CARDIOPROTECTIVE EFFECTS OF BUNGA KANTAN (*Etlingera elatior*) IN HYPERCHOLESTEROLAEMIC MYOCARDIAL INFARCTION SPRAGUE DAWLEY RATS

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by

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DECLARATION

I hereby declare that this dissertation is the result of my investigations, except otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research, and promotional purposes.

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(SHARRAN A/L SELVAPAANDIAN)

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LIST OF SYMBOLS

º	Degree
ºC	Degree Celsius
÷	Divided by
=	Equals
g	Gram
mg/dL	Milligram per deciliter
mg/kg	Milligram per kilogram
mg/ml	Milligram per milliliter
mm	Millimeter
×	Multiplied by
ng/L	Nanogram per litre
%	Percentage
±	Plus/Minus

LIST OF ABBREVIATIONS

ASCVD	Atherosclerotic cardiovascular disease
BK	Bunga Kantan/ Kantan flower
BP	Blood pressure
CABG	Coronary Artery Bypass Grafting
COX	Cyclooxygenase
CVDs	Cardiovascular diseases
ECG	Electrocardiogram
EEAE	*Etlingera elatior* flower aqueous extract
FH	Familial Hypercholesterolaemia
HC	Hypercholesterolaemia
HCD	High Cholesterol Diet
HC-MI	Hypercholesterol-Myocardial Infarction
HDL	High-density lipoprotein
IHD	Ischaemic Heart Diseases
IP	Intraperitoneal
LDL	Low-density lipoprotein
LIMA	Left Internal Mammary Artery
MI	Myocardial Infarction
PCI	Percutaneous Coronary Intervention

SD	Sprague-Dawley
SVG	Saphenous Vein Grafts
Tn-T	Troponin-T
TXA2	Thromboxane A2
UAE	Ultrasonic-assisted extraction

KESAN KARDIOPROTEKTIF BUNGA KANTAN (Etlingera elatior) PADA TIKUS SPRAGUE DAWLEY HIPERKOLESTEROLEMIA INFARKSI MIOKARDIUM

ABSTRAK

Penyakit kardiovaskular (CVDs), terutamanya penginfarkan miokardium (MI), kekal sebagai cabaran kesihatan global dengan kadar kematian yang tinggi. Walaupun terdapat kemajuan dalam intervensi pembedahan dan farmakologi, prevalen MI terus meningkat, sering dikaitkan dengan faktor risiko seperti kolesterol tinggi. Terapi alternatif yang menggunakan herba semulajadi dengan sifat kardioprotektif menawarkan pendekatan yang berpotensi untuk menangani kekurangan ini. Kajian ini menyiasat potensi ekstrak akues *Etlingera elatior* (EEAE) sebagai rawatan pencegahan terhadap MI yang disebabkan oleh kolesterol tinggi. Kajian ini menggunakan diet tinggi kolesterol (HCD) yang menyebabkan hiperkolesterolemia dalam tikus Sprague-Dawley, diikuti dengan pemberian isoprenaline untuk mencetuskan MI. Tikus yang dirawat dengan EEAE pada dos 1000 mg/kg dinilai selama 12 minggu. Parameter seperti tekanan darah dan tahap kolesterol diambil. Analisis histopatologi dijalankan untuk memerhati penambahbaikan struktur pada miokardium. Hasil kajian menunjukkan bahawa EEAE tidak dapat menurunkan tahap kolesterol dan tekanan darah dengan signifikan, tetapi mampu meningkatkan mengekalkan integriti jantung. Kumpulan yang dirawat menunjukkan pemulihan histologi yang lebih baik berbanding kumpulan yang tidak dirawat. Penemuan ini mencadangkan bahawa EEAE berpotensi sebagai agen terapeutik semula jadi untuk mencegah MI dalam keadaan kolesterol tinggi. Kajian lanjut diperlukan untuk meneroka mekanisme dan aplikasinya dalam konteks klinikal

CARDIOPROTECTIVE EFFECTS OF BUNGA KANTAN (*Etlingera elatior*) IN HYPERCHOLESTEROLAEMIC MYOCARDIAL INFARCTION SPRAGUE DAWLEY RATS

ABSTRACT

Cardiovascular diseases (CVDs), especially myocardial infarction (MI), remain a global health challenge with high mortality rates. Despite advancements in surgical and pharmacological interventions, the prevalence of MI continues to rise, often linked to risk factors such as hypercholesterolaemia. Alternative therapies utilising natural compounds with cardioprotective properties offer a promising alternative for addressing these limitations. This study investigates the potential of *Etlingera elatior* aqueous extract (EEAE) as a preventive treatment against hypercholesterolaemia-induced MI. The research employed a high-cholesterol diet (HCD) to induce hypercholesterolaemia in male Sprague-Dawley rats, followed by isoprenaline administration to induce MI. Rats treated with EEAE at 1000 mg/kg were evaluated over 12 weeks. Parameters, including blood pressure and cholesterol levels, were taken. Histopathological analyses were conducted to observe structural improvements in the myocardium. Results demonstrated that EEAE had no significant effect in lowering cholesterol levels and blood pressure but showed capability in preserving cardiac integrity. EEAE-treated groups exhibited enhanced histological recovery compared to untreated controls. These findings suggest that EEAE holds potential as a natural therapeutic agent for preventing MI in hypercholesterolemic conditions. Further research is needed to explore its mechanisms and applicability in clinical settings.

CHAPTER ONE: INTRODUCTION

1.1 Research Background

Myocardial infarction (MI) is a type of ischaemic heart disease that causes a decrease or complete cessation of blood flow towards the myocardium of the heart. MI has a worldwide distribution and can either be unnoticed or lead to severe effects. These include a reduction in heart function or instant death. The major cause of MI is an underlying condition known as coronary heart disease. (Ojha, 2023)

Cardiovascular diseases (CVDs) are considered the leading cause of mortality globally, with an estimated total of 17.9 million deaths in the year 2019, which is considered as 32% of all global deaths. MI caused 85% of those deaths, with three-fourths of the deaths having occurred predominantly in low and middle-income countries (World Health Organization, 2021). Furthermore, in the year 2021, the total number of fatalities by CVDs has increased to 20.5 million (Cesare et al., 2024). According to Chong et al. (2024), it is predicted that there will be a 90.0 % rise in cardiovascular prevalence and a 73.4% increase in crude mortality between 2025 and 2050, with 35.6 million cardiovascular deaths predicted in that year.

Various risk factors have the potential to develop into CVDs; these include hypertension, smoking, diabetes, and even hypercholesterolaemia (Muthiah Vaduganathan et al., 2022). Hypercholesterolaemia, particularly high levels of low-density lipoprotein (LDL), significantly affects the development of CVDs (Jung et al., 2022). A study performed by Ference et al. (2017) stated that high levels of LDL are not just a risk factor but also a causal factor to Atherosclerotic Cardiovascular Disease (ASCVD) such as MI.

The current treatment towards MI includes surgical procedures such as coronary artery bypass grafting (CABG) with downsides such as leading to infections or prolonged

healing time, percutaneous coronary intervention (PCI), **w**hich can cause myocardial injury, and conventional medications such as antiplatelet therapy or thrombolytics which may lead to bleeding (Sachdeva et al., 2023).

One of the significant public health concerns is the ongoing rise in MI rates and worldwide disease burden. This shows that current medical interventions towards managing MI are still proving to be insufficient, and more efficient treatment needs to be researched not only to treat but also to prevent MI. In this research, *E. elatior* flower is used as a traditional remedy due to its wide variety of health benefits. These benefits include anti-oxidant properties (Jackie et al., 2011), anti-diabetic properties (Nor et al., 2020), anti-cancer properties (Ghasemzadeh et al., 2015), and anti-hypercholesterolaemic properties (Nor et al., 2020). Despite all the stated properties above, the research done for *E. elatior* flower is still insufficient. More studies need to be done to determine its efficacy and therapeutic potential in humans conclusively.

This research utilises Sprague-Dawley (SD) rats induced with Hypercholesterolaemia and MI (HC-MI) to investigate the potential cardioprotective effects of *E. elatior* flower. This research aims to provide a preliminary insight into *E. elatior* flower's potential as a preventive intervention for hypercholesterolaemic patients with MI.

1.2 Problem Statement

Despite numerous research and the introduction of new medical interventions, CVDs still have a significant disease burden, which is still increasing and is considered the leading cause of mortality and morbidity worldwide. In Malaysia, there was a total of 109,155 deaths were recorded in the year 2020, with 17.0% of those deaths being caused by MI (CVSKL, 2020). The current treatments for MI include a mixture of surgical intervention and even long-term medications such as CABG, PCI, antiplatelet therapy and so on. However, there are unwanted side effects to the patient, such as further injury to the heart, prolonged healing time or even excessive bleeding. In this study, We aim to adopt an alternative approach by utilising a natural remedy, specifically *E. elatior* flower, which offers a promising alternative or adjunt to preventing MI.

1.3 Rationale of Study

MI poses a significant global challenge, with mortality rates steadily rising despite advancements in medical interventions. Current medical advancements in treating MI include CABG, PCI, antiplatelet therapy, and even thrombolytic therapy. The gold standard for treating MI is PCI; however, it comes with disadvantages, such as coronary complications such as a slow or no flow of blood, abrupt vessel closure, and perforation of the blood vessels. Vascular complications like haematoma, may also occur at the site of catheter insertion (Mahilmaran, 2023).

According to a study by Noordin et al. (2022), the *E. elatior* flower contains cyanidin-3-O-glycosides, an anthocyanin. This compound plays an important role towards the antioxidant properties of the flower. In another study by Sapian et al. (2022), it was mentioned that anthocyanin also has anti-hypercholesterolaemic properties, and its ingestion has been shown to lead to improved cholesterol efflux, reducing levels of LDL, known as bad cholesterol and increasing levels of high-density lipoprotein (HDL), which is the good cholesterol. Besides this, *E. elatior* flower has been used as a traditional medicine to treat coughs, sprains, postpartum care and even earaches (Saudah et al., 2022). Given its reported properties, *E. elatior* flower shows potential for preventing MI. However, as no studies have evaluated its effectiveness in this context, this research aims to provide essential insights into its impact on MI.

1.4 Objectives

1.4.1 General Objective

To evaluate the cardioprotective effects of *E. elatior* flower aqueous extract (EEAE) in preventing myocardial infarction (MI) in hypercholesterolaemic (HC) Sprague Dawley rats.

1.4.2 Specific Objectives

I. To determine the nutritional contents of the self-prepared HCD
II. To evaluate the effects of EEAE on systolic blood pressure in HC.
III. To determine the effects of EEAE on cholesterol levels.
IV. To evaluate the histopathology improvements by EEAE on heart structures.
V. To determine the effect of EEAE on troponin-T levels.

1.5 Hypothesis

1.5.1 Null Hypothesis (H0)

There is no cardioprotective effect of EEAE towards preventing myocardial infarction in hypercholesterolaemic rats.

1.5.2 Alternative Hypothesis (HA)

There is a cardioprotective effect of EEAE towards preventing myocardial infarction in hypercholesterolaemic rats.

CHAPTER TWO: LITERATURE REVIEW

2.1 Hypercholesterolaemia

2.1.1 Overview of Hypercholesterolaemia

Hypercholesterolaemia has been established as one of the significant risk factors for CVDs (Malone et al., 2024; Paquette et al., 2021). Cholesterol is insoluble in water and must be transported in the presence of proteins. These proteins are known as lipoproteins, which play a crucial role in the transport of both cholesterol and lipids within the whole body (Feingold and Grunfeld, 2024).

Ahangari et al. (2018) reported that the global prevalence of hypercholesterolaemia is 39% among adults. In Malaysia, the prevalence of hypercholesterolaemia has fluctuating trends, with an increase from 35.1% in 2011 to 47.7% in 2015 and a decrease to 38.1% in 2019 (Ministry of Health Malaysia, 2020). While the current trend is currently improving, the prevalence of 38.1 is still high. More attention should be paid to hypercholesterolaemia, to reduce the disease burden further.

One type of lipid disorder includes hypercholesterolaemia, which is characterised by abnormal levels of cholesterol in the bloodstream. Specifically, there is an elevated level of low-density lipoprotein (LDL) cholesterol. Lipoprotein disorders are clinically important because of their contribution to atherogenesis and can lead to an increased risk of developing atherosclerotic cardiovascular disease (ASCVD) (Ibrahim et al., 2023). Atherogenesis refers to the formation of plaques within the intimal layer of arteries and it is agreed that this process can be exacerbated by the accumulation of LDL cholesterol (Wang et al., 2023). Hypercholesterolaemia can be defined as having an LDL cholesterol

level of more than 190 mg/dL, more than 160 mg/dL with one significant risk factor, or more than 130 mg/dL with two cardiovascular risk factors) (Ibrahim et al., 2023).

2.1.2 Causes of Hypercholesterolaemia

Hypercholesterolaemia can be divided into two major categories: primary and secondary hypercholesterolaemia. The latter category refers to a group of factors that can promote the development of hypercholesterolaemia, including dietary factors, a sedentary lifestyle, and even underlying health conditions. One of the more common causes is an unbalanced diet. LDL cholesterol is the major risk factor for CVDs (Schoeneck & Iggman, 2021). According to their study, foods such as eggs, coffee, and free sugars can increase LDL cholesterol levels in the body.

A sedentary lifestyle is another significant causal factor, defined as not meeting the existing guidelines for physical exercise. A study by Ford and Caspersen (2012) shows evidence that greater sedentary time has increased the risk of CVDs. It also mentioned that higher levels of physical activity, along with lower sedentary behaviour were strongly associated with higher levels of HDL cholesterol. In contrast, individuals leading a more sedentary lifestyle were reported to have a higher level of LDL cholesterol (Crichton & Ala'a Alkerwi, 2015).

Other risk factors such as smoking, diabetes, hypertension and even age-related factors may lead to hypercholesterolaemia. For age, the risk increases for males above 45 years old and females above 55 years old. Primary cholesterolemia, often termed familial hypercholesterolaemia (FH), refers to the genetic causes of a mutation in the gene which encodes for LDL-receptor. 85% of FH is due to a loss of function mutation in the low-density lipoprotein receptor (LDLR) gene. This mutation reduces the activity of the

receptor, which can further decrease the clearance rate of LDL cholesterol and eventually lead to elevated levels of LDL cholesterol in the circulation. Other causes of FH include defective apolipoprotein B, which reduces binding to the LDL receptor. Also, a gain-of-function mutation in the proprotein convertase subtilisin/kexin type 9 (PCSK9) gene where the serine protease PCSK9 will target the LDL-receptor for degradation, which ultimately increases the level of LDL cholesterol (Ibrahim et al, 2023).

2.1.3 Signs and Symptoms of Hypercholesterolaemia

There are many potential signs of hypercholesterolaemia, which can be divided into atherosclerotic complications and physical manifestations. Hypercholesterolaemia is one of the significant risk factors towards atherosclerosis. This condition occurs at the intimal layer of arteries and causes its thickening and fat accumulation, forming atherosclerotic plaque. This plaque acts as the pathological foundation towards causing ischaemic heart disease (IHD) (Wang et al., 2017). This leads to the narrowing of the arteries and reduces the blood flow. A lack of blood flow requires the heart to pump more blood throughout the body and causes hypertension (Mahmoud Rafieian-Kopaei et al., 2014). Hypertension can lead to cardiovascular diseases such as myocardial infarction and stroke. The symptoms are often seen as angina, shortness of breath, muscle weakness, nausea, and even dizziness (Lu et al., 2015).

The physical manifestations of hypercholesterolaemia are often found in individuals with FH, which has a genetic basis. These signs include xanthomas and corneal arcus. Xanthomas are lipid deposits that can occur anywhere in the body and organs; however, they are more common on the skin (Varghese, 2014). Xanthomas can be categorised as eruptive, tuberous and tendinous. Eruptive xanthomas occur rapidly in

groups of papules and are considered acute inflammatory lesions that can disappear after a certain period (Figure 2.1 (a)).

Tendinous xanthomas are lesions that gradually progress and can affect the extensor, elbows and Achilles tendons (Figure 2.1b). Tuberous xanthomas are also associated with hyperlipoproteinemia and appear red with inflamed papules that coalesce (Figure 2.1 (c)). These structures may occur around the elbows or palms and usually resolve after a few months. (Bell and Shreenath, 2020). The corneal arcus is another physical manifestation characterised as a lipid-rich extracellular deposit that forms around the cornea (Figure 2.1 (d)). It is present as incomplete white or grey rings. Its formation relies on the limbus, the junction between the sclera and cornea. Due to higher permeability, the arcus initially forms at the superior and inferior regions of the cornea (Munjal & Kaufman, 2023).

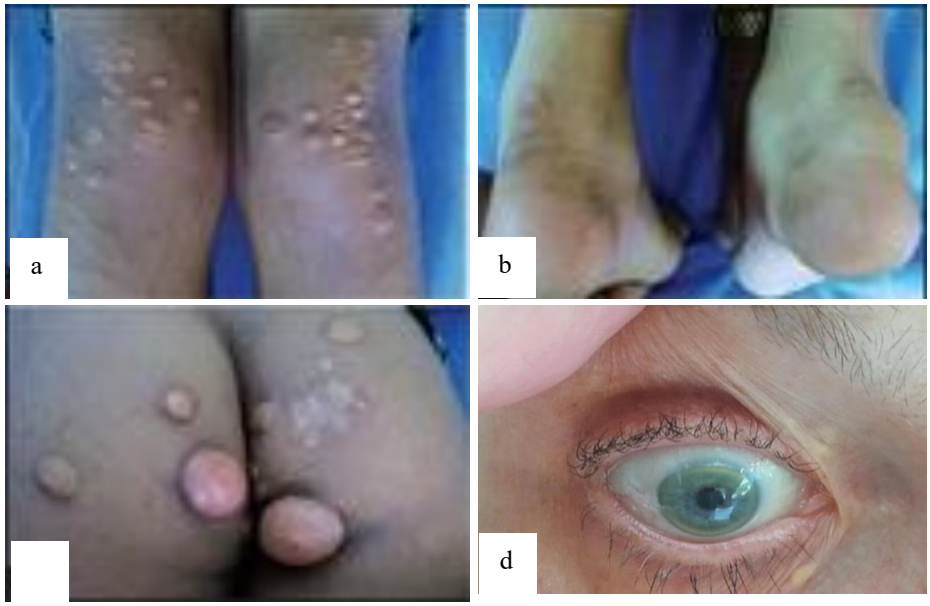

Figure 2.1: Physical manifestations of FH. (a) Eruptive xanthoma, (b) Tendinous xanthoma, (c) Tuberous xanthoma (Bell and P.Shreenath, 2020). (d) Corneal Arcus (Munjal & Kaufman, 2023).

2.2 Myocardial Infarction

2.2.1 Overview of Myocardial Infarction

Myocardial Infarction (MI) or the commonly used term “heart attack”, is defined as a decreased or cessation of oxygen delivery to a portion of the myocardium. This results in the death of the heart muscle cells due to ischemia. MI can go undetected in minor cases or may even lead to sudden death in severe cases, with the majority of MI caused by underlying coronary artery diseases (Ojha & Dhamoon, 2023).

MI is considered one of the leading causes of death globally. As of 2020, it was estimated that the prevalence of IHD, which includes MI, is approximately 244.1 million (Tsao et al., 2022). The global prevalence of IHD has been projected to reach approximately 510 million cases by the year 2050 (Shi et al., 2024). On the other hand, The global prevalence of MI is estimated to be around 3.8% for individuals below 60 and 9.5% for those above 60 (Salari et al., 2023).

According to WHO (2021), it was stated that in the year 2019, there were 17.9 million deaths attributed to CVDs, which represents 32% of total global deaths in the year. MI caused three-fourths of those deaths, which occurred mainly in low and middle-income countries. In the year 2021, the mortality of CVDs rose to 20.5 million deaths (Cesare et al., 2024). The global mortality of CVDs was predicted to rise to 35.6 million deaths in the year 2050 (Chong et al., 2024). In Malaysia, there were a total of 109,155 deaths reported in the year 2020, with 17.0% of the mortality attributed to MI (CVSKL, 2020).

MI can be categorised based on the electrocardiographic presentations: ST-segment elevation myocardial infarction (STEMI) and non-ST segment elevation myocardial infarction (NSTEMI). STEMI refers to a complete blockage of the coronary artery, whereas NSTEMI occurs as a partial blockage or occlusion of the coronary artery

(Kingma, 2018). Out of all MI cases, it is estimated that 70% of acute MI cases are due to blood vessel blockage with atherosclerotic plaque. Modifiable risk factors of MI are attributed to 90% of acute MI cases in men and 94% in women. These modifiable risk factors include a sedentary lifestyle, hypertension, obesity, cigarette smoking, and elevated LDL cholesterol levels. In contrast, age, sex and family history are a few examples of nonmodifiable risk factors for acute MI (Mechanic et al., 2023).

2.2.2 Pathophysiology of Myocardial Infarction

Coronary arteries are composed of endothelial cells, which form the lumen exposed to the bloodstream. Below this layer, the intima layer consists of collagen and glycosaminoglycans. This is followed by the media layer, which consists of smooth muscle cells (SMCs) (Ewelina Młynarska et al., 2024). MI may occur when a blockage within the coronary arteries lasts longer than 20 to 40 minutes. This blockage is usually thrombotic and is associated with an atherosclerotic plaque (Ojha & Dhamoon, 2023).

The formation of atherosclerosis indicates the narrowing and hardening of arteries and is the most common cause of MI (Ewelina Młynarska et al., 2024). This development consists of a few main stages and starts with endothelial dysfunction. This condition refers to the damage to the intima layer, which can be caused by oxidised cholesterol, components of cigarette smoke and even altered blood flow, which is usually caused by hypertension. This damage will lead to the permeation of circulating lipoprotein particles into the intima layer and leads to its trapping (Gimbrone & García-Cardeña, 2016).

Restoration of blood flow is essential in saving the ischemic myocardium, which is commonly done through percutaneous coronary intervention (PCI). At the same time, there will be an increase in reactive oxygen species, elevated inflammatory response, and

calcium overload, leading to oxidative stress. These factors contribute to further myocardial damage, which is classified as myocardial ischemia-reperfusion injury (MIRI) (Zhang et al., 2024).

The next phase is attributed to the lipid-streak stage, where LDL cholesterol builds up inside the artery's walls (Ewelina Młynarska et al., 2024). This build-up leads to macrophages infiltrating the intima layer, and the cells take up LDL cholesterol, facilitated by CD36 and scavenger receptors. The macrophages accumulate cholesterol and form foam cells (Lee-Rueckert et al., 2022).

This triggers the fibrous plaque phase, whereby vascular SMCs from the media layer migrate to the intima layer to form a fibrous cap surrounding the foam cells. The fibrous cap acts as a supporting structure to the plaque (Ewelina Młynarska et al., 2024). Foam cells will continue accumulating within the plaque, and their death can be due to macrophage apoptosis, secondary necrosis and impaired efferocytosis. The dying cells will release their intracellular contents, which play a role in the necrotic core formation (Gonzalez & Trigatti, 2016).

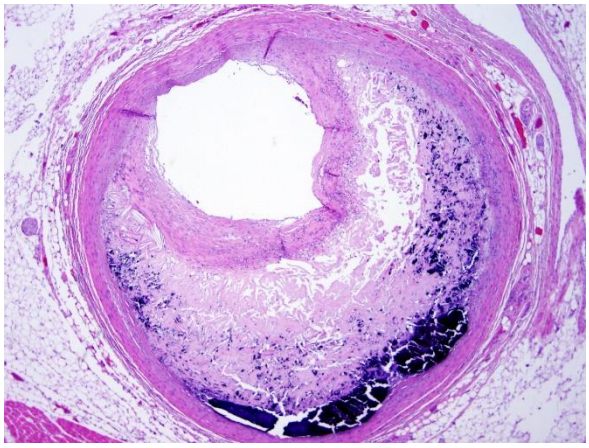

Figure 2.2: Coronary artery with atherosclerosis (Ospina-Romero and Schulte, 2022)

Eventually, the fibrous cap will weaken, and the necrotic core will expand, which occurs in the advanced lesion and thrombosis phase. Damage towards the atherosclerotic plaque will lead to its rupture and release of its inner content (Ewelina Młynarska et al., 2024). An inflammatory response encompassing monocytes and macrophages will be initiated once the rupture occurs, triggering the formation of a thrombus. There will be less oxygen delivery within the coronary arteries, leading to an inadequate or a cessation of blood flow to the myocardium. Inadequate oxygenation will trigger an ischemic cascade, and if ischemia takes place for more than 20 to 40 minutes, there will be apoptosis in the myocardial cells, leading to MI (Mechanic et al., 2023).

2.2.3 Diagnosis of Myocardial Infarction

There are several methods of diagnosing MI, and one of them is performing an electrocardiogram (ECG), which records the electrical activity of the heart. This procedure should be performed in patients presenting symptoms such as chest pains, shortness of breath, dizziness or heart palpitations. ECG is highly specific in diagnosing MI, with a specificity of 95% to 97%. However, it has a low sensitivity of approximately 30%. Diagnosing MI in ECG depends on the T-waves; a peak in the T-waves indicates ischemia, which can progress towards ST elevation; if the elevations are greater than 2mm, this indicates STEMI. However, not all patients with MI may present a diagnosis of ST elevation. If a patient exhibits symptoms of chest pain without any apparent ST elevation, this is usually indicative of NSTEMI.

Due to the limited sensitivity of ECG in diagnosing MI, cardiac biomarkers are commonly employed. These biomarkers include cardiac troponin I (cTnI), cardiac troponin T (cTnT) and creatine kinase-myocardial band (CK-MB) (Mechanic et al., 2023).

Cardiac markers, particularly cTnT, are proteins that regulate contraction in cardiac muscle fibres. Damage to the heart muscle, such as during MI, will release cTnT into the bloodstream (Starnberg et al., 2020). According to Thygesen et al. (2018), diagnosing MI must be done using the 99th percentile of the normal upper reference limit as a threshold. A normal cTnT is below the threshold of 14 ng/L, whereas values above this threshold were considered elevated cTnT values (Liu et al., 2023). During MI, the levels of troponin will peak on the 12th day, and this elevation may persist for up to one week (Mechanic et al, 2023).

2.2.4 Histological Features of Myocardial Infarction

In the histology of heart muscle cells (cardiomyocytes), they are mononucleated (Figure 2.3 (a)). However, binucleated and multinucleated cells have been reported. The nuclei of the cardiomyocytes are centrally located and the sarcoplasm has a high number of mitochondria. Cardiomyocytes are connected to the adjacent cell through intercalated discs containing desmosomes, gap junctions and fascia adherens. Figure 2.3 (b) shows the histological findings of MI; cell death due to ischemia is the first change observed in a cell after having MI (Ghafoor et al., 2020). This is followed by an influx of neutrophils observed 12 to 24 hours after MI. Between days 1 and 3, there is nuclei loss; at days 3 to 7, the necrotised cells are phagocytosed by macrophage. Fibrosis formation starts around 2 weeks, and a complete scar formation is completed within 2 months. Masson's Trichrome Staining (MTS) is usually employed to aid in visualising fibrosis. This stain identifies collagen fibres by staining it blue and differentiates normal tissue by staining red (Sridharan et al., 2022).

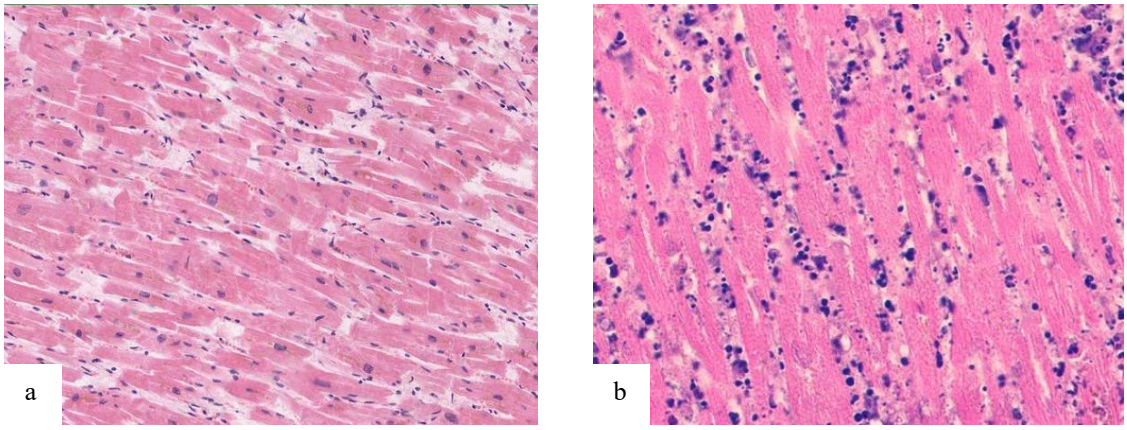

Figure 2.3: H&E staining of heart tissue under 40x magnification, A: normal heart tissue section (Crumbie, 2023), B: MI heart tissue section (Ghafoor et al., 2020).

2.2.5 Current Treatment and Prevention of Myocardial Infarction

There are several treatments along with specific prevention that can be performed towards MI, which include CABG, PCI, antiplatelet therapy and even the use of statin. CABG is a surgical procedure whereby blockages in a patient's coronary arteries are bypassed by harvested blood vessels from the patient's body. Usually, the left internal mammary artery (LIMA) and saphenous vein grafts (SVG) are taken. This restores blood flow to the myocardium and relieves symptoms such as angina. CABG has potential complications such as graft failure, renal failure, wound infection, stroke and even a chance of perioperative death (Bachar & Manna, 2023).

Another important surgical procedure is PCI, an invasive procedure used in treating the narrowing of the coronary arteries to restore blood supply to the ischemic myocardium. This procedure is typically performed by threading a catheter with a balloon attached to the blood vessels. The balloon, when inflated, will compress the surrounding atherosclerotic plaque to ensure the artery remains open. PCI includes complications such as coronary artery rupture, local or systemic infection, renal failure or even stroke, which can occur due to blood clots that will form during the procedure (Ahmad et al., 2023).

Another major complication of PCI is stent thrombosis, which may occur from incorrect stent positioning, fracture of the stent or even a hypercoagulable condition due to patient-related factors (Abubakar et al., 2023).

Antiplatelet therapy is another method of treating MI and can reduce the potential risks of thrombosis. The primary aim of this therapy is to reduce platelet activation and aggregation, which is pivotal in thrombus formation. One example is aspirin, an irreversible cyclooxygenase inhibitor (COX) (Abubakar et al., 2023). COX is an enzyme that plays an important role in producing thromboxane A2 (TXA2); TXA2 is a platelet activator that promotes platelet aggregation and vasoconstriction. Therefore, inhibiting the COX enzyme will prevent platelet aggregation (Bruno et al., 2023). The adverse effects of aspirin include gastrointestinal bleeding that may result in ulcers and haemorrhage, and these effects can be exacerbated when nonsteroidal anti-inflammatory medicines (NSAIDs) like ibuprofen are taken (Brazier, 2020).

Statins have also been utilised to prevent MI by reducing the overall cholesterol levels in the blood. Statin is a specific inhibitor towards hydroxymethylglutaryl-CoA reductase (Han et al., 2018). This enzyme acts as the rate-limiting step for the biosynthesis of cholesterol. By inhibiting this enzyme, LDL receptors will be upregulated, reducing the overall LDL cholesterol in the blood (Daniels & Couch, 2014). The majority of statins come with musculoskeletal adverse effects; here, myalgia is often seen, and myositis is reported periodically. Statins are also associated with an increased risk of diabetes mellitus. This is done by interrupting the insulin signalling pathways or may even contribute to insulin resistance (Ramkumar et al., 2016).

2.3 Animal Model

Animal models used in research can range from small sizes, such as rats and mice, to bigger sizes, such as monkeys, rabbits and horses. These models provide a preliminary understanding of our biology, physiology, and behaviour and assist in elucidating complex mechanisms of diseases, pharmacological responses, genetic studies, etc. Animal studies can control environmental factors and have precise experimental manipulations to accurately determine the factors an animal is exposed to and analyse its possible effects (Phillips & Roth, 2019).

Rodent models (Figure 2.4) are more commonly used for research on cardiovascular diseases due to their effective induction of human-related cardiovascular diseases, anatomical and physiological similarities, high reproductive capacity and cost-effectiveness (Jia et al., 2020). Both mice and rats have their pros and cons for research. The use of mice comes with the benefits of numerous genetically modified strains. This relates to their widespread use in studying the underlying mechanism of MI. Mice are also relatively inexpensive for maintenance, making them viable for large-scale research. However, their smaller size presents a challenge in surgical procedures as it is harder to determine the coronary arteries than larger rodents like rats (Lindsey et al., 2021).

On the other hand, rats are larger, making it easier to perform surgical procedures. Besides this, rats have been proven to be more similar anatomically to humans than mice (Porto et al., 2010; Kimitaka Kogure et al., 1999; Hryn VH et al., 2018). Rats also have a higher rate of survival, and according to a study done by Srikanth et al. (2009), they reported an 87% survival after inducing MI in 24 rats. For the rodent models, the left anterior descending (LAD) coronary artery ligation is the most common method of inducing MI. The procedure involves ligating the LAD coronary artery, which blocks

blood flow to a portion of the heart. This leads to a lack of oxygen and stimulates MI in rodents (Curaj et al., 2015).

Isoprenaline (ISO) is a sympathomimetic drug which acts on the beta-adrenergic receptor. This drug is used to increase heart rate for patients with severe bradycardia, a low heart rate, or even as a temporary treatment for heart blocks. ISO-induced MI in animals has been shown to produce abnormal morphology in the myocardial tissues of rats, which is similar to the morphology observed in human MI (Siddiqui et al., 2016). ISO is capable of increasing the intracellular Calcium ion (Ca^{2+}) through the usage of L-type Ca^{2+} channels. Calcium plays an important role as a signalling molecule in many reactions, such as in oxidative stress and necrosis, and an overload of calcium is known to lead to cardiac dysfunction (Ahmed et al., 2023). Besides this, ISO is capable of causing oxidative stress by increasing oxidative stress markers such as cardiac troponin-T (cTnT), creatine phosphokinase (CPK), creatine kinase-MB (CK-MB), and Lactate Dehydrogenase (LDH) whereas reduces the levels of superoxide dismutase (SOD) a type of antioxidant enzyme. Lastly, ISO is capable of damaging the cardiac cells by inducing hypoxia in the myocardium (Hosseini et al., 2023).

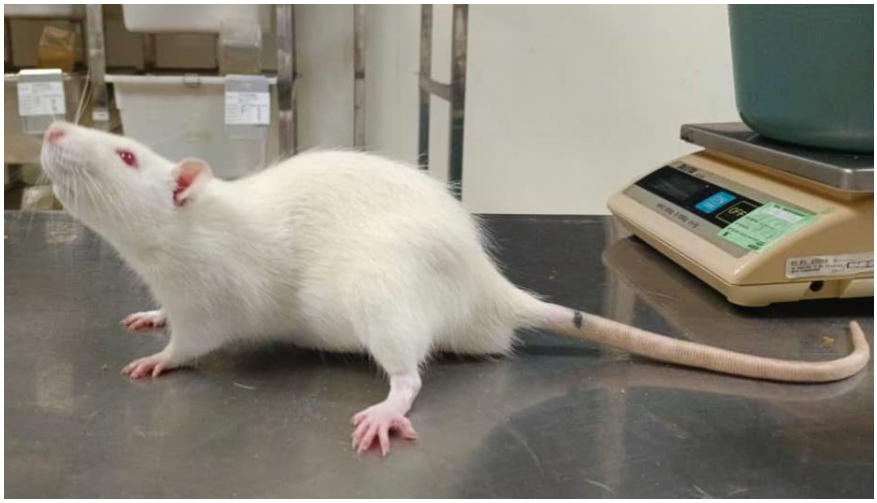

Figure 2.4: Sprague-Dawley rat used as an animal model

2.4 *Etlingera elatior*

2.4.1 Characteristics

Etlingera elatior (BK), also known as torch ginger or locally termed “Bunga Kantan”, is a flower grown for its decorative function and culinary applications. BK is commonly used in local Malay and Nyonya cuisine, such as “asam laksa”, “nasi kerabu”, “asam pedas”, or even “nyonya laksa” as a way to enrich their flavour (Julius, 2022). BK is native to Thailand, Malaysia, Indonesia and even the Philippines. In Malaysia, BK can be found in the Peninsular region, Sabah and Sarawak. In Indonesia, they are commonly found in Sumatra, Java and Kalimantan (Sanna & Botanic, 2018).

BK is a perennial herb that lives for up to 2 years and can grow up to two to six meters tall. The flower has closely grouped pseudostems, which are false stems formed by overlapping leaf sheaths. BK has an inflorescence of one to two meters, which arises from the base of the leafy shoot. Inflorescence refers to a group of flowers clustered on one branch. The flower contains a receptacle where the floral organs are attached and elongate for 10 cm. This structure can hold up to 320 flowers, however, only 10 to 20 may bloom at one time (Julius, 2022).

Figure 2.5: *Etlingera elatior* (BK)

2.4.3 Application in Traditional Medicine

BK has been utilised as a traditional medicine in many ethnic groups in Southeast Asia. According to a study done in the Aceh province of Indonesia (Saudah et al., 2022), 12 reported ethnic groups have knowledge of using different plants in traditional medicine, and one such flower includes BK. These tribes utilised the stems and fruits of various flowers in their concoctions of traditional medicines such as “pareng”, “lampok” or “oukup”. One example of the concoction, “pareng”, was made by macerating the young stems of the BK flower, which has functions such as preventing infections by cleaning wounds. The macerated stems, mixed with salt, are used for cough medicine, and a decoction of the young stems is produced to function as an antiseptic. Besides this, different parts of the flower have been demonstrated to have the ability to treat various ailments, according to Ismail & Ridzuan (2023). They reported that the pseudostem of BK could reduce swelling, cough and diarrhoea. The leaves were used for post-partum treatment, and cleaning wounds, the flowers for reducing bad breath and body odour, and lastly, the fruits were used to treat cough, heartburn, relieving fatigue and treating bruises.

2.4.4 Anti-Oxidant Properties of *Etlingera elatior*

It is well established that anthocyanin is an antioxidant found in BK (Lestari et al., 2018; Noordin et al., 2022; Ramasamy et al., 2016). Anthocyanin is a water-soluble pigment classified under the subgroup of secondary plant metabolites known as flavonoids (Bindhu Alappat and Jayaraj Alappat, 2020) (Figure 2.6). The consumption of flavonoids has been shown to produce a positive effect towards preventing damage from ROS. This follows the basis of the antioxidant mechanism whereby flavonoids are oxidised by free radicals, which form a more stable radical. The highly reactive hydroxyl groups of flavonoids, such as anthocyanin, can scavenge free radicals. This is capable of improving the overall antioxidant status within cardiomyocytes by preventing oxidative damage. (Khan et al., 2021). The neutralisation of ROS by antioxidants also contributes to preventing lipid oxidization. This is crucial because oxidised cholesterol plays a major role towards atherosclerosis formation (Kiokias et al, 2018)

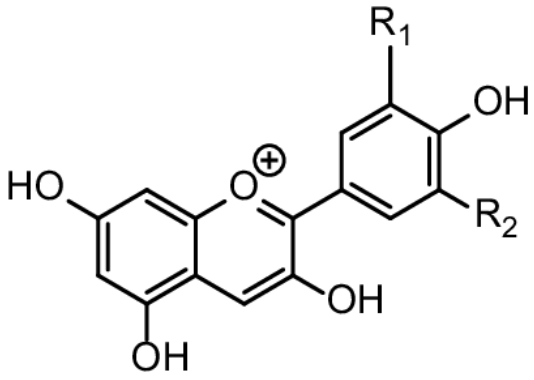

Figure 2.6: The basic structure of anthocyanin (Deepti Dabas, 2018)

2.4.4 Anti-Hypercholesterolaemic Properties of *Etlingera elatior*

Cholesterol is one of the major modifiable risk factors for MI, particularly LDL. According to Sapian et al, (2022), anthocyanin has the potential for anti-hypercholesterolaemic properties by improving cholesterol efflux. This improvement is capable of reducing the LDL levels while at the same time improving the HDL levels.

Besides this, Osadee et al. (2011) mentioned the presence of saponin in BK inflorescence based on a phytochemical analysis. Saponins are another group of secondary metabolites called glycosides, which consist of a hydrophobic aglycon that is linked to a hydrophilic sugar moiety; this nature gives saponin a surfactant property (Timilsena et al., 2023). Saponins in the body will cause the aglycon moiety to bind to steroids such as cholesterol to form an insoluble complex. Due to being insoluble, the absorption of cholesterol by the intestinal mucosal cells is reduced, leading to a further reduction in blood cholesterol levels (Osadee et al., 2011).

However, there is a lack of evidence that EEAE can improve overall cholesterol levels that correlate to damage in cardiomyocytes when given to hypercholesterolaemic individuals to prevent MI. Therefore, this study aims to evaluate the cardioprotective effect of EEAE in HC-MI-induced rats.

2.5 High Cholesterol Diet

Hypercholesterolaemia is one of the major modifiable risk factors of MI that has been studied in animal models. Feeding a high-cholesterol diet (HCD) to rats is one of the ways to mimic the MI pathology in humans to induce high cholesterol levels. This is a crucial component in atherosclerosis formation (Cunha et al., 2021). Hassan et al. (2023) mentioned that rodents are frequently used to create animal models of human diseases related to the induction of hypercholesterolaemia. Most studies utilised cholesterol in the diet to induce hypercholesterolaemia in rodents, and there are also cholesterol substitutes such as saturated fat and maize oil. These diets could raise total cholesterol levels, LDL, VLDL and even triglycerides. A prominent component of the diet was cholic acid, which induces hypercholesterolaemia.

According to Sahoo et al. (2021), cholic acid is a type of bile acid synthesised in the liver with a rich concentration in humans. Cholic acid plays a role in digesting foods by forming micelles with lipids and cholesterol to enhance their absorption further, thus leading to elevated serum cholesterol levels. The mixture of cholic acid and cholesterol is an effective method of inducing hypercholesterolaemia compared to substitutes such as oil and fat Hassan et al., (2023).

CHAPTER THREE: MATERIALS & METHODOLOGY

3.1 Study In General

Figure 3.1 summarises the experimental design that was used to evaluate the effects of *E. elatior* aqueous extract (EEAE) on hypercholesterolaemia-induced myocardial infarction (HC-MI) male Sprague-Dawley rats. The study involved three groups: a normal control group receiving a standard pellet diet, an untreated HC-MI group, and a treated HC-MI-EEAE group. The experiment began with an initial cholesterol measurement followed by a six-week high-cholesterol diet (HCD) induction phase to induce hypercholesterolaemia in the HC-MI groups. In the treatment phase, cholesterol measurement was initially performed on all groups on Week 6. All groups received biweekly blood pressure (BP) measurements. Furthermore, the treatment group received EEAE treatment at 1000 mg/kg during this phase. Following HCD induction, myocardial infarction was induced in all groups with isoprenaline (85 mg/kg subcutaneously). They were euthanised to obtain the heart organ, which was for histopathological analysis, and blood was collected for renal function tests. Data from all groups were statistically analysed to evaluate the efficacy of EEAE as a potential therapeutic agent.