNA expression was observed in CAL induced myocardial infarct animals. However, upregulation in mRNA expression of Nrf2, HO-1, and Bcl2 has shown by treatment of telmisartan and quercetin.

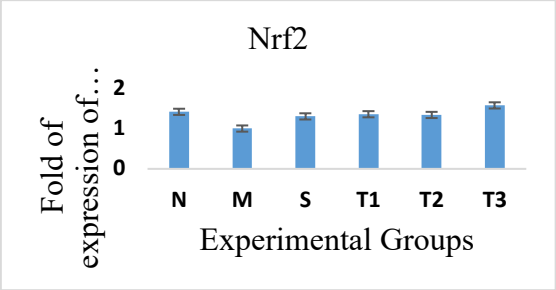


Figure 13 Effect of quercetin on Nrf2 mRNA expression

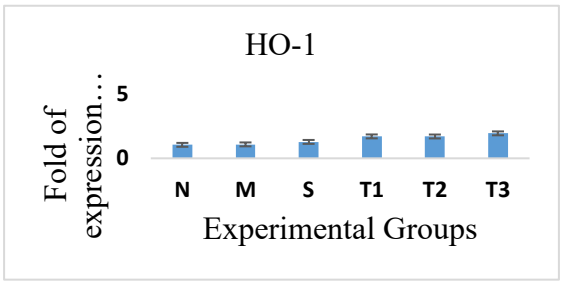


Figure 14 Effect of quercetin on HO-1 mRNA expression

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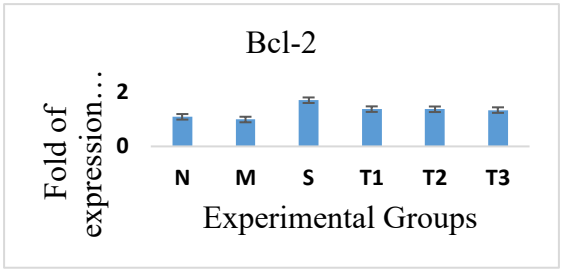

Figure 15 Effect of quercetin on Bcl-2 mRNA expression

CAL induced myocardial infarct animals showed upregulation in gene expression of Nf-Kβ, IL-6, and TNF-alpha. The level of these genes was downregulated by telmisartan and quercetin treated animals.

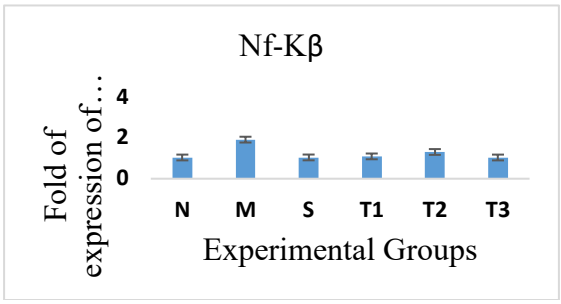

Figure 16 Effect of quercetin on Nf-kβ mRNA expression

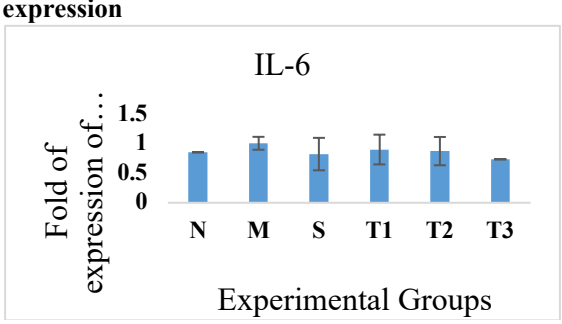

Figure 17 Effect of quercetin on IL-6 mRNA expression

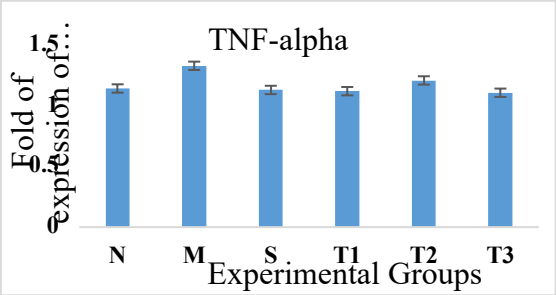

Figure 18 Effect of quercetin on TNF-alpha mRNA expression

CAL induced myocardial infarct animals showed insignificant upregulation in gene expression of Bax and Caspase-3. The level of these genes was downregulated by all treatments.

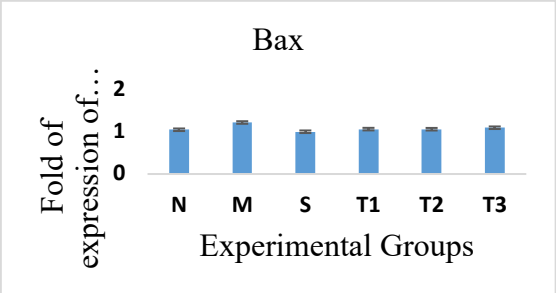

Figure 19 Effect of quercetin on Bax mRNA expression

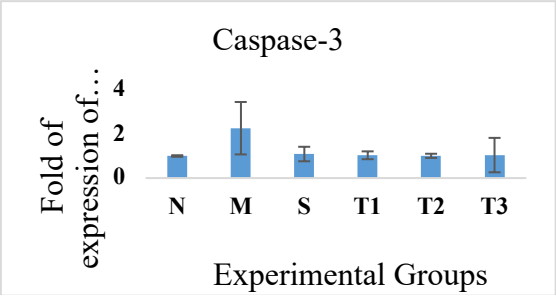

Figure 20 Effect of quercetin on Caspase-3 mRNA expression

4. DISCUSSION

Oxidative stress is one of the most important pathogenic process that accelerates the onset and progression of cardiovascular diseases, including atherosclerosis, MI, heart failure, and stroke. Strategies aimed at reducing oxidative stress may have therapeutic potential in the prevention and treatment of these diseases. These strategies may include lifestyle modifications, such as diet and exercise, as well as pharmacological interventions targeting oxidative stress.

In MI, acute oxidative stress damages the heart muscle cells, leading to cell death and impairing heart function. Targeting oxidative stress in these conditions may therefore have therapeutic benefits. One such potent molecule, Quercetin, a natural flavonoid, has been shown to possess potent antioxidant and anti-inflammatory properties. Hence, this study focused to assess cardioprotective effects in animal models of hypertension and myocardial infarction. Also, this study focuses on the modulation of nuclear factor erythroid 2-related factor 2 (Nrf2) gene responsible for antioxidant responses.

The potential of quercetin to attenuate oxidative stress induced myocardial infarction which is caused by a blockage of blood flow to the heart muscle because MI is a major consequence of CVD need to be explored. Exploring this pathophysiology could offer a more comprehensive understanding of how quercetin influences the cardiovascular system. Hence, quercetin has also been studied in coronary artery ligation (CAL) induced myocardial infarction rat model. This model has been shown to closely mimic the pathological and physiological features of human MI, making it a valuable tool in preclinical research.[21,22,23]

Decreased heart weight is a common finding in the CAL induced myocardial infarction (MI) rat model.[24] This significant reduction in heart weight of Model control group animals was due to the loss of cardiomyocytes, which occurs following the CAL surgery and subsequent MI. While, all doses of quercetin restored the cardiomyocyte loss as evident by increasing the weight of heart, which may be attributed to the ability of quercetin to reduce oxidative stress and inflammation, which are known to contribute to cardiomyocyte loss after MI.

Several studies has been conducted to investigate the effects of quercetin on blood pressure in the CAL induced myocardial infarction (MI) model. While the results have been mixed, some studies have suggested that quercetin may have beneficial effects on blood pressure in this context.[25] In consistent with the same, we found significant changes in blood pressure caused by treatment with quercetin compared to rats that received no treatment, suggested that this effect may be due to the ability of quercetin to enhance the production of nitric oxide, a vasodilator that helps to regulate blood pressure.[26]

In line with the previous study, significant increase in level of serum calcium was observed in MI rats as compared with that of control group, may be due to calcium release from damaged cardiac cells. It is certain that lowering in nitric oxide levels by excessive generations of ROS leading to vasoconstriction, and hence a rise in blood pressure; myocardial calcium homeostasis is negatively altered by ROS, leading to arrhythmias.[27] This calcium homeostasis in cardiac cells was significantly regulated by treatment with quercetin 50 mg/kg, compared to rats that received no treatment.

CAL induced myocardial infarction (MI) in rat models is linked to considerable alterations in cardiac biomarkers such as, creatine kinase-MB

(CK-MB) and lactate dehydrogenase (LDH) are two major cardiac biomarkers commonly used to assess myocardial injury, while troponin is another widely used biomarker for detecting MI. Several studies have reported increased CK-MB and LDH levels, but no change in troponin levels, following CAL-induced MI in rat models. A lack of change in troponin levels may be due to the timing of the measurements or the extent of myocardial injury or sensitivity of the assay used.[28,29] Consistent with the previous study, we found significant elevated levels of CK-MB and LDH without affecting troponin level in serum of Model control group animals than that of control animals. Conversely, quercetin treated animals presented significant decrease in serum levels of CK-MB and LDH, compared to rats that received no treatment. However, there was no significant difference in troponin levels between the groups, this may be since troponin release is delayed compared to CK-MB and LDH release.

LVEDP, dp/dt max and min are important measures of cardiac function and are commonly used as indicators of myocardial contractility.[30] CAL-induced MI rats exhibited significantly impaired LV function, as demonstrated by an increase in LVEDP and decrease in coronary flow rate, compared to control rats. However, treatment with quercetin significantly improved LV function, as evidenced by a decrease in LVEDP and increase in coronary flow rate, compared to rats that received no treatment. Furthermore, quercetin treatment also improved dp/dt max and min in rats with CAL-induced MI, compared to untreated rats. This improvement in cardiac function by quercetin may be due to the ability of quercetin to improve mitochondrial function and reduce oxidative stress.

The rats with CAL-induced MI exhibited significant myocardial damage as evidenced by the presence of myocardial necrosis, inflammatory cell infiltration, and degenerate myocardial fibres. These changes were more pronounced in the areas surrounding the ligation site owing to increased oxidative stress and inflammatory response in this region. However, treatment with quercetin was found to significantly reduce these histopathological changes, specifically, a decrease in myocardial necrosis and a reduction in inflammatory cell infiltration. These findings suggest that quercetin may have a protective effect on the heart in CAL-induced MI rat models.

CAL-induced MI in rat models is associated with a significant increase in infarct area, which is typically assessed using triphenyltetrazolium chloride (TTC) staining.[31] The current findings observed that rats with CAL-induced MI had a significantly larger infarct area as visually apparent by its color as compared to control rats. While, treatment with quercetin significantly reduced infarct area compared to rats that received no treatment. The reduction in infarct area by quercetin treatment in this study is likely due to its antioxidant and anti-inflammatory properties. Quercetin has been shown to reduce oxidative stress and inflammation in the heart, which can contribute to the pathophysiology of MI.

Myocardial infarction (MI) is associated with alterations in several molecular pathways as well, including those related to oxidative stress, inflammation, and apoptosis.[32] As discussed earlier, Nrf2 is a transcription factor that plays a key role in the cellular response to oxidative stress. Several studies have demonstrated that Nrf2 activation can protect against CAL-induced MI in rat models by activating antioxidants.[33,34]

Consistent with data found in DOCA model, treatment with quercetin, a potent activator of Nrf2, improved cardiac function and reduced infarct area in rats with CAL-induced MI. This was associated with increased expression of HO-1, a downstream target of Nrf2, which has been shown to have anti-inflammatory and antioxidant effects. Furthermore, we also observed that rats treated with quercetin also displayed anti-oxidant effects by significantly increasing SOD and catalase activities and decreasing MDA levels compared to rats that received no treatment. These effects shown its protective effects by modulating Nrf2 in the heart.

Additionally studied the inflammatory genes such as, Nf-kb, transcription factor that plays a key role in the inflammatory response during injury.[35] It has been shown that excessive ROS production can lead to mitochondrial dysfunction and the release of pro-inflammatory molecules, which can trigger necrosis. Our study found that treatment with quercetin, a natural compound with anti-inflammatory effects, inhibited Nf-kb activation, which is known to promote apoptosis in myocardial tissue. Inflammatory cytokines such as IL-6 and TNF-alpha also play a role in CAL-induced MI, which can further exacerbate tissue damage and contribute to adverse cardiac remodelling.[36,37] The current pharmacological intervention with quercetin, a natural compound with anti-inflammatory effects, improved cardiac function in rats with CAL-induced MI. This was associated with decreased expression of IL-6 and TNF-alpha.

In response to oxidative stress, cells may activate signalling pathways that lead to apoptosis, thereby eliminating damaged or potentially harmful cells from the body. Therefore, role in apoptosis is also a key contributor to CAL-induced MI, a form of programmed cell death that plays a role in tissue remodelling and repair. The BCL2 family of proteins plays a critical role in regulating apoptosis.[38] An increased expression of BCL2 and decreased expression of BAX and caspase-3, a pro-apoptotic protein was displayed by quercetin

treatment suggesting improvement in cardiac function by protecting myocardial cells.

5. CONCLUSION

In conclusion, quercetin has been shown to have potential therapeutic effects in the treatment of cardiovascular diseases, specifically in the contexts of CAL-induced myocardial infarction.

In the CAL-induced myocardial infarction model, quercetin (50 mg/kg) was found to have cardioprotective effects by reducing infarct area, improving cardiac function, and decreasing apoptosis of cardiomyocytes. These effects were mediated by its antioxidant and anti-apoptotic properties, as well as modulation of various signalling pathways involved in inflammation and apoptosis.

Taken together, these findings suggest that quercetin may be a promising candidate for the treatment of hypertension and myocardial infarction. Specifically, quercetin has been shown to activate Nrf2 by promoting its nuclear translocation and enhancing its binding to AREs. However, further research is needed using effective dose of quercetin to fully elucidate this underlying mechanism and also need to explore the dark side of Nrf2 in the heart.

Overall, the results of this study provide important insights into the potential therapeutic benefits of quercetin in the context of cardiovascular diseases, and highlight the need for further investigation in this area.

Author Contributions

Methodology, Visualization, Writing – original draft, Data curation, Formal analysis, Writing – review & editing: all authors, *Conceptualization, supervision*: S.K, T.G.

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Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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