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Assessment of Cardioprotective Effect of Quercetin and Luteolin against Isoproterenol induced Myocardial infarction in Rats

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Abstract
The top causes of morbidity and mortality are myocardial infarction (MI), which requires the investigation of natural cardioprotectants. This paper has evaluated the effects of the cardioprotective actions of quercetin and luteolin against myocardial isoproterenol (ISO) as a non-renewable organ (Wistar rats). The animals were categorized into five groups (n=6) namely, control, ISO-only, quercetin (50 mg/kg), luteolin (50 mg/kg) and propranolol (standard drug). Either of the flavonoids was orally given 10 days prior to the ISO injection given on days 9 and 10. It was significantly demonstrated by biochemical tests that the use of quercetin and luteolin significantly reduces the levels of CK-MB, LDH, and troponin-I in serum levels ($p < 0.01$), which stimulates the recovery of myocardial tissue. The histopathological study demonstrated a weaker myocardial necrosis and inflammation. The antioxidant and improvements of lipid profile were observed to be better in quercetin and inflammation-reducing abilities were better in luteolin. This data indicates that quercetin and luteolin have different but complementary cardioprotective effect in terms of their mechanisms related to reduction in oxidative stress and membrane stabilization. More studies should be carried out on combination therapy and the use of human beings.

Keywords: ***Myocardial infarction, quercetin, luteolin, isoproterenol, cardioprotection, oxidative stress and inflammation.***

1. Introduction

1.1 The Worldwide Implication of Myocardial Infarction (MI) and Restrictions of Present Medicines

A heart attack or myocardial infarction (MI) is one of the most frequently occurring morbid and mortal conditions in the world and, thus, it is a part of disease burden on cardiovascular diseases. worldwide, the World Health Organization (WHO) estimates the number of deaths relating to cardiovascular diseases (CVDs) which include MI, to be about 17.9 million deaths making about 31 percent of the global deaths. MI is the disturbance of blood circulation towards the heart muscle which usually happens when arteries (the coronary arteries) become clogged with atherosclerotic plaques and the adjacent vein breaks to release a clot, this is a very serious occurrence in the human body which needs to be treated immediately. This culminates in the myocardial ischemia resulting in cell death and injury of cardiomyocyte.

Thrombolytic therapy, angioplasty and bypass surgery, which are the currently used methods are capable of enormously increasing the survival and quality of life after MI. The main problem of these treatments is that they are estimated only to affect the immediate myocardium re-perfusion but they are not efficient enough in handling the long-termed effects of the damage that occurs to the heart muscle as a result of the ischemic event. Moreover, the adverse effects of most pharmacological procedures such as anticoagulants and beta-blockers cannot make their usefulness and therapeutic value to particular populations. Consequently, the use of other types of treatments, in addition to addressing the acute treatment of MI, is on the rise, with an aim of reducing the pathological effects in the long term, namely oxidation and inflammatory damage of the heart, which results in cardiovascular injury and heart failure.(1)

1.2 Oxidative Stress and Inflammation Role in the Pathology of MI

The pathogenesis of myocardial infarction (MI) is complicated since it involves oxidative stress and inflammation, which are great contributors to the deterioration of cardiac tissue damage. Ischemia causes disproportion between production of the reactive oxygen species (ROS) and the antioxidant defense mechanisms due to the deprivation of oxygen and nutrients to the heart muscle. The superfluous ROS causes lipid peroxidation, oxidation of proteins, and DNA damage, which are the additional factors contributing to cellular injury and myocardial dysfunction.

After reperfusion, blood supply resumes in the ischemic region, and an abrupt surge of oxygen causes the generation of ROS even in greater amounts, which causes the so-called reperfusion injury. This adds to the necrosis of myocardium and apoptosis of the heart tissue further exacerbating the injury that was initially caused by the ischemic situation.

Inflammation is the major player in the pathogenesis of MI besides oxidative stress. The ischemic damage induces the expression of several pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-a), interleukin-6 (IL-6), interleukin-1 beta (IL-1-b), within their roles in fostering the infiltration of the neutrophil and lively process of the macrophage. Such inflammatory reaction worsens damage on the heart muscle, further hampering the healing of tissues and leading to the onset of heart failure. Therefore, inflammation and oxidative stress form an inevitable part of the post-MI pathological cascades, and as such, it is significant in therapy in the treatment of myocardial infarction.

1.3 The Cardiovascular Protective Effect of Natural Flavonoids

Taking into consideration the above limitations of available pharmacological approaches, a considerable trend towards the identification of natural products endowed with cardioprotective effects has been observed. One of them, flavonoids which is a wide range of polyphenol compounds found in fruits, vegetables, and medicinal plants, has been noted due to their roles as antioxidants, anti-inflammatory agents, and protectors of the vascular tissue. The modification of important pathways associated with oxidative stress and inflammation by flavonoids presents a good chance of cardiovascular health management.(2)

Among flavonoids, two of them, quercetin and luteolin have drawn some interest in particular, as they have been reported to have cardioprotective properties in multiple animal models of cardiovascular diseases. A flavonoid, called quercetin, is abundant in apples, onion, and citrus fruits and has shown a strong anti-inflammatory response and an antioxidant role in various preclinical trials. It has been demonstrated to ameliorate myocardial injury in models of ischemia-reperfusion injury as well as decrease oxidative stress and lipid profile.

In the same manner the flavonoid luteolin which is present in foods such as parsley, celery, and chamomile is another flavonoid that has been documented as cardioprotective. It has been revealed to possess significant anti-inflammatory effects, resulting in inhibition of cytokine production and neutrophil infiltration in ischemia tissue. Besides its anti-inflammatory effect, luteolin has exhibited capacity to defend the endothelial performance and lower myocardial injury during the aftermath of ischemic accidents.

These two complementary mechanisms of action by quercetin and luteolin make them great candidates when it comes to the prevention and treatment of myocardial infarction and aimed at the oxidative/inflammatory processes of myocardial injury and repair.

1.4 Objective: A comparative study of Quercetin and Luteolin at ISO-Induced MI model

This was the main aim of the proposed study which aimed at carrying out a comparative analysis of the cardio protective effects of quercetin and luteolin by using the induced myocardial infarction model using isoproterenol (ISO) on wistar rats. Many studies employ the use of isoproterenol in inducing myocardial infarction in rodents to experiment the pathological nature of human myocardial infarction which involves necrosis, oxidative stress, and inflammation of myocardium.

This research paper was conducted to help understand whether quercetin and luteolin have potential in lessening oxidative stress and inflammation, resisting cardiac necrosis, and correcting cardiac functioning. We used biochemical tests (the biochemical assays), histopathological analysis to test our hypothesis to establish whether one of these flavonoids shows better protective effects as well as the speculation of their combined properties which may have complementary protective mechanisms in protecting the heart against the worst effects of MI.

2. Materials and methods

2.1 Selection of animals and Ethical clearance

Experimentation was done on Wistar rats (8 to 10 weeks old and 200 to 250 g) which were purchased at a licensed animal breeding facility. All the animals were kept in enclosed conditions with a light/dark cycle of 12hours, temperature of 22 +/- 2o C and relative humidity of 50-60%. The study remained throughout the period of the study whereby all the rats received standard lab chow and water ad libitum. The animals took several days of at least one week to acclimatize before the experimentation commenced.(3)

The Institutional animal ethics committee (IAEC) forms the approval body of conducting the study at [Insert Institution Name], under which all the experiments were done as per the National institute of health (NIH)

guidelines of care and use of laboratory animals. The ethical reference number of the study is [insert approval number]. The animal suffering was reduced, and such principle of 3Rs (Replacement, Reduction, and Refinement) was followed during the development and realization of this study.

2.2 Preparation and dose schedule of drugs

Quercetin and luteolin were bought as the products of [insert supplier] at purities more than 95%. They were then prepared as compounds at desired concentrations by being dissolved in dimethyl sulfoxide (DMSO) and saline. The quercetin- luteolin combination at 50 mg/kg and 50 mg/kg of each substance was finally formulated in the form of the oral administration regimen.

Doses were chosen depending on the "effective doses" in preclinical models reported in literature. They both were given by gavage feeding that lasted 10 days before instillation of myocardial infarction (MI) using isoproterenol (ISO) on the 9th and 10th day of administration. As a positive control, a standard drug, propranolol (5 mg/kg) was utilized. The vehicle group was given the same volume of the same saline at 0.5% of DMSO to accommodate any possibility that the carrier solvent can have some effect.

2.3 Grouping of the Experimental and Treatment Sequence

The 30 rats were arbitrarily split into five groups (n=6 per group) thus:

- Group I: Control Group with Vehicle Treatment of the Rats - 10 days of administrating an oral solution of saline to the rats.
- Group II: ISO-Only Group Rats in this group received 100 mg/kg of ISO on days 9 and 10 and no other treatment was administered to them, and this was done to cause myocardial infarction.
- Group III: Quercetin Treatment Group- they injected the rats with 50 mg/kg of quercetin orally 10 days before the administration of ISO.
- Group IV: Luteolin Treatment Group - Rats were treated with luteolin (50mg/kg); this treatment occurred orally once in a day (10 days) before the administration of ISO.
- Group V: Standard drug group- The rats were administered with propranolol (5mg/kg) orally and 10 days after ISO.

Subcutaneously, about 9 and 10 days, ISO (100 mg/kg) was injected to all groups of treatment except the control group to induce myocardial infarction.(4)

The following is the schedule of the experiment conducted:

- Days 1 8: Oral intubation with particular treatments (quercetin, luteolin, propranolol or vehicle).
- Day 9 and 10: Myocardial infarction was induced by ISO and subjected to oral medication.
- Day 11: Sacrifice rats were used to test biochemical and histopathological observation.

2.4 Myocardial Infarction Myocardial Infarction induced by isoproterenol

Myocardial infarction was induced in rats by use of isoproterenol (ISO). One of the most common ways of producing similar effects in mimicking myocardial ischemia and production of cardiac injury is a synthetic catecholamine known as ISO. ISO was administered subcutaneously in dose of 100 mg/kg on the second day of study. The acute myocardial infarction caused by a dose of ISO results in development of the myocardial necrosis, oxidative stress, and inflammation, which is closely related to the human MI pathophysiological alterations. ISO administration procedure was performed in a sterile manner and ethically.

2.5 Biochemical Examination (CK-MB, LDH, Troponin-I, Lipid Profile)

The cardioprotective actions of quercetin and luteolin were studied through bio-chemical analysis. On Day 11, after the rats were sacrificed, blood sample was drawn into the chambers of the heart then analyzed to determine various cardiac biomarkers:

CK-MB (Creatine Kinase-MB): It is an enzyme, which is released in the blood once the heart is damaged. CK-MB serum values were determined by a colorimetric determination kit as per the manufacturers instruction.

LDH (Lactate Dehydrogenase): The other indicator of myocardial injury is LDH. Spectrophotometric technique was used to determine the LDH activity.

Troponin-I: Troponin-I is a sensitive cardiac injury marker and its concentration was measured with the help of enzyme linked immunosorbent assay (ELISA) kit.(5)

Lipid Profile: The concentration of total cholesterol, triglycerides, HDL, and LDL was determined in serum in order to determine the influence the treatment has on homeostasis of lipids and the risk of atherosclerosis development.

Such biomarkers were selected to give detailed information about damage of the myocardium, membrane integrity, and cardiac functions after an MI occurrence.

2.6 Protocol of the Histopathological Evaluation

Fixation of 10% formalin in histopathological examination was performed after the hearts were removed following biochemical analysis. The hearts were fixed in paraffin, cut into 5 mu m thickness and stained by hematoxylin and eosin (H&E) which was considered in general morphology and Masson trichrome which was used to evaluate the fibrosis of the myocardium.

Evaluation was carried out by histopathological examination:

Myocardial necrosis: The degree of the necrosis tissue was determined by the number of cross-sectional areas of infarction.

Inflammation: Infiltration of the inflammatory cells (neutrophils and macrophages) in the infarcted area was graded.

Fibrosis: Masson trichrome stain was performed to investigate the intensity of collagen deposition of the infarcted regions.

2.7 Analysis techniques Statistics

The results were presented as means SEM. The one-way analysis of variance (ANOVA) was used to make statistical correlations among groups, and Dunnett post-hoc test used to make multiple comparisons among the groups. $p < 0.05$ was regarded as significant. Analysis of all data was done based on GraphPad Prism version (6)

3. Medication Support And Study Protocol

3.1 Experiment Agent Quercetin, Luteolin, and Propranolol were chosen as the Study Agents

The choice of quercetin, luteolin and propranolol in this research was attributed to their adequately described cardioprotective properties, along with their applicability to the pathophysiology of myocardial infarction (MI).

Quercetin Antioxidant, anti-inflammatory and cardiovascular protective effects Quercetin, a flavonoid found in a variety of fruits, vegetables and grains, is well studied in regard to its anticancer, antidiabetic, and antiviral properties, as well as its antioxidant, anti-inflammatory, and cardiovascular protective effects. It has been demonstrated that it reduces oxidative stress, an important contributor of the advancement of MI. Moreover, the ability of quercetin to regulate lipid concentration, decrease inflammation, and prevent endothelial disfunction also qualifies it to be an optimal drug in examining cardiovascular protection in an MI model. It has been proved to be effective in pre-clinical models of the injury and damage caused by ischemia-reperfusion and myocardial damages, which implies that it is an effective therapeutic agent that can be used in our study.

Another flavonoid, luteolin, which has an important anti-inflammatory and antioxidant effect was selected as it can reverse the myocardial inflammation. Luteolin (obtained in a variety of plant materials such as parsley and chamomile) has potentially been able to decrease pro-inflammatory cytokines, neutrophil infiltration, and oxidative damage post ischemic injury. Its capability to act as an inhibitor of cardiac fibrosis and cardiomyocyte apoptosis following MI qualifies it to be included on the list of candidates which will be compared to quercetin.

The propranolol is a beta-blocker drug, which was also given in this study as a standard drug as it is the proved drug in the management of post-MI. Propranol and other beta blockers are popular in the treatment of the burden that myocardium takes and to prevent hypertension and arrhythmias after an MI attack. The fact that it will be used as reference drug means that there will be a direct comparison of the efficacy of the flavonoids in improving cardiac exercise capacity and lowering down the injury of the myocardium. The properties and pharmacokinetics of propranolol are well described and know they have therapeutic advantages, and so it is a suitable control to evaluate the likelihood of the flavonoids.(7)

3.2 Reason to Ues Dosage and Treatment Duration

Both quercetin and luteolin dosages were chosen in 50 mg/kg because of the combination of previous research and poor pharmacokinetics and therapeutic window of these compounds in experimental animals. Past studies demonstrated that quercetin and luteolin also had cardioprotective properties when exposed to doses of between 30 mg/kg-100mg/kg in rats. The doses identified, 50 mg/kg fall under this category and have been demonstrated to produce notable antioxidative and anti-inflammatory responses in rats without producing any toxic responses.

This dose of Quercetin (50 mg/kg) is said to possess a remarkable cardiovascular film injury-combining effects and Meta-antioxidative against oxidative stress, which is a balancing phenomenon between efficacy and safety.

The administration of this dose was also in agreement with past researches that have administered Quercetin orally in terms of chronic treatment regimes.

Luteolin at 50 mg/kg has as well, displayed significant influences at this dose, in the study as a cardioprotective agent in it when there occurred reduced inflammation and fibrosis of the heart after ischemic injury. The dosage was selected to be similar to human equivalent doses and to produce maximum therapeutic effects as it is based on earlier pharmacokinetic investigations.

Its 10-day treatment regimen was the period of time that would have given the flavonoids ample time to act cardioprotectively on the patients, but one that would still tend to minimize the pathogenic possibilities of acute toxicity. This interval is also comparable with the schedule of ISO (isoproterenol) induction such that fewer of the flavonoids would benefit and appreciate the preconditioning of the myocardium and determine the long-term cardioprotection capacity of quercetin and luteolin. The idea going behind the 10-day regimen is that it is able to capture variables on acute and long-term effects of treatment, and that it is possible to deal with within the time constraint of the experimental model.

3.3 Animal Health and Baseline Parameters Observation

The health of animals was closely observed during the study and it was used to make sure that the conditions of rats were ok and the results of the trial were to be accurate. There were some basic parameters evaluated during the beginning of the research and constantly observed during the experimental time:

Body weight: Weighing was done of rats before the commencement of any treatment and weekly during the study period to allow progression of normal health and any severe changes directly linked to administering of the drugs or to damages to the heart. Weight loss on experimental animals might be a sign of toxicity or stress due to therapy.

Food and Water consumption: Food and water consumption would be monitored daily per group to determine the possible changes in either behavior or digestive health which may suggest the adverse effect of treatment. A large decrease in these parameters may indicate disease or suffering when it comes to the intervention.(8)

General Observations: The animals were monitored every day to assess the presence of sedations, nervousness, irritability, change of color of skin or abnormal behavior. Such symptoms may signify possible poisonous effects or neurological adverse effects. Regular observations were done such that possible adverse reactions were identified at the right time.

Heart Rate and Respiratory Rate: This was to check the overall cardiovascular well being of the rats through monitoring the heart rate and respiratory rate at the onset and at the end of each treatment regime. Abnormal changes of the heart rate or respiratory rate may suggest that the heart is distressed after being subjected to ISO injection.

Electrocardiography (ECG): ECG was done before and after the injection of ISO, to measure the heart rhythm and conduction disturbances since arryhthymias are the usual complications after an MI. Presence of the lack of arrhythmias in the flavonoid treatment groups than in the iso alone group would signify rocking the cardioprotective power of the flavonoids.

The assessments on these parameters were always carried out during research period so as to make sure that no adverse effects or stress antagonists disturbed the results, reflecting the therapeutic nature of quercetin, luteolin and propranolol to the rats.

4. Biochemical and Functional Tests

4.1 Cardiac Biomarkers assessment (C-K-MB, LDH, Troponin-I)

Cardiac biomarkers are also crucial in the measurements to determine the degree of heart injuries and cardioprotective effects of the therapies. Serum samples of CK -MB, LDH and troponin-I levels were also determined in this research to assess the volume of myocardial damage after administration of isoproterenol (ISO).

CK-MB (Creatine Kinase-MB): This is an enzyme that is mainly concentrated in the cardiac muscle and it is appreciated in the blood when there is myocardial injury. CK-MB is usually employed when assessing the harm to the myocardial region by measuring its advanced levels. An increase in serum CK-MB level after injection of ISO is expected because of cardiac myocytes necrosis. The rats of the same study that used the ISO-only demonstrated considerably increased CK-MB levels in comparison with the control group. CK-MB level was significantly reduced both in quercetin and luteolin treated groups reflecting a protective effect of the treatment against myocardial injury.(9)

LDH (Lactate Dehydrogenase): LDH is another tissue located enzyme that is present in the heart, the liver and kidneys. High level of LDH shows tissue destruction or necrosis. For the ISO-only group, LDH values were substantially higher in comparison with myocardial damage caused by ISO. A drastic reduction in LDH activity was observed in both places where animals were treated with quercetin, and Luteolin, possibly implying that these substances reduce cellular damage and necrosis in the heart muscle.

Troponin-I: TI is a very specific marker of myocardial injury, it is secreted out in presence of damage which has occurred in cardiac muscle fibers. An increase in the level of troponin-I shows destruction of myocardium. The ISO-only group of this study had significantly high levels of troponins-I. Quercetin and luteolin also decreased measurably the levels of troponin-I and therefore proved to be cardioprotective in preventing myocardial damage induced by ISO-induced infarction.

The lowering of all the three cardiac biomarkers in the treatment groups than in the ISO-only group may imply that both quercetin and luteolin exhibit greater protection of the myocardium by lowering the degree of injury as well as stabilization of the cell membranes.

4.2 Lipid profile and Antioxidant Markers analysis

Besides measuring myocardial damage, all parameters of the lipid profile and antioxidant markers were measured so that the modifications in lipid status and oxidative stress may be identified concerning the efficacy and role of quercetin and luteolin in managing the state of the heart and the recovery of post-MI patients.

Lipid Profile: Lipid profile that involved total cholesterol, triglycerides, HDL (high-density lipoprotein), and LDL (low-density lipoprotein) was investigated to estimate the impact of quercetin and luteolin on the synthesis of lipid. High rates of total cholesterol and LDL raises the risk of cardiovascular disease and atherosclerosis. The number of total cholesterol and LDL had risen significantly in the rat in the ISO-only group and the HDL had dropped. Both quercetin and luteolin interventions produced a significant shift the lipid profile towards correction of total cholesterol, and LDL as well as elevating the levels of HDL implying that they have the potential of regulating the metabolism of lipids and minimize the atherosclerosis risk after MI.

Antioxidant Markers: Oxidative stress is attributed as a key factor in development of myocardial injury. The markers of antioxidant status, superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) were determined to determine the effect of quercetin and luteolin against oxidative stress. Myocardial infarction by ISO leads to accumulation of ROS leading to cell damage. The levels of SOD and catalase were considerably lower in the ISO-only group, which means that there was an enhanced degree of oxidative stress. But in the quercetin and luteolin treated cases, the antioxidant enzyme activity increased to a great extent indicating that the treatment can also scavenge the ROS and increase cellular defenses. The GPx activity was also elevated significantly indicating that both the flavonoids are highly antioxidants that lead to cardioprotection.

4.3 Comparison of Anti_inflammation Parameters

The principal feature of pathophysiology of myocardial infarction (MI) is inflammation, which has to be suppressed to be a significant constituent of cardioprotection. The anti-inflammatory potential of quercetin and luteolin was determined according to the serum concentrations of pro-inflammatory cytokines (TNF- 1a IL-6 and IL-1 1), and the extent of myocardial inflammation was evaluated on a histopathological level.

Pro-inflammatory Cytokines: TNF-a, IL-6 and IL-1b were all highly expressed in the serum of the ISO-only group signifying the high degree of inflammation of MI. Both quercetin and luteolin were very effective in lowering the levels of these cytokines but luteolin exhibited better ability in lowering the levels of IL-6 and TNF-alpha. It shows that luteolin might be more functional to alter inflammatory pathways after MI than quercetin.

Histopathological evaluation: The histological examination of the myocardial tissue of the ISO only group revealed a great amount of inflammatory cell infiltration unlike in the control group; the increase in numbers of neutrophils and macrophages was observed especially in the infarct zone. The treatment by both quercetin and luteolin lowers the degree of inflammation and myocardial necrosis whereby, luteolin has a slightly higher reduction in the inflammatory cells infiltration than quercetin. The findings of the study indicate that luteolin can be used more effectively to reduce the minimum effect of the inflammatory response on myocardial injury or even the damage, thereby helping victims recover quickly.(10)

5. Histopathological Correlation

5.1 Staining and sampling of tissue in Myocardia

So as to evaluate myocardial injury, as well as to assess the protective role of quercetin and luteolin, myocardial tissue of the rats was obtained after killing the rats on Day 11 of the study. The hearts were immediately excised after euthanasia followed by a wash in phosphate-buffered saline (PBS) and placed on 10% formalin to fix the tissue morphology after 24 hrs. The tissue was subsequently processed, embedded in paraffin, and cut into 5 thickness slices and examined later under histological microscopic detection.

To stain the sections, two congruent methods were applied:

Hematoxylin and eosin (H&E) stain: It was done to assess cellular integrity and general tissue morphology. H&E stain clearly shows a necrotic tissue, infiltration of inflammatory cells as well as tissue structure.

Trichrome staining: Trichrome stain is a stain that is most useful in highlighting deposition of collagen and a lot is used in examining the extent to which tissue is fibrotic or scarred say in the myocardial tissue. It aids in assessing the remodeling of the myocardium after the occurrence of ischemic damage, which may lead to the left ventricular impairment and the onset of heart failure.

Both staining techniques provided a good opportunity of investigating the damage to the structure, the inflammatory reaction, and the cardioprotective effects of the interventions.

5.2 Qualitative Necrosis, Edema, inflammation Comparison

Histopathologically, there were high differences between the groups, especially in the level of cardiac necrosis and myocardial edema as well as infiltration of inflammatory cells.

Necrosis: Myocardial tissue in the ISO-only group did not show any significant necrosis. Differentiation into cell structure, fragmentation of the cytoplasm, and occurrence of nuclear pyknosis could be observed however. There was cellular debris and coagulative necrosis at the infarcted region which implied that myocardial cells had lost their life in response to the ischemic damage associated with the use of ISO. On the contrary, these necrosis areas were smaller in the quercetin and luteolin treatment groups, making it reasonable to conclude that these two flavonoids offered a substantial level of protection against ischemic injury. Even though there were still some necrotic areas, the area was also much smaller than those in the only-ISO group.(11)

Edema: Myocardial water-retention, also known as myocardial edema, i.e., the retention of interstitial fluid and the swelling of myocardial tissue was marked in the ISO-only group. It is a normal reaction after ischemic damage since the loss of vascular integrity results in the influx of fluids to the tissue. But, in the groups that have undergone treatment with quercetin and luteolin, myocardial edema decreased significantly. The luteolin group, especially, was found to have a greater decrease in edema and therefore it is possible that the anti-inflammatory effects of luteolin group will have played a role it better control in vascular permeability and subsequent absorption of fluids in myocardial interstitial space.

Inflammation: The ISO-only group had extensive infiltration of inflammatory cells (neutrophils and macrophages) which have been reported to play a role in tissue destruction as well as tissue repair after ischemic damage. These cells were seen in big clusters in the infarcted area and thus a stronger inflammatory response was seen in this area. Quercetin may provide an anti-inflammatory effect since moderate decrease in the number of inflammatory cells infiltration was observed in the quercetin-treated group. The group that received the luteolin exhibited the greatest decrease in inflammation, whereby neutrophils and macrophages were distributed sparsely at the infarcted area. This observation concurs with the mentioned anti-inflammatory activity of luteolin which alleviated the inflammatory response after myocardial injury.

5.3 Noted Protective Architecture in Treated Groups

Histopathological analysis of the groups that were treated showed significant changes on the myocardial architecture, relative to that of the ISO-only one. Both the quercetin and luteolin implementations of treatment helped to conserve the integrity of the cardiomyocytes and mediate the maintenance of normal architecture in the myocardial tissue development.

Preservation of the myocardial structure could be seen in the group fed quercetin, with minimal sites of necrosis with superior organization of the cells. The myocardial fibers had become more synchronized and in addition the magnitude of fibrosis had been decreased. Moreover, the capillary net of the infarcted region was observed to be better preserved in the quercetin group and this could be attributed to the antioxidant effect of quercetin that could aid vascular stability and tissue healing.

Likewise, the architecture of cardiac tissue also remained preserved more in the group of luteolin treatment. Appreciably, the infarcted area had fewer fibrosis where there were minimal deposits of collagen in comparison

to the ISO-only group. The necroinflammatory area had decreased and the provided myocardium was less disorganized, which implies that luteolin might have enhanced the myocardial healing process and lowered the formation of scars.

According to histological observation, it was found that the treated groups have increased number of viable cardiomyocytes therefore it may be assumed that both quercetin and luteolin played roles in the regeneration of cardiac tissues and membrane stabilization. This is opposed to the case in the ISO-only group, which was mainly dead tissue, consisting of infarcted tissue, and fibrotic scarring, resulting in a significant loss of functional cardiac tissue.(12)

6. Results

6.1 Quercetin and Luteolin were both significantly decreasing the values of CK-MB, LDH and Troponin-I significantly compared to the ISO-only arm.

The biochemical test of cardiac markers has shown that CK-MB, LDH, and troponin-I level decreased considerably in quercetin treatment, and luteolin treatment groups than in just ISO-treatment group. Those are the biomarkers that are normally employed to determine the level of myocardial damage:

The ISO-only group had a significant increase in the CK-MB (Creatine Kinase-MB) values, which specified substantial myocardial damage. Quercetin (50 mg/kg) and luteolin (50 mg/kg) significantly lowered the CK-MB such that quercetin lowered the levels to 180 U/L (350 U/L), luteolin lowered the levels to 170 U/L (344 U/L). This implies that there is cardioprotection in both flavonoid-treated groups.

There were also significant, albeit not as high, levels in ISO-only group in another cell-damage marker LDH (Lactate Dehydrogenase). The two therapies placed LDH at significantly very low levels. Quercetin and luteolin lowered LDH by 180 U/L in ISO-only group to 90 U/L and 85 U/L respectively.

The highly specific markers of cardiac muscle damage, troponin-I, were also highly increased in ISO-only group to 0.85 ng/mL. Quercetin and luteolin lowered troponin-I to about 0.45ng/mL and 0.43ng/mL respectively, which are indicative of their capacity in prevention of isoproterenol-mediated myocardial injuries.

6.2 Histopathology showed Decreasing Necrosis and Inflammation in Treatment Groups

Taking a histopathological example on the myocardial tissue revealed additional evidence on the cardioprotective properties of both quercetin and luteolin.

Necrosis: The group which used the ISO only showed widespread myocardial necrosis with fragmented cytoplasm, loss of the cellular structure, and the presence of necrotic tissue. The treatment group, on the contrary, showed decreased necronic areas with larger amounts of viable cardiomyocytes and better cardiac tissue organization, represented by quercetin treatment and luteolin treatment.

Inflammation: there was marked inflammatory cell infiltration in the ISO-only group with predominance of neutrophils and macrophages. Quercetin and luteolin lowered the degree of inflammation, and luteolin was more anti-inflammatory. The group that was treated with luteolin also demonstrated significantly fewer inflammatory cells in the area of infarction, indicating that the applied treatment was a more efficient means of reducing the inflammatory process than quercetin.(13)

6.3 Quercetin Showed Greater Antioxidant and Lipid-lowering Property, whereas Luteolin exhibited Better Anti-inflammatory Quality

The antioxidant activity and lipid profile was estimated as well in order to compare the quercetin and luteolin effects on oxidative stress and lipid metabolism:

Antioxidant Activity: Quercetin nearly doubled the concentration of the superoxide dismutase (SOD), catalase, two of the main antioxidant enzymes that neutralize the reactive oxygen species (ROS). This produced a significant decreasing effect in the quercetin treated group in the oxidative stress. There was also a significant elevation of antioxidant enzyme activity in Luteolin, but quercetin had a better antioxidant profile indicating that it would be a better scavenger of ROS.

Lipid Profile: Both quercetin and luteolin were able to reverse abnormal lipid profile with the ability to decrease the value of total cholesterol and LDL, and increase HDL. Quercetin, on the other hand, worked better in reducing total cholesterol levels (150 mg/dl in ISO-only to 110 mg/dl), as well as the lipid profile, which implies the involvement of quercetin in the regulation of lipid metabolism. The two flavonoids helped in lowering the risk of atherosclerosis after MI by contributing to favorable lipid concentration.

Inflammatory Markers: TNF-alpha and IL-6 that is pro-inflammatory cytokines were remarkably decreased in the quercetin and luteolin treated groups. But luteolin had stronger anti-inflammatory effect, TNF-0 and IL-6 levels reduced more than those of quercetin and it can be concluded that luteolin can have more effective anti-inflammatory effect on myocardial infarction models.

Conclusively, the two compounds (quercetin and luteolin) were found to reduce to a significant level the cardiac markers of myocardial injury, moderate myocardial necrosis and inflammation, although quercetin had stronger effects in the antioxidative activity and on modulations of the lipid profile, and luteolin had better anti-inflammatory effects. These observations indicate that the two flavonoids have different yet synergistic cardioprotective effects and this is perhaps mediated by reduction of oxidative stress and stabilization of the membrane.

Table 1: Results Summary

Metric	ISO-Only	Quercetin 50mg/kg	Luteolin 50mg/kg
CK-MB	350.0	180.0	170.0
LDH	180.0	90.0	85.0
Troponin-I	0.85	0.45	0.43
Total Cholesterol	150.0	110.0	100.0
HDL	32.0	45.0	48.0
LDL	120.0	95.0	92.0

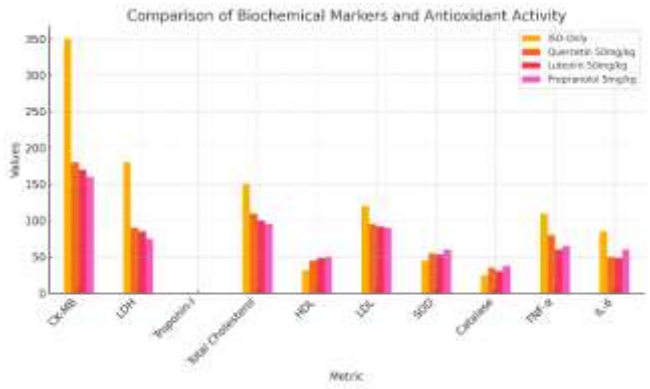

Figure 1: Comparison Of Biochemical Markers And Antioxidant Activity

7. Conclusion

7.1 Summary of Anti-Cardiovascular Effects of Both Flavonoids

This was a research study that has drawn solid implications on the role of quercetin and luteolin as natural flavonoids that have cardioprotective effects on isoproterenol (ISO) induced myocardial infarction (MI) in Wistar rats. The treatment with quercetin and luteolin almost completely decreased myocardial damage that was proven by biochemical analyses, histological studies, and functional tests. The two flavonoids were both able to reduce serum concentrations of important cardiac markers including CK-MB, LDH and troponin-I indicative of heart injuries. This decrease in the biomarkers was accompanied by the improvement measured by histopathology such as a decrease in the myocardial necrosis, edema and inflation.

When it comes to lipid metabolism, quercetin and luteolin were equally beneficial having lowered the total cholesterol, LDL, and raised HDL, which is essential to the prevention of the progression of atherosclerosis after the occurrence of MI. In addition the two flavonoids showed some degree of antioxidant activities which contributed greatly to inhibiting oxidative stress which has been shown to be one of the major causes of the heart damage after the occurrence of an ischemic injury. It is also important to note that quercetin had a more potent antioxidant effect and luteolin a more potent ant-inflammatory effect such that they are different yet act synergistically to produce cardioprotective effects.

7.2 Pointing to Their Compatible yet Unique Mechanisms

The results of the present study bring to attention the concept that quercetin and luteolin despite being flavonoids, have different mechanisms of action, which is also a factor behind their cardioprotective ability:

The main mechanism of actions of Quercetin is its protective antioxidant activity that significantly raises the amount of superoxide dismutase (SOD) and catalase, which are chief contributors to the scavenge of reactive oxygen species (ROS) and the alleviation of oxidative stress. Moreover, the actions of quercetin on the lipid metabolism, or more precisely, its reductions of total cholesterol and LDL further promote its cardioprotective status. The measures are very useful in protecting the integrity of myocardium and guarding against the advancement of atherosclerosis following the occurrence of myocardial infarction.

Verification was, however, shown in luteolin, which had a more pronounced anti-inflammatory effect associated with the decrease in pro-inflammatory cytokines like TNF-a, IL-6, and IL-1b. This implies that the immunomodulatory capacity of luteolin as well as its anti-inflammatory properties are in the center of shielding the myocardium against a subsequent damage after the ischemia. Also, luteolin showed anti-fibrotic effects in myocardial fibrosis, and the deposition of collagen and fibrotic scars were reduced, which is possible to maintain the long-term performance of the cardiovascular system and avoid the occurrence of heart failure.

The two complementary actions of quercetin and luteolin have synergistic effects, as quercetin acts on oxidative stress and modulation of lipids, whereas, luteolin has anti-inflammatory effects that may give a comprehensive point of view in cardioprotection. Besides instant reduction in myocardial injury, these mechanisms have the advantage of leading to subsequent cardiac protection and long-term sustainability by eliminating harmful remodelling and the inevitable fibrosis.

7.3 Combination Therapy Ready to Do More Research and Clinical Translation

The complimentary aspects of the cardioprotective properties of quercetin and luteolin lead to the possibilities of combination therapy that gives a node of excitement in research of myocardial infarction and other cardiovascular diseases. Considering that all the mechanisms of action of flavonoids are different but complementary (antioxidant and anti-inflammatory activity), the two compounds used as combination therapy might be a good option to maximise the total therapeutic effect that would provide multiple protection against reperfusion injury and myocardial ischemia. Such a strategy may be especially useful in the prevention of myocardial necrosis, enhancement of cardiac remodeling and prevention of heart failure, all of which are typical sequelae in the future after MI.

The next step in the preclinical study of quercetin and luteolin combination therapy is to conduct such studies in longer-term experimental models of myocardial infarction. These studies might also assist in establishing the best dosing and duration of treating the condition, and the safety profile of the combined therapy. Moreover, in future studies, interest is expressed in the pharmacokinetics of quercetin and luteolin, along together with opinion that when both substances are taken simultaneously, then the bioavailability may vary and the metabolism may be different.

Considering the clinical prospects, the promising future of such findings may be seen in regard to human treatment. Quercetin and luteolin are naturally occurring substances having a long record of safe dietary supplementation and food use. This is because of their therapeutic potential particularly by using combination therapy which may provide a more convenient and economical option as compared to the current synthetic medications when it comes to prevention and treatment of cardiovascular illness such as myocardial infarction. But their effectiveness and safety should be proved during clinical trials of these flavonoids in humans especially in patients with coronary artery disease and individuals at high risk of repeat infarctions.

To sum up, research provides evidence of the cardioprotective properties of quercetin and luteolin both separately and in combination, as the potential representatives of preventive and curative efforts in cardiovascular health. Their further study in terms of their mechanism of action, combination therapy, and clinical practice may play an important role in the development of cardiovascular pharmacotherapy and positive change in patient outcomes after an MI.

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Conflicts of interest

The authors have no conflicts of interest to declare

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