EVALUATION OF *Hibiscus sabdariffa* L. CALYX AS CARDIOPROTECTIVE AGENTS

NUR AIN MOHD ASRI

UNIVERSITI SAINS MALAYSIA

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EVALUATION OF *Hibiscus sabdariffa* L. CALYX AS CARDIOPROTECTIVE AGENTS

by

NUR AIN MOHD ASRI

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LIST OF SYMBOLS

%	percent
°C	degree Celsius
>	greater than
<	less than
≥	greater than or equal to
≤	less than or equal to
µA	microampere
µL	microlitre
µm	micrometre
I.E.	International Units
g	gram
mg	milligram
mg/kg	milligram/kilogram
mm	millimetre
mmHg	millimetre mercury
mL	millilitre
mmol/L	millimoles per litre

LIST OF ABBREVIATIONS

AEHS	Aqueous Extract *Hibiscus sabdariffa* L. calyx
AMI	Acute Myocardial Infarction
ApoB	Apolipoprotein B
BMI	Body Mass Index
CAD	Coronary Artery Disease
CK-MB	Creatine Kinase Myocardial Band
CVD	Cardiovascular Disease
ECG	Electrocardiogram
HC	Hypercholesterolaemic
HCA	Hibiscus Acid
HCD	High-Cholesterol Diet
HFD	High-Fat Diet
HC-MI	Hypercholesterolaemic-Myocardial Infarction
HDL	High-Density Lipoprotein
HMG-CoA	3-Hydroxy-3-Methylglutaryl Coenzyme A
HMGCR	3-Hydroxy-3-Methylglutaryl-CoA Reductase
IDL	Intermediate-Density Lipoprotein
IP	Intraperitoneal
ISO	Isoprenaline
LCx	Left Circumflex Coronary Artery
LDL	Low-Density Lipoprotein
LDLR	Low-Density Lipoprotein Receptor
LFT	Liver Function Test

MetS	Metabolic Syndrome
MI	Myocardial Infarction
NAFLD	Non-Alcoholic Fatty Liver Disease
NHMS	National Health and Morbidity Survey
PPSK	Pusat Pengajian Sains Kesihatan
RFT	Renal Function Test
SBP	Systolic Blood Pressure
SC	Subcutaneous
SD	Sprague Dawley
STEMI	Short Term-Elevation Myocardial Infarction
TC	Total Cholesterol
USM	Universiti Sains Malaysia
VLDL	Very Low-Density Lipoprotein
WHO	World Health Organisation

PENILAIAN KALIS *Hibiscus sabdariffa* L. SEBAGAI AGEN PELINDUNG-KARDIO

ABSTRAK

Penyakit kardiovaskular (CVD), terutamanya infarksi miokardium (MI), adalah punca utama mortaliti dan morbiditi di seluruh dunia. Hiperkolesterolemia (HC) meningkatkan risiko mendapat CVD. *Hibiscus sabdariffa* L. (Roselle) telah digunakan secara tradisional untuk mengurangkan tahap kolesterol. Walau bagaimanapun, bukti tentang pelindung-kardio tumbuhan ini adalah sedikit. Kajian ini menilai kesan ekstrak akuas kaliks *H. sabdariffa* (AEHS) pada tikus hiperkolestrolemia-infarksi miokardium (HC-MI). Analisis proksimat dilakukan untuk menentukan komposisi nutrisi diet-kolesterol-tinggi buatan sendiri (HCD). Terdapat tiga fasa dalam kajian haiwan. Fasa pertama (fasa induksi) bertujuan untuk membangunkan model tikus HC menggunakan HCD selama 6 minggu. Fasa kedua (fasa intervensi) untuk menilai kesan pemberian AEHS selama 30 hari ke atas indeks jisim badan (BMI), paras gula darah berpuasa (FBS), kadar kolesterol berpuasa (FC) dan tekanan darah sistolik (SBP) pada tikus. Fasa ketiga di mana tikus diaruh infarksi miokardium oleh isoprenalin (HC-MI) pada hari ke-29 dan 30. Walaupun tahap kolesterol adalah normal dalam tikus HC-MI, terdapat perubahan lemak dalam hati (steatosis). HC-MI dapat diwujudkan berdasarkan tahap troponin T kardiak yang meningkat dan perubahan nekrotik pada tisu ventikel kiri. Walaubagaimanapun, AEHS tidak meningkatkan parameter-parameter secara ketara semasa fasa intervensi. Tambahan pula, AEHS tidak menurunkan tahap troponin T kardiak dengan ketara berbanding dengan HC-MI-tidak terawat, seiring dengan perubahan histolgi ventrikel kiri. Akan tetapi, ujian fungsi

renal (RFT) dan ujian fungsi hepar (LFT) adalah normal dalam HC-MI-terawat AEHS. Secara keseluruhan, kajian lanjutan diperlukan untuk menilai *H. sabdariffa* sebagai agen pelindung-kardio.

EVALUATION OF *Hibiscus sabdariffa* L. CALYX AS CARDIOPROTECTIVE AGENTS

ABSTRACT

Cardiovascular diseases (CVD), particularly Myocardial Infarction (MI), are the leading cause of mortality and morbidity worldwide. Hypercholesterolaemia increased the risk of developing CVD. *Hibiscus sabdariffa* L. (Roselle) has been used traditionally to reduce cholesterol levels. However, there is limited evidence of the plant's cardioprotective effects. The present study evaluated the effects of the aqueous extract of *H. sabdariffa* calyx (AEHS) on hypercholesterolaemia-myocardial infarction (HC-MI) rats. The proximate analysis was conducted to determine the nutritional composition of a self-made high-cholesterol diet (HCD). There are three phases in the animal study. The first phase (induction phase) was aim to develop an HC rat model using HCD for 6 weeks. The second phase (intervention phase) was to evaluate the effects of 30 days of AEHS treatment on body mass index (BMI), fasting blood sugar (FBS), fasting cholesterol (FC) and systolic blood pressure (SBP) in the rat. The third phase is where isoprenaline induced the myocardial infarction in rats (HC-MI) at day- 29 and 30. Although the cholesterol level was normal in HC-MI rats, there was a fatty change in the liver (steatosis). The HC-MI rat was also established based on raised cardiac troponin T level and the necrotic changes on the left ventricle tissue. However, AEHS did not significantly improve the parameters in the intervention phase. Moreover, AEHS did not reduce the cardiac troponin T significantly compared to the untreated-HC-MI, which is concurrent with the histology of the left ventricle. However, RFT and LFT were normal in AEHS-treated HC-MI.

Collectively, further studies are needed to elucidate *H. sabdariffa* as a cardioprotective agent.

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Cardiovascular diseases (CVD) are the leading cause of death and disability worldwide. Coronary heart disease, cerebrovascular illness, rheumatic heart disease, and other heart and blood vessel disorders are classified as CVD (Roth et al., 2020) and this includes ischemic heart disease (IHD). IHD is the leading cause of death in Malaysia, responsible for 15% of the 109,164 medically certified deaths in 2019 (DOSM, 2020). IHD was identified as the cause of myocardial infarction (MI), also known as a 'heart attack' (Dominguez, 2021; Roth et al., 2020). Among the key risk factors identified for CVDs are high blood sugar, high blood pressure and high cholesterol (NHMS, 2019).

Several prospective studies have found that plasma cholesterol concentration is strongly associated with the risk of cardiac morbidity and mortality (Girod et al., 1999). Generally, the cholesterol hypothesised in atherosclerosis development is based on early discoveries that cholesterol is a fundamental component of arterial plaques (Linton et al., 1950). Atherosclerosis is the substrate for MI, and it occurs at the location of pre-existing atherosclerotic lesions, frequently after local plaque disruption and occlusive thrombosis (Wu, 2019). The growing number of individuals suffering from MI around Malaysia emphasises the need for more effective prevention and treatment techniques.

The most effective measures for reducing disease burden remain health promotion and disease prevention. They allow for the prevention of diseases earlier when they are more likely to respond to therapy.

Hibiscus sabdariffa Linn, or, Roselle is a tropical plant commonly taken as a hot tea or juice due to its wide variety of nutraceutical advantages. A previous study has shown *H. sabdariffa* supplementation in rats significantly reduced total cholesterol (TC), low-density lipoprotein (LDL), and high-density lipoprotein (HDL), but not triglycerides (TG) (Barter, 2011). HDL has been shown to protect against atherosclerosis, and a reduction in HDL levels affects vascular protection (Barter, 2011). Interestingly, most animal experiments showed that *H. sabdariffa* or its extracts could not increase HDL or had no significant effect, instead of lowering it (Zhang et al., 2020). *H. sabdariffa* calyx is high in polyphenols and may reduce the risk of cardiovascular disease by working as an antioxidant (Budin et al., 2019). Previous research had shown that *H. sabdariffa* aqueous extract (AEHS) could improve cardiac function by reducing myocyte hypertrophy and cardiac fibrosis, as well as reducing oxidative stress (Si et al., 2017). Therefore, it merits further evaluate the potential of AEHS in the prevention and improving MI complications.

1.2 SCOPE OF STUDY

This study was conducted to evaluate the cardioprotective effects of Aqueous Extract *Hibiscus sabdariffa* L. calyx (AEHS) supplementation in hypercholesterolaemia-myocardial infarction (HC-MI) Sprague Dawley rats. This study has three phases: the hypercholesterolemia induction phase, treatment phase, and MI induction phase.

In this experiment, the *in-vivo* approach has been utilised in the HC-MI rat model. AEHS was prepared using the sonication method. The nutritional content of a self-made high cholesterol diet (HCD) used in hypercholesterolaemia induction were evaluated using proximate analysis standard methods, based on the Association of Official Analytical Chemists (AOAC).

The treatment phase was conducted for 4 weeks by oral gavage to assess the AEHS cardioprotective effects. Atorvastatin was used as a controlled drug. For the MI-induction phase, isoprenaline was administered by peritoneal injection in 2 single doses for 2 days. The parameters recorded include body weight, body mass index (BMI), fasting cholesterol (FC), fasting blood sugar (FBS), and systolic blood pressure (SBP). These parameters were measured every two weeks from the hypercholesterol induction phase until the MI-induction phase. At the end of the study, the cardiac Troponin enzyme was analysed by ELISA to confirm the occurrence of MI. In addition, kidney and liver function tests were also done to evaluate the organ function. Lastly, the heart tissue, specifically, left ventricle, were sorted out for histology study. In summary, this study was conducted to evaluate the cardioprotective effects of *H. sabdariffa* on MI and its complication on HC-MI rats.

1.3 PROBLEM STATEMENT AND RATIONALE OF STUDY

CVD, particularly MI, continues to be a major issue since it is the leading cause of mortality worldwide. According to WHO reports, both developed and developing nations are experiencing increased mortality (17.9 million) and cardiovascular disease-related disability (WHO, 2020).

CVD caused 98.9 deaths per 100,000 people in Malaysia in 2012, or 29,400 deaths, or 20.1% of all deaths (WHO, 2017). Even more so, from 2005 to 2014, CVD has remained the leading cause of death in Malaysia (DOSM, 2016). In comparison to their counterparts in other regions, Malaysians acquire heart disease at a younger age. For instance, Malaysians developed MI-specific CVDs at 58, compared to Thailand at 65, mainland China at 63, Western countries at 66, and Canada at 68 (Ahmad et al., 2018). Numerous significant efforts over the previous decades have been made to improve existing therapeutic alternatives. The surge in finding the therapies resulted from ineffective conventional therapy, causing the number of deaths by MI to keep rising.

IHD, especially acute MI, is linked to hypercholesterolaemia. For example, familial hypercholesterolaemia (FH) is one of the most prevalent major hereditary disorders of cholesterol metabolism, raising levels of LDL-C and increasing the risk of early atherosclerosis, including MI and coronary artery disease (CAD) (Onorato and Sturm, 2016). The global prevalence of FH is estimated to be between 1:200 and 1:500. Unfortunately, the incidence of early CAD is rising in Malaysia, with the average age of beginning of CAD being lower than in neighbouring nations and among the western population (Razman et al., 2019).

In both primary and secondary prevention, moderate-and high-intensity statin medication has been shown to reduce the risk of atherosclerotic cardiovascular disease (Adhyaru and Jacobson, 2018). Although current treatment methods, such as statins, are available to combat elevated cholesterol that contributes to MI, the choice of drugs or combinations of drugs cannot guarantee full efficacy because the treatment is

dependent on several factors, such as personal risk factors, age, health, and the possibility of adverse drug reactions.

Researchers' interest in nutraceutical goods, on the other hand, has expanded in the previous decades. This is consistent with the effort on evaluating *H. sabdariffa* as an alternative to alleviate MI complications. The research findings may cast doubt on bioactive *H. sabdariffa* compounds that have cholesterol-lowering properties. Since high cholesterol level is a major risk for MI, reducing cholesterol level will reduce or prevent MI complications.

1.4 RESEARCH QUESTION

Do AEHS will decrease cholesterol levels and reduce or prevent the development of MI in HC-MI induced Sprague Dawley rats?

1.5 OBJECTIVES OF STUDY

1.5.1 General Objective

To evaluate the cardioprotective effects and anti-hypercholesterol effects of AEHS in rats.

1.5.2 Specific Objectives

The specific objectives of this study are as below:

1. To evaluate the self-made high cholesterol diet (HCD) nutritional content
2. To determine the effects of HCD on BMI, FBS, FC, and SBP
3. To determine the effects of AEHS on BMI, FBS, FC and SBP in HC-MI rats

4. To evaluate the effects of AEHS on lipid profiles, renal and liver function tests in HC-MI rats
5. To determine the effects of AEHS on cardiac histology in HC-MI rats
6. To determine the effects of AEHS on cardiac enzyme, Troponin level in HC-MI rats

1.6 RESEARCH HYPOTHESES

The hypotheses of the study are as follows:

1. Self-made HCD will give constant BMI and high FBS, FC and SBP after the HC induction phase
2. AEHS will decrease BMI, FBS, FC and SBP in HC-MI rats
3. AEHS will show improvement in lipid profiles, and renal and kidney functions in HC-MI rats
4. AEHS will improve the morphology of the cardiac tissue in HC-MI rats
5. AEHS will decrease the cardiac enzyme, Troponin level in HC-MI rats

CHAPTER 2

LITERATURE REVIEW

2.1 CHOLESTEROL

2.1.1 Overview of Cholesterol

Cholesterol is a lipophilic substance required for human survival. It performs a variety of functions that help cells function appropriately. Cholesterol, for example, is an essential component of the cell membrane. It contributes to the membrane's structural makeup and regulates its fluidity (Huff et al., 2021). In addition, cholesterol is a precursor molecule for vitamin D and steroid hormone production (Huff et al., 2021). Cholesterol is also a component of bile salt, which aids in the absorption of fat-soluble vitamins A, D, E, and K during digestion (Ciaula et al., 2017). As cholesterol is primarily lipophilic, it is carried through the bloodstream inside lipoprotein particles, along with triglycerides (Huff et al., 2021). In a clinical setting, these lipoproteins can be detected to estimate cholesterol in the blood.

Lipoproteins have a lipid core (which can contain cholesterol esters and triglycerides) and a hydrophilic outer membrane (which includes phospholipid, apolipoprotein, and free cholesterol) (Wang et al., 2017). This structure allows lipid molecules to travel throughout the body through the bloodstream and be delivered to cells that require them. High-density lipoproteins (HDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and very-low-density lipoproteins (VLDL) are the different types of lipoproteins (Huff et al., 2021).

LDL particles, in particular, are assumed to be a primary cholesterol transporter, with at least two-thirds of circulating cholesterol residing in LDL and being transported to peripheral tissues (Wang et al., 2017). HDL molecules, on the other hand, are hypothesised to have the opposite effect. They remove excess cholesterol from the body and return it to the liver, which is excreted (Wang et al., 2017). These two lipoproteins are clinically important because high LDL and low HDL enhance the risk of atherosclerotic vascular disease in individuals (Sacks et al., 2017).

2.1.2 Functions of Cholesterol

Cholesterol plays various biological roles in membranes and lipid metabolism, including cell signalling, morphogenesis, lipid digestion and absorption in the intestines, reproduction, stress reactions, sodium and water balance, and calcium and phosphorus metabolism (William, 2021).

One of the cholesterol's key functions is to regulate membrane fluidity by interacting with the membrane's complex lipid components, particularly phospholipids like phosphatidylcholine and sphingomyelin (William, 2021). This fluidity can affect the ability of some tiny molecules to permeate through the membrane, changing the cell's internal environment (Dotson et al., 2017). Cholesterol's role is to improve membrane order (cohesion and packing), resulting in a liquid-ordered phase (William, 2021). Cholesterol is also found in oestrogen, testosterone, and adrenal hormones (Moon et al., 2016). Moreover, vitamin D, cortisol, aldosterone, progesterone, oestrogen, testosterone, and bile salts, among other hormones, all require cholesterol

as a precursor molecule (Javitt, 1994). Cholesterol controls several pathways that are important for brain health (Ghulam et al., 2019).

In the same way, it is crucial for nerve regeneration. Although cholesterol and its metabolites have a positive impact on neuron regeneration, it is also important to highlight that metabolic dysregulation of cholesterol is thought to be a cause of several major brain illnesses, as seen in Figure 2.1 (Ghulam et al., 2019).

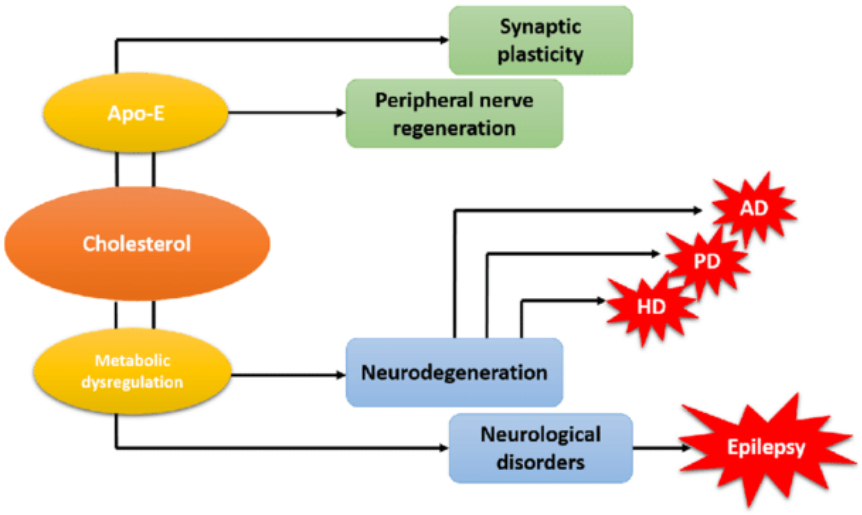

Figure 2.1 Cholesterol's role in brain health and disease. AD: Alzheimer's disease, PD: Parkinson's disease, HD: Huntington's disease

2.2 HYPERCHOLESTEROLAEMIA

2.2.1 Overview of Hypercholesterolaemia

While cholesterol is necessary for many important cell processes, it may also be harmful to the body if it reaches dangerously high blood levels. Specifically, when LDL cholesterol levels are very high, a condition known as hypercholesterolemia, increases the risk of early atherosclerotic cardiovascular disease (ASCVD) (Rosenblit, 2019). Doctors can order a lipid panel (lipid profile) to determine the cholesterol levels

in a patient's blood. The concentrations of HDL, LDL, triglycerides, and total cholesterol are all included in a typical test result (Huff et al., 2021).

Hypercholesterolemia (high LDL cholesterol) is one of the key risk factors for atherosclerotic plaque formation. These plaques raise the risk of a variety of poor clinical outcomes, including coronary artery disease. In epidemiological studies, an increased HDL blood concentration has been linked to lower risk, while clinical trials using HDL-cholesterol-raising medicines have given adverse outcomes (Huff et al., 2021). As a result, one of the main goals of patient management is to lower LDL levels. Hypercholesterolaemia can be caused by both inherited and acquired factors (Ibrahim et al., 2021). Familial hypercholesterolaemia is a hereditary condition caused by mutations in the LDL-receptor gene, which results in LDL-C levels of more than 190 mg/dl (10.6 mmol/L) in heterozygotes and more than 450 mg/dl (25.0 mmol/L) in homozygotes (Dainis and Ashley, 2018). A malfunction in the LDL receptor causes at least 85% of familial hypercholesterolaemia. Loss-of-function mutations in the gene encoding the LDL receptor cause familial hypercholesterolaemia. Due to a decrease in LDL receptor activation in the liver, the rate of clearance of LDL from the circulation is slowed (Mytilinaiou et al., 2018).

The increased LDL particles are thought to permeate the vascular intima and become caught by proteoglycans in the intima. LDL is oxidatively changed in the intima, promoting inflammation and the development of fatty streaks. Atherogenesis progresses from a fibrous plaque to a mature lesion, culminating in plaque rupture and a cardiovascular event (Ibrahim et al., 2021). Subclinical atherosclerosis includes calcified, inflammatory, and unstable atheromatous plaques, as well as first reactions

to risk exposure, particularly endothelial dysfunction (Figure 2.2) (Macedo and Faerstein, 2017). Endothelial injury is the first step in the formation of atherosclerotic plaques. Endothelial damage causes endothelial cell dysfunction, which increases the number of LDL particles that can pass through the arterial wall (Huff et al., 2021).

Lipoproteins, particularly LDL, are deposited within the vessel wall, confined in the intima by the cellular matrix. LDL is then modified and taken up by macrophages via scavenger receptors, resulting in the production of foam cells. Smooth muscle cells migrate into the lesion when more lipid accumulates within the artery wall. These smooth muscle cells eventually encase the newly formed plaque, forming the fibrous plaque, keeping the lipid core exposed to the artery lumen (Huff et al., 2021). Atherosclerotic plaques can lead to occlusion of the vessel that decrease blood flow distally and causing ischemia. The development of plaques are mainly due to abundant lipid and macrophages (vulnerable plaque) rupture, inducing the formation of a thrombus that can completely block the flow of blood. Thus, hypercholesterolemia acts as a pre-conditioning factor for atherosclerosis, leading to infarction.On the other hand, the strength of the link between hypercholesterolemia and acute myocardial infarction should be weaker than the link between calcified atheromatous plaque and infarction (Macedo and Faerstein, 2017).

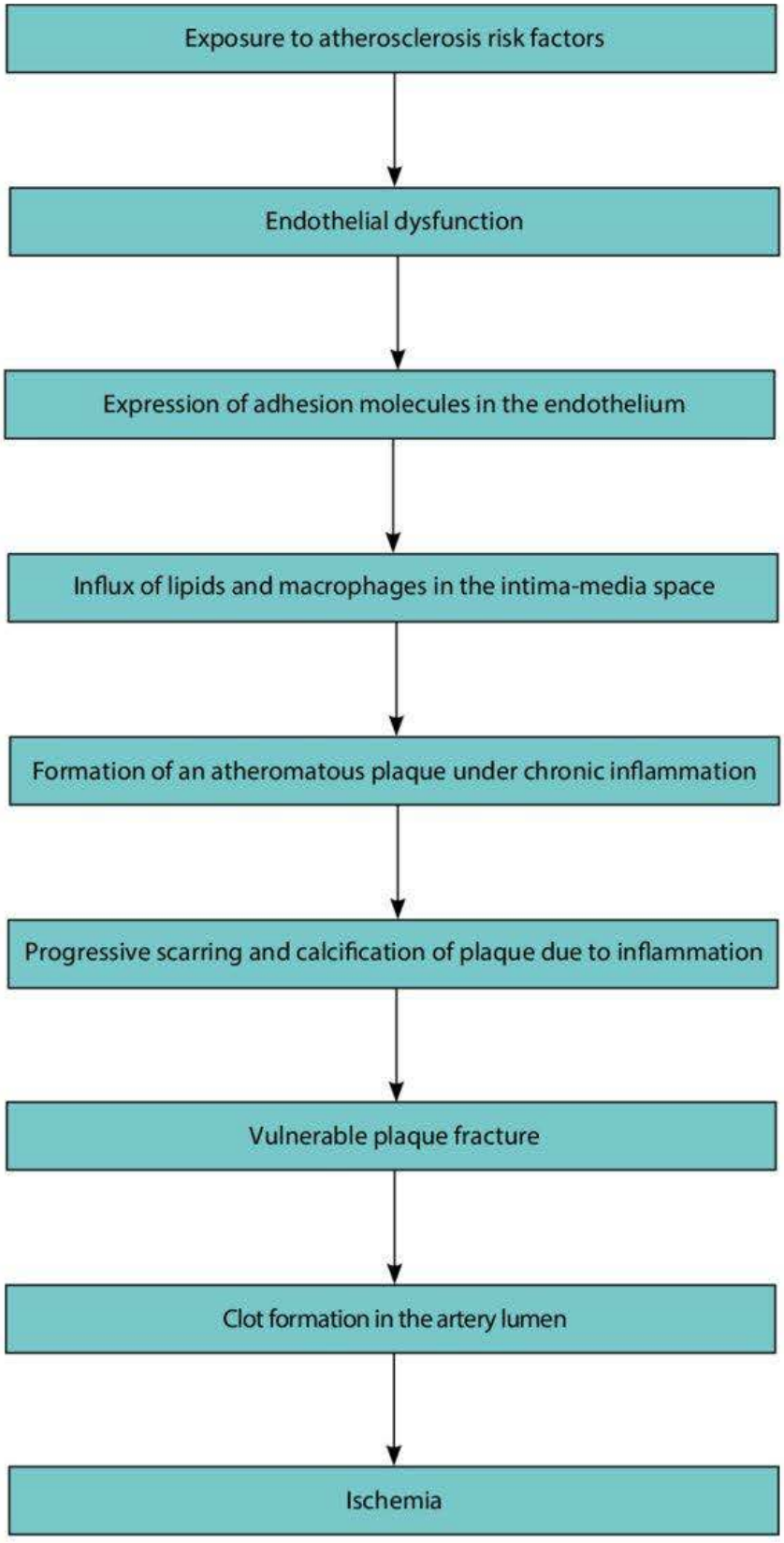

Figure 2.2 The sequence of events in the pathogenesis of atherosclerotic cardiovascular disease (Macedo and Faerstein, 2017).

2.2.2 Clinical Significance of Hypercholesterolaemia

Hypercholesterolaemia is a condition in which a patient's blood LDL cholesterol levels are abnormally high. High LDL cholesterol is of particular clinical concern; however, hypercholesterolaemia can also contain VLDL and IDL cholesterol, which are non-HDL cholesterol (Huff et al., 2021). The absent pulses, bruits, arcus senilis (younger than 50 years old), tendon xanthoma, xanthelasma, and aortic stenosis on clinical examination are all signs of very high cholesterol levels, as seen in familial hypercholesterolaemia (Huff et al., 2021). Genes that regulate LDL receptors in the liver are among the genes that cause elevated LDL levels in the blood. Thus, patients with a hereditary predisposition to high cholesterol can be prescribed statins or other cholesterol-lowering drugs to reduce their risk (Lloyd-Jones et al., 2016).

2.3 MYOCARDIAL INFARCTION (MI)

2.3.1 Overview of MI

Acute myocardial infarction (AMI), often known as a heart attack, is caused by a reduction or complete cessation of blood supply to a segment of the heart, resulting in heart muscle necrosis (Saleh and Ambrose, 2018). A blood clot in the epicardial artery, which supplies that area of the heart muscle, is usually the cause. It is now widely acknowledged that, depending on how AMI is defined, not all occurrences of AMI require a blood clot (Saleh and Ambrose, 2018). The blood supply must fulfil the oxygen demands of every living tissue, including heart muscle, known as the supply-demand ratio. Thus, an imbalance in this ratio, such as that caused by a fast heart rate or a drop in blood pressure, can cause myocardial injury without the presence of a blood clot.

MI causes irreparable damage to the heart muscle due to a shortage of oxygen. MI can decrease diastolic and systolic function, making the patient vulnerable to arrhythmias (Mechanic et al., 2021). Furthermore, MI can result in a variety of significant consequences. For a better prognosis, the treatment should start early. The aim is to restore blood flow and reperfuse the heart. Acute MI still has a significant fatality rate, with most deaths occurring before the patient arrives at the hospital. Within the first 12 months after a MI, at least 5% to 10% of survivors die, and 50% require hospitalisation within the same year. The prognosis is determined by the level of muscle injury (Mechanic et al., 2021).

2.3.2 Pathophysiology of MI

Atherosclerotic rupture causes a monocyte and macrophage inflammatory cascade, thrombus development, and platelet aggregation (Mechanic et al., 2021). Low-grade inflammation, as indicated by an elevated C-reactive protein level, has also been found to contribute to cardiac events, regardless of LDL levels, and is thus thought to contribute to the formation and progression of atherosclerotic disease (Bohula et al., 2018). As a result, oxygen transport through the coronary artery is reduced, resulting in diminished myocardial oxygenation. The inability of the mitochondria to make ATP triggers the ischaemia cascade, which results in endocardial apoptosis (cell death) or MI (Mechanic et al., 2021). Coronary arteries have distinct and diagnostic territorial distributions, with significant exceptions due to genetic variation. The interventricular septum, anterolateral wall, and ventricular apex, for example, are all supplied by the left anterior descending coronary artery.

The right coronary artery supplies oxygen and nutrients to the right ventricle. The left circumflex or right coronary artery supplies the inferior wall (Heusch and Gersh, 2017). Therefore, the pathogenesis of MI includes the various process from endothelial injury to AMI (Figure 2.3) (Sabesan and Narasimhan, 2015).

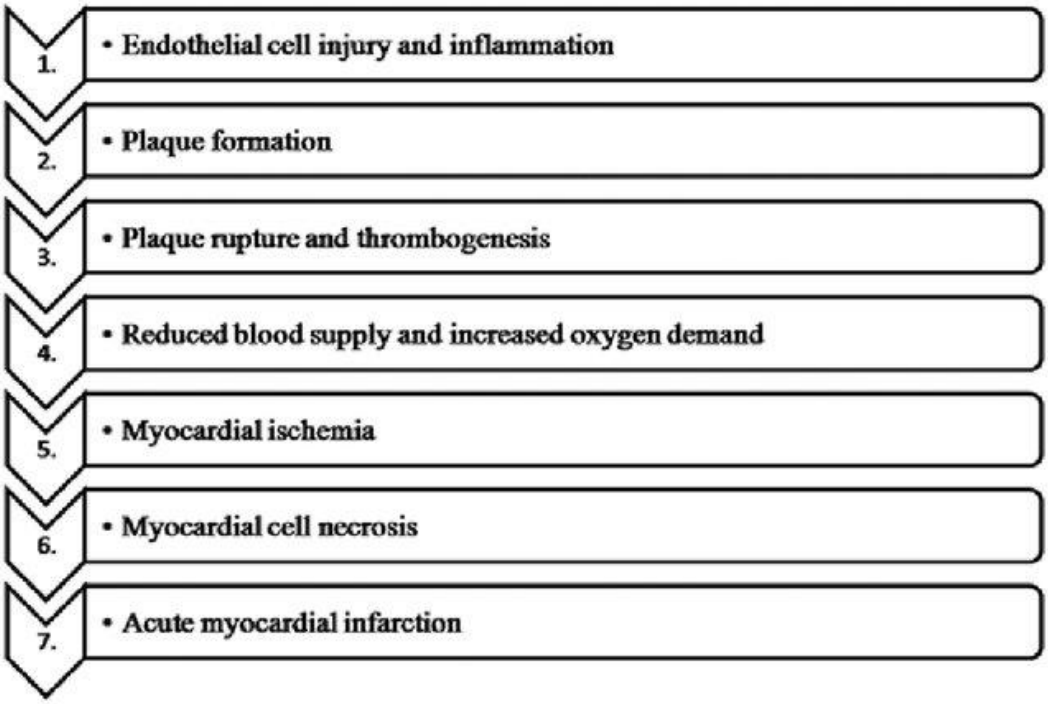

Figure 2.3 A diagram outlining the pathogenesis of a myocardial infarction (Sabesan and Narasimhan, 2015).

2.3.3 Toxicokinetics and Histopathology of MI

Acute myocardial infarction (AMI), particularly non-ST-elevation MI (non-STEMI), can be diagnosed using cardiac biomarkers. The ideal markers for myocardial damage are cardiac troponins T and I, which offer the highest sensitivity and specificity for the diagnosis of AMI. Troponin contains two isoforms, I and T, and is the most specific lab test (Mechanic et al., 2021). Troponins reach their peak after 12 hours and last for

seven days (Wu, 2019). Creatinine kinase-MB peaks at ten hours in MI, but it returns to normal in two to three days (Mechanic et al., 2021). MB has limited specificity for the myocardium, hence not employed clinically anymore. Moreover, lactate dehydrogenase (LDH) rises after 72 hours to normal after 10 to 14 hours.

After extensive research and use in Europe, high-sensitivity troponin was recently licenced for use in the United States (Alaour et al., 2018). It is more sensitive than standard troponin, but it is also less specific. Thus, several false-positive interpretations could pose a problem (Alaour et al., 2018). Troponin levels, as well as changes over time, are valuable for measuring, diagnosing, or ruling out MI, and troponin testing's diagnostic accuracy is improving over time (Figure 2.4) (Brambi, 2018).

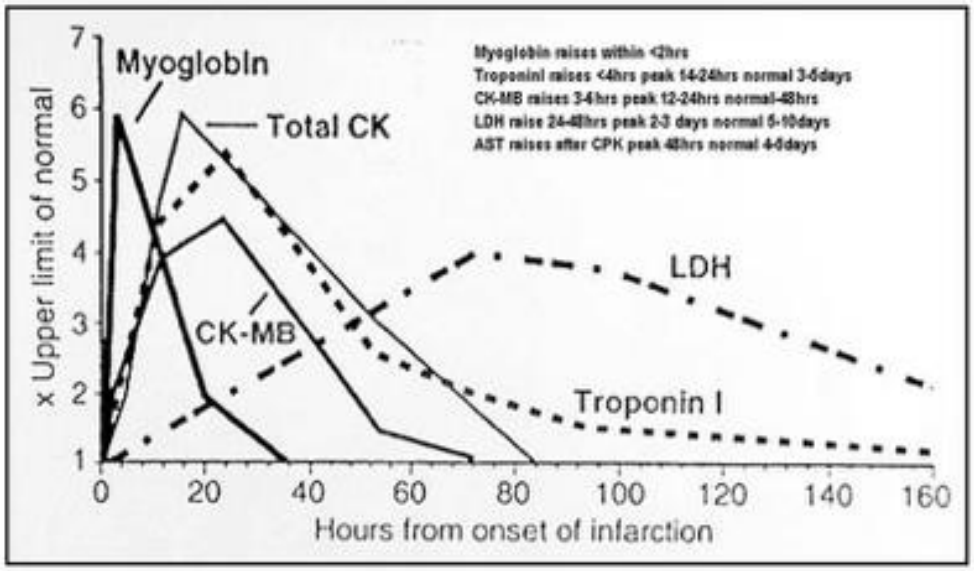

Figure 2.4 Various biomarker elevations during a confirmed myocardial infarction (Brambi, 2018). CK-MB: Creatine kinase-MB, LDH: Lactate dehydrogenase, AST: Aspartate aminotransferase, CPK: Creatine phosphokinase

The histology of MI changes as the disease progresses. Within 0.5 to 4 hours, the waviness of fibres at the tissue's periphery can be noticed under light microscopy. At this time, glycogen levels have dropped. The myocardium develops coagulation necrosis and oedema after 4 to 12 hours. The gross specimen turns black and mottled after 12 to 24 hours. There is also contraction band necrosis and a neutrophil predominance. There is a decrease of nuclei in cardiac cells after 1 to 3 days, and macrophages appear to eliminate apoptotic cells after 3 to 7 days. Granulation tissue occurs after 7 to 10 days. Collagen deposition begins at 10 days and onwards. The myocardium scarred after two months (Mechanic et al., 2021). Moreover, other heart conditions, such as hypertension, valve disease, and hereditary abnormalities such as cardiomyopathies, induce sporadic myocyte loss. In contrast, MI causes large-scale loss of cardiac muscle (Figure 2.5) (Laflamme and Murry, 2005).

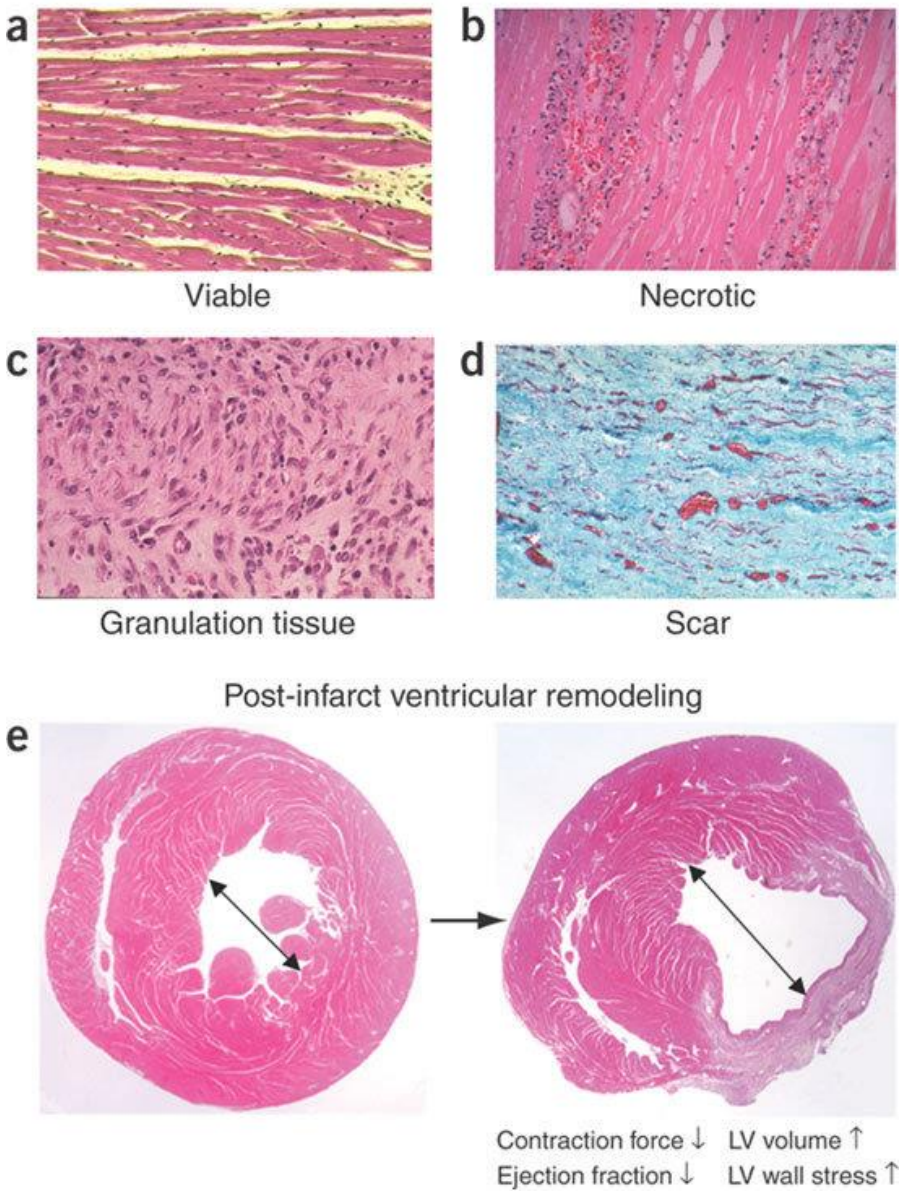

Figure 2.5 Histological changes of MI. (**a**) Within 20 minutes of ischemia, the myocardial begins to suffer permanent damage, and a wavefront of cell death sweeps from the inner layers to the outer layers of myocardium during a three- to six-hour period. (**b**) A strong inflammatory response is elicited by myocardial necrosis. (**c**) Granulation tissue, a hydrophilic provisional wound repair tissue rich in proliferating fibroblasts and endothelial cells, invades the infarct zone from the surrounding tissue. (**d**) Granulation tissue transforms into highly collagenous scar tissue over time. (**e**) At the organ level, a myocardial infarction causes the damaged wall to thin and the ventricular chamber to dilate, a process known as ventricular remodelling (Laflamme and Murry, 2005).

2.4 STATIN

2.4.1 Overview of Statin

Statins, also known as 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, are a type of lipid-lowering drug that helps people at risk high risk of cardiovascular disease survive better. They are the most commonly prescribed cholesterol-lowering medications. Statins block the enzyme HMGC reductase from converting HMG-CoA to mevalonate, a crucial step in cholesterol production (Pinal-Fernandez et al., 2018). Statins have a potent lipid-lowering impact as a result, which lowers cardiovascular risk and death. Like any medications, statins can cause side effects, such as musculoskeletal problems, an increased risk of diabetes, and a higher risk of haemorrhagic stroke (Pinal-Fernandez et al., 2018). However, the incidence of side effects is exceedingly low, and the advantages of statins much outweigh the dangers in some patient populations.

Current European and American guidelines recommend starting statin therapy as soon as possible in individuals with AMI (Ibanez et al., 2018). The National Cholesterol Education Program Adult Treatment Panel (ATP) formerly suggested that lipid profiles be measured on admission or within 24 hours, but did not highlight the timing of statin medication commencement (ATP, 2001). There are limited studies on the timing of statin medication in AMI. According to the Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering trial, atorvastatin 80 mg/day given within four days of an acute coronary syndrome (ACS) episode significantly reduced the incidence of major adverse cardiovascular events (MACEs) compared to placebo (Cannon et al., 2004).

Similarly, the Pravastatin or Atorvastatin with Aggressive Cholesterol Lowering (PROVE-IT) trial found that giving atorvastatin 80 mg/day instead of pravastatin 40 mg/day within 10 days after an ACS episode reduced MACEs significantly (Schwartz et al., 2001).

2.4.2 Pros and Cons of Statin

Statins were found to be far more effective than previously known approaches in lowering LDL levels, and the benefits of lowering cholesterol levels with statins were supported in studies evaluating primary and secondary CVD prevention (Baigent et al., 2005). Statins also inhibit additional downstream products of the mevalonate pathway, causing pleiotropic effects, in addition to decreasing cholesterol levels (Pinal-Fernandez et al., 2018). Statins control nearly all known processes of atherosclerosis and have therapeutic benefits outside of the cardiovascular system due to their pleiotropic actions, dysfunction and vascular smooth muscle proliferation, and lowering platelet activity.

However, statins also provide a few of adversity prior to their usage. To date, evidence suggests that statins can produce either self-limited myotoxicity (due to statins' direct effect on muscle) or an autoimmune myopathy (due to autoantibodies targeting HMGC reductase) (Pinal-Fernandez et al., 2018). However, direct myotoxicity is a relatively uncommon side-effect, with an annual incidence of about 10–20 cases per 10,000 statin-treated patients (Ganga et al., 2014). Patients on statins also have an increased risk of acquiring diabetes mellitus, based on large randomised clinical trials (Pinal-Fernandez et al., 2018). Diabetes has an attributable excess risk of

10–20 per 10,000 patients treated per year, which is comparable to the chance of having a severe myopathy (Sattar et al., 2010).

2.5 ANIMAL MODELS

2.5.1 Animal Model in Pre-Clinical Studies

Animal models have been used in various tests and research to answer multiple scientific problems and gain insight into the medical industry. Pre-clinical animal testing aims to provide knowledge or reasonable evidence for human testing and clinical trials. The better the predictive value for clinical trials, the closer the model is to the resemblance of human-acquired diseases. The ideal animal model would have symptoms and illnesses that are similar to those seen in humans. The collection of animal models for pre-clinical studies has become more difficult as the current understanding of illness or disorder stratification and aetiology have grown. The ideal animal model is still being established, yet it is critical for understanding all disease-related pathophysiology.

However, many of the existing animal models,including small and large creatures, are valuable in answering specific issues. An appropriate animal model should be chosen after understanding both the context of the study topic and the qualities of the animal model.

2.5.2 Hypercholesterolaemic Animal Model

Many animals develop atherosclerosis after being exposed to induced hypercholesterolaemia for a long time. In humans, serum cholesterol level is a significant risk factor for coronary heart disease. This cause-and-effect relationship

has driven the exploration of cholesterol metabolism and regulation. To learn more about human cholesterol metabolism, selecting a suitable animal model is critical.

The amount of cholesterol in the body is regulated by the balance of intake and outflow, primarily determined by dietary cholesterol absorption, endogenous cholesterol synthesis, and faecal excretion of neutral steroids and bile acids (Beynen, 1988). Increased cholesterol intake will trigger changes in one or more of the homeostatic systems of whole-body cholesterol metabolism.

The amount of dietary cholesterol consumed will determine what compensatory mechanisms take hold by the animal species tested. Thus, the establishment of hypercholesterolaemia in animal models is strongly associated with modifying diets used in the study. The mouse has long been utilised in medical research. This model's features include a well-known genetic background, ease of breeding, and cheap care costs. Small size and other physiological traits, on the other hand, could be considered limiting factors. The plasma lipoprotein profile of mice, for example, differs significantly from that of humans.

In mice, circulating cholesterol is primarily found in HDL particles, but in humans, it is primarily in LDL particles (Kapourchali et al., 2014). In this study, Sprague-Dawley (SD) rats were used as hypercholesterolaemic model. Despite other well-known benefits such as easy handling and cheap, SD rats were able to adapt well to a variety of diets. Wistar and SD outbred rats are the most commonly utilised rodents for developing metabolic syndrome models because they are prone to diet-induced obesity and resistance with individual features (Gunawan et al., 2021). In addition, SD

rats were discovered to be more responsive to a high-fat, high-cholesterol diet than mice (Fukuda et al., 2019).

For cardiovascular and cerebrovascular disease studies, a hypercholesterolaemia rat model can be generated by giving rats a 0.5% - 1.0% cholesterol-supplement diet for many weeks (Wang et al., 2010). In rats, dietary cholesterol of 0.5% to 1.0% can significantly raise blood VLDL and LDL levels. In addition, cholic acid is known as a hypercholesterolaemia inducer. Compared to a diet supplemented with merely high fat and cholesterol, a cholic acid-supplemented HFC diet, or atherogenic diet, is known to imitate human steatosis pathophysiology and cause early fibrosis (Ibrahim et al., 2015).

2.5.3 Myocardial Infarction (MI) Animal Model

In conjunction with prior hypercholesterolaemia, MI induction should establish a model that could mimic the pathology of diseases based on high cholesterol dietary intake. The animal model of MI is important for human MI prevention, diagnosis, and treatment.

The closure of a major coronary artery in small rats, followed or not by reperfusion, has shown to be a successful model for assessing pathophysiological importance and pharmacological effects in myocardial ischemia. *Ex vivo* animal models for MI, such as Lengerdoff heart preparations, are used to learn how specific organs and tissues work when isolated from other body stimuli (Kumar et al., 2016). This model makes the molecular mechanism, electrophysiology, and pharmacological drug target location simpler and easier to comprehend. Furthermore, *in vivo* MI animal

models are useful to mimic the total effect of medicines on an actual patient (Kumar et al., 2016).

Isoproterenol (ISO)-induced MI in rats is a model used to investigate the effects and efficacy of various cardio-related drugs. ISO is a non-selective beta-adrenergic agonist that is utilised in the myocardium to cause severe oxidative stress. ISO at 85 mg/kg for two days (Hassan et al., 2017) cause cardiac contractile dysfunction and myocardial injury by releasing excess free radicals, such as reactive oxygen species (ROS). This lead to infarcted necrosis in cardiac tissue (Ardjmand et al., 2019). Moreover, ISO is in charge of alterations in hemodynamic, biochemical, histopathologic, and oxidative stress markers (Kumar et al., 2016). Histologically, intramyocardial lipid accumulation and moderate fibrosis in the myocardium were seen in the ISO-MI model (Ardjmand et al., 2019). Furthermore, heart tissue samples can be taken, and an *ex vivo* experiment can be carried out to assess the effects of MI on tissue.

2.6 Hibiscus sabdariffa L. (HS)

2.6.1 Overview of HS

Hibiscus sabdariffa L. (HS), which has a distinctive flower, is widely planted in many developing countries. This plant is commonly utilised in traditional medicine because it is high in phytochemicals such as polyphenols, particularly anthocyanins, polysaccharides, and organic acids, all of which have significant therapeutic potential in modern medicine (Riaz and Chopra, 2018). It was a tropical or subtropical annual or biannual shrub with a height of up to 2 m from Africa. The flowers are 8–10 cm in diameter, white to pale yellow with a dark red mark at the base of each petal, and have