CARDIO- AND NEUROPROTECTIVE EFFECTS OF ALPHA CYCLODEXTRIN/MORINGIN COMPLEX IN SPRAGUE DAWLEY RATS WITH ISOPROTERENOL - INDUCED MYOCARDIAL INFARCTION

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

January 2023

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DEDICATION

This thesis is dedicated to my lovely parents (Prof. Aliyu Kamal & Haj. Batulu Nasir Ahmad), my caring husband (Dr. Usman Sunusi), my ever-patient children (Muhammad Kamal and Muhammad Sunusi) and my wonderful siblings.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Myocardial infarction (MI) is a severe form of coronary artery disease resulting from ischemic pathological changes of the myocardium. MI presents with signs of heart and brain affectation, but these are treated independently with different drugs. There is need for alternative treatment options that can target both cardiovascular and behavioral alterations due to MI. Isothiocyanates (ITCs) are bioactive compounds resulting from myrosinase catalyzed hydrolysis of glucosinolates (GLs). ITCs are derived from plants and have been shown to protect the heart and brain from oxidative stress in various cardiovascular and neuronal diseases' models. In previous studies, ITCs normalized hemodynamic variables, behavior as well as heart and brain redox status and morphology. *Moringa oleifera* Lam. seeds are rich in glucomoringin (a GL) the precursor of moringin (an ITC). The cardio- and neuroprotective effects of the ITC moringin (MG) as well as its novel formulation (alpha-cyclodextrin/moringin complex (α-CD/MG)) have not been studied on MI. *M. oleifera* was chosen because of its powerful antioxidant and cardioprotective effects and is believed to provide benefits in MI. This study was designed to investigate the potential of MG and α-CD/MG in protecting the heart and brain against MI-induced oxidative stress in isoproterenol-induced MI rat model. MG obtained from myrosinase catalyzed bioactivation of glucomoringin (GMG) isolated from *M. oleifera* seeds, was characterized with high performance liquid chromatography (HPLC) and nuclear magnetic resonance spectroscopy (NMR). Similarly, newly formulated α-CD/MG was characterized using one- and two-dimensional NMR. HPLC revealed successful bioactivation of GMG to MG after 15 min of incubation at 37 ℃ while NMR confirmed the molecular structures of MG and α-CD/MG. Male Sprague Dawley rats were grouped into 4 groups of eight. MG and α-CD/MG groups were respectively pretreated via oral gavage with MG and α-CD/MG at 20mg/kg and 42 mg/kg body weight diluted in 2 ml phosphate buffered solution (PBS) for seven days. While control and MI groups received only PBS. In the last two days of pretreatment, MI was induced by the subcutaneous

administration of isoproterenol hydrochloride (85 mg/kg body weight) in 2 ml of normal saline 24 hr apart. Induction of MI in rats was successful as confirmed with serum diagnostic cardiac markers. Data were analyzed and presented as mean ± standard error of means. The difference between means was determined using one-way analysis of variance (ANOVA), and the level of significance was reported at $p \leq 0.05$. Pretreatment of rats with MG and α-CD/MG significantly modified hemodynamic alterations, behavior, heart and brain oxidative stress and architectural distortions compared to vehicle-pretreated MI rats. Non-invasive CODA readings showed significant modification in tachycardia (heart rate of 345±15 vs 466±4) and hypotension (systolic blood pressure 115±3 vs 77±2) while Open Field Test (OFT) parameters revealed reduced anxiety-like behaviors (number of lines crossed 29 vs 8) in α-CD/MG compared to vehicle-pretreated MI rats. Significant difference in serum cardiac troponin I (270±1.46 vs 407±0.52) and creatine kinase-MB (93±2.16 vs 155 ±7.58), hippocampal dopamine (2.35±0.27 vs 0.88±0.04) and serotonin (0.4 ±0.02 vs 0.25±0.02) levels, oxidative stress markers as well as microscopic abnormalities were favorably modified with α-CD/MG pretreatment compared to vehicle-pretreated MI rats. Conclusively, and for the first time MG and α-CD/MG exhibited significant suppression of myocardial and brain oxidative stress evidenced by alleviation of behavioral, hemodynamic, biochemical, and histological alterations from isoproterenol-induced MI. Hence, α-CD/MG appears to be a novel alternative in the prevention of cardiovascular and behavioral manifestations of MI. Nonetheless, there is need for further studies to gain more insight into the mechanism of action of α-CD/MG in MI.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN KARDIO- DAN NEUROPROTEKTIF KOMPLEKS ALFA-SIKLODEKSTRIN/MORINGIN PADA TIKUS SPRAGUE DAWLEY DENGAN INFARKSI MIOKARDIUM YANG DIARUH OLEH ISOPROTERENOL

Oleh

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Januari 2023

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Infarksi miokardium (MI) adalah bentuk penyakit arteri koronari yang teruk akibat perubahan patologi iskemia miokardium. MI hadir dengan tanda-tanda terjejas jantung dan otak, tetapi ini dirawat secara bebas dengan ubat yang berbeza. Terdapat keperluan untuk pilihan rawatan alternatif yang boleh menyasarkan kedua-dua perubahan kardiovaskular dan tingkah laku akibat MI. Isothiocyanates (ITCs) adalah sebatian bioaktif yang terhasil daripada hidrolisis glukosinolat (GLs) yang dimangkinkan oleh myrosinase. ITC berasal daripada tumbuh-tumbuhan dan telah ditunjukkan untuk melindungi jantung dan otak daripada tekanan oksidatif dalam pelbagai model penyakit kardiovaskular dan neuron. Dalam kajian terdahulu, ITC menormalkan pembolehubah hemodinamik, tingkah laku serta status dan morfologi redoks jantung dan otak. *Moringa oleifera* Lam. benih kaya dengan glucomoringin (a GL) prekursor moringin (ITC). Kesan kardio dan neuroprotektif ITC moringin (MG) serta rumusan novelnya (alpha-cyclodextrin/moringin complex (α-CD/ MG)) belum dikaji pada MI. *M. oleifera* Lam. dipilih kerana kesan antioksidan dan kardioprotektifnya yang kuat dan dipercayai memberi manfaat dalam MI. Kajian ini direka bentuk untuk menyiasat potensi MG dan α-CD/MG dalam melindungi jantung dan otak daripada tekanan oksidatif yang disebabkan oleh MI dalam model tikus MI yang disebabkan oleh isoproterenol. MG yang diperoleh daripada bioaktivasi bermangkin myrosinase bagi glucomoringin (GMG) yang diasingkan daripada biji *M. oleifera* Lam., dicirikan dengan kromatografi cecair prestasi tinggi (HPLC) dan spektroskopi resonans magnetik nuklear (NMR). Begitu juga, α-CD/MG yang baru dirumuskan telah dicirikan menggunakan NMR satu dan dua dimensi. HPLC mendedahkan kejayaan bioaktivasi GMG kepada MG selepas 15 minit pengeraman pada 37 ℃ manakala NMR mengesahkan struktur molekul MG dan α-CD/MG. Tikus jantan Sprague Dawley dikumpulkan kepada 4 kumpulan lapan. Kumpulan MG dan α-CD/MG masing-masing telah diprarawat melalui gavage oral dengan MG dan α-CD/MG pada 20 mg/kg dan 42 mg/kg berat badan yang dicairkan

dalam 2 ml larutan buffer fosfat (PBS) selama tujuh hari. Manakala kumpulan kawalan dan MI hanya menerima PBS. Dalam dua hari prarawatan terakhir, MI telah disebabkan oleh pentadbiran subkutaneus isoproterenol hidroklorida (85 mg/kg berat badan) dalam 2 ml garam biasa dengan jarak 24 jam. Induksi MI dalam tikus berjaya seperti yang disahkan dengan penanda jantung diagnostik serum. Data dianalisis dan dibentangkan sebagai min ± ralat piawai bagi min. Perbezaan antara min ditentukan menggunakan analisis varians sehala (ANOVA), dan tahap keertian dilaporkan pada $p \leq 0.05$. Prarawatan tikus dengan MG dan α-CD/MG mengubahsuai secara ketara perubahan hemodinamik, tingkah laku, tekanan oksidatif jantung dan otak dan herotan seni bina berbanding tikus MI prarawatan kenderaan. Bacaan CODA tidak invasif menunjukkan pengubahsuaian ketara dalam takikardia (kadar jantung 345±15 vs 466±4) dan hipotensi (tekanan darah sistolik 115±3 vs 77±2) manakala parameter Ujian Medan Terbuka (OFT) mendedahkan tingkah laku seperti kebimbangan yang berkurangan (bilangan garisan melintasi 29 vs 8) dalam α-CD/MG berbanding tikus MI prarawatan kenderaan. Perbezaan ketara dalam troponin jantung serum I (270±1.46 vs 407±0.52) dan creatine kinase-MB (93 ±2.16 vs 155±7.58), hippocampal dopamine (2.35±0.27 vs 0.88±0.04) dan serotonin (0.24 vs 0.04) dan ±0.02), penanda tegasan oksidatif serta keabnormalan mikroskopik telah diubah suai dengan baik dengan prarawatan α-CD/MG berbanding tikus MI prarawatan kenderaan. Secara konklusif, dan buat pertama kalinya MG dan α-CD/MG mempamerkan penindasan ketara terhadap tekanan oksidatif miokardium dan otak yang dibuktikan dengan pengurangan perubahan tingkah laku, hemodinamik, biokimia, dan histologi daripada MI yang disebabkan oleh isoproterenol. Oleh itu, α-CD/MG nampaknya merupakan alternatif baru dalam pencegahan manifestasi kardiovaskular dan tingkah laku MI. Walau bagaimanapun, terdapat keperluan untuk kajian lanjut untuk mendapatkan lebih banyak maklumat tentang mekanisme tindakan α-CD/MG dalam MI.

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LIST OF ABBREVIATIONS

13C-NMR	Carbon 13 nuclear magnetic resonance spectroscopy
1H-NMR	Proton nuclear magnetic resonance spectroscopy
5HT	Serotonin
ABI	Agro Biotechnology Institute
AITC	Allyl isothiocyanate
ANOVA	Analysis of variance
BCA	Bicinchoninic acid
BITC	Benzyl isothiocyanate
BP	Blood pressure
CABG	Coronary artery bypass graft
CAD	Coronary artery disease
CAT	Catalase
CDs	Cyclodextrins
CK-MB	Creatine kinase myocardial band
COMeT	Comparative Medicine and Technology unit
COSY	Correlation spectroscopy
cTnI	Cardiac troponin I
cTnT	Cardiac troponin T
CVDs	Cardiovascular diseases
D_2O	Deuterium oxide
DA	Dopamine
DALYs	Disability-adjusted life years
DBP	Diastolic blood pressure

DNA	Deoxyribonucleic acid
EAE	Experimental autoimmune encephalomyelitis
EDTA	Ethylene diamine tetra-acetic acid
ELISA	Enzyme linked immunosorbent assay
EPIC	European Prospective Investigation into Cancer and Nutrition
ER	Erucin
GER	Glucoerucin
GL	Glucosinolate
GMG	Glucomoringin
GRA	Glucoraphanin
GSHPx	Glutathione peroxidase
GST	Gluconasturtiin
GTL	Glucotropaeolin
H & E	Hematoxylin and eosin
H_2O_2	Hydrogen peroxide
H_2S	Hydrogen sulfide
hGMSCs	Human gingival mesenchymal stem cells
HMQC	Heteronuclear multiple quantum correlation
HO-1	Heme oxygenase 1
HPLC	High performance liquid chromatography
HR	Herat rate
HSQC	Heteronuclear single quantum correlation
IACUC	Institutional Animal Care and Utilization Committee
iNOS	Inducible nitric oxide synthase

ITC	Isothiocyanate
LDH	Lactate dehydrogenase
LPS	Lipopolysaccharide
MAP	Mean arterial pressure
MDA	Malondialdehyde
MG	Moringin
MG	Moringin
MI	Myocardial infarction
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MYR	Myrosinase
NF-κB	Nuclear factor kappa beta
NMR	Nuclear magnetic resonance spectroscopy
NQO1	NADPH quinone oxireductase 1
Nrf2	Nuclear factor erythroid 2-related factor 2
OFT	Open Field Test
PBS	Phosphate buffer solution
PCI	Percutaneous coronary intervention
PEITC	Phenethyl isothiocyanate
PI	Protease inhibitor
RNA	Ribonucleic acid
S.E.M	Standard error of mean
SBP	Systolic blood pressure
SC	Subcutaneous
SFN	Sulforaphane

SIN	Sinigrin
SOD	Superoxide dismutase
TBAR	Thiobarbituric acid reagent
TOCSY	Total correlation spectroscopy
T-SOD	Total superoxide dismutase
TSP	Trimethylsilylpropionic acid
WHO	World Health Organization
WST-1	Water-soluble tetrazolium salt
α-CD	Alpha-cyclodextrin
α-CD/MG	Alpha-cyclodextrin/moringin complex

CHAPTER 1

INTRODUCTION

1.1 Research background

Heart and nervous system diseases are significant causes of morbidity and mortality worldwide. The diseases are most encountered in the elderly but also affect younger generations. There is rising mortality rate in the elderly population (Global Health Metrics, 2018). Myocardial infarction (MI) is a severe form of coronary artery disease (CAD) characterized by death of myocardial tissue associated with ischemic pathological changes that if not arrested or intervened immediately can lead to deterioration of cardiac function (Reed et al., 2017). MI usually results from rupture of coronary atheromatous plaque where the affected myocardial area becomes necrosed from oxidative stress-induced injury resulting in leakage of cardiac enzymes and decline in myocardial functioning (Kotecha & Rakhit, 2016; Zhao et al., 2022). These cardiac enzymes include cardiac troponins and creatine kinase myocardial band (CK-MB) that are utilized as diagnostic markers of MI (Kotecha & Rakhit, 2016). Various hemodynamic, biochemical and morphological alterations ensue and as a consequence of brain-heart interactions, behavioral abnormalities like anxiety symptoms manifest (Tkachenko et al., 2018). The brain is highly subjective to oxidative stress damage due to high basal oxygen consumption compounded with low antioxidant enzymes (Tarozzi et al., 2013). Moreover, the synthesis of monoamines like dopamine and serotonin are altered as a result of acute stress (Hou et al., 2021). The morphological architecture of the heart and brain become distorted (Abu-elfotuh et al., 2022).

Coronary artery diseases have global incidence of 18.2 million with MI affecting more than seven million people (Global Health Metrics, 2018). In the United States, it was reported that every 40 seconds, someone gets a heart attack (i.e. MI), amounting to 1-1.5 million annually (Reed et al., 2017). Further, the prevalence of MI in the young population (less than 45 years) has risen (6.1%) as reported in a study done in Malaysia (Hoo et al., 2016). In 2019, the global mortality from coronary artery diseases reached 360,900. MI and related complications are the main causes of death throughout the world (Shah et al., 2019): these accounted for four million in the Europe and Northern Asia, 2·4 million in the USA, and mortality rate of 7.1-11.5% in Iran (Mohseni et al., 2017). A study by (Hoo et al., 2016) reported 20-25% mortality in young adults following MI (Hoo et al., 2016). MI has a tremendous cost implication on health allocations; the US spends about US$450 billion annually (Reed et al., 2017).

The World Health Organization (WHO) has prompted for more research especially on plants to generate alternative treatment options. In the history of medicinal use, plant-based medicines have proven effective in many heart and neuronal diseases (Li et al., 2015). Many studies have reported the effective use

of plant-based compounds, often known as phytochemicals as therapies to help reduce the impact of disease symptoms. They are used to treat various diseases as well as to encourage overall health (Global Health Metrics, 2018). Plants synthesize phytochemicals as secondary defense mechanism, and they possess biological properties that are beneficial to human health. Many of these phytochemicals are antioxidants and are increasingly used to defend against a wide range of cardiovascular and neurological diseases (Global Health Metrics, 2018). Glucosinolates (GLs) and isothiocyanates (ITCs) are phytochemicals that have been found to protect both heart and brain. These metabolites are commonly found in the Cruciferae family (Finsterer & Wahbi, 2014) but recently reported in Moringaceae family (Waterman et al., 2014). Cabbage, kale, broccoli, cauliflower, Brussels sprouts, kohlrabi, rape, black and brown mustard, turnips and rutabagas are all Cruciferous vegetables (Finsterer & Wahbi, 2014) rich in various GLs and ITCs including sulforaphane, erucin, allyl isothiocyanate, phenyl isothiocyanate etc. (Guzman-Martinez et al., 2019). On the other hand, glucomoringin (GMG) and moringin (MG) – a GL and ITC respectively – are rich in *Moringa oleifera* Lam. (Moringaceae) which is a tropical tree first described by Jean-Baptiste Lamarck in the year 1785 (Fahey et al., 2019; Waterman et al., 2014).

M. oleifera Lam. is a tropical plant native of the Asian subcontinent regarded as the “tree of life” with reported antioxidant, anti-inflammatory, cardioprotective, neuroprotective, antibiotic, antitumor, wound healing to mention a few of its bioactivities (Vergara-Jimenez et al., 2017). Over the years, various components of the plant have been extracted and used on different models of human and animal diseases with interesting results (Chhikara et al., 2020). The GL of the plant that is GMG gives rise to the bioactive ITC MG upon hydrolysis by the enzyme myrosinase which is found in a separate compartment in the plant. Many of the plant’s effects have been ascribed to MG (Ghimire et al., 2021). MG possesses significant antioxidant, anti-inflammatory, and neuroprotective activities as shown in recent studies (Chiricosta et al., 2019; Giacoppo, Galuppo, Montaut, et al., 2015; Silvestro et al., 2021). Literature search revealed limited studies on the cardioprotective effects of MG or α-CD/MG and none on MI. Though, few studies reported the effect of *M. oleifera* Lam. extract on reducing serum cardiac injury markers, myocardial oxidative stress, and histopathological damage due to MI. Even though previous studies showed the neuroprotective effects of MG or α-CD/MG but effect on serotonin is lacking. Serotonin is a monoamine neurotransmitter that gets dysregulated in the hippocampus following MI and takes part in the behavioral manifestations of the disease.

MG has poor solubility and stability in aqueous medium. As a result, it was complexed with alpha-cyclodextrin (α-CD) and the formulation (alpha-cyclodextrin-moringin complex, α-CD/MG) rendered MG more biologically effective (Mathiron et al., 2018). The elucidation of cardio- and neuroprotective effects of the stable formulation of MG i.e., α-CD/MG was achieved in the rat model of isoproterenol-induced MI.

1.2 Problem statement

MI contributes the greatest quota of disability-adjusted life years (DALYs) of cardiovascular diseases (CVDs) and this is compounded by inaccessibility and non-affordability of drugs especially in low resource settings (Kishore et al., 2018; Peng et al., 2019). The World Health Organization (WHO) called for research into natural medicines – plant based – which are cheaper and documented to improve the health status of people with CVDs and neuronal diseases (Lopez-Rodriguez et al., 2020). In addition, there is greater requirement for candidate drugs in the treatment of MI because many agents fail translation from "bench to bedside" (Pullaiah et al., 2021).

Researchers' interest has been lured towards the use of ITCs from plants to curtail this problem. *M. oleifera* Lam. is a plant with diverse bioactive phytochemicals including ITCs derived from GLs as well as flavonoids, phenolic acids, carotenoids, and tocopherols which confer the benefits of the plant in various human diseases (Lopez-Rodriguez et al., 2020). The seeds have higher content of GMG and hence MG compared to the leaf or other parts of the plant. MG showed greater antioxidant and anti-inflammatory activity compared to sulforaphane, a related ITC compound derived from broccoli. Unfortunately, MG lacks good solubility and stability in aqueous medium. The novel formulation of MG with alpha cyclodextrin – alpha cyclodextrin moringin complex, α-CD/MG – has better pharmacokinetic profile than non-complexed MG but it is yet to be explored for cardio- and neuroprotective effect in MI. Hence, α-CD/MG was studied in the setting of MI-induced oxidative stress to unveil these effects.

Further, cardiovascular and behavioral symptoms of MI are treated independently, using multiple drugs to target each symptom (Ge et al., 2020). Most of the current treatment options for MI primarily target the cardiovascular manifestations of the disease and not the behavioral symptoms. Patients present with these behavioral symptoms that are not effectively suppressed by regular MI medications. Therefore, patients are treated with different drugs like benzodiazepines and morphine. Morphine is the most commonly prescribed medication for chest pain and serves to allay anxiety. However, the use of morphine may be associated with a higher risk of adverse clinical outcomes or even death. In addition, multiple drugs pose the risk of drug interaction(s) and failure of compliance. On the other hand, naturally derived compounds have multiple bioactivities, and a single agent may be beneficial against multiple signs and symptoms and lessen these untoward effects. α-CD/MG is a strong antioxidant which has been reported to have neuroprotective activity (Mathiron et al., 2018) and may also provide myocardial protective effect; so it is important to explore the cardio- and neuroprotective effects of α-CD/MG on cardiovascular and behavioral abnormalities of MI.

1.3 Justification

MI presents with cardiovascular and behavioral features. As the myocardial tissues become necrosed and release their contents, the heart suffers from depressed function, and it cannot effectively meet the metabolic demands of the body. Areas with highest sensitivities include water shed areas throughout the body, and deep brain structures like the hippocampus, thalamus, and basal ganglia. In particular, the hippocampus is susceptible to oxidative damage due to extensive production of reactive oxygen species, relatively low antioxidant capacity, high rate of oxidative metabolic activity, high content of polyunsaturated fatty acids, low repair mechanism, and poor plasticity (Kirisattayakul et al., 2013). Current MI drugs effectively manage the cardiovascular features, but the neurological symptoms are treated independently with different drugs.

Understanding and research for drugs that can target both cardio- and behavioral features of MI will go a long way in providing disease alleviation (Li et al., 2015). Alternative therapies, such as phytotherapy, are considered to improve the health status of people with CVDs and neuronal diseases (Lopez-Rodriguez et al., 2020). Since antiquity, natural medicines have been used to prevent and treat various illnesses due to their remarkable efficacy and high degree of safety. In recent times, natural medicines have become popular worldwide and widely accepted as supplements and/or alternative therapies. Accumulating scientific evidence has shown that many natural drugs have achieved good results in the treatment of various diseases including cardiovascular and neuronal diseases. Hence, natural agents derived from plants are undoubtedly invaluable resources for identifying candidate drugs (Zhang et al., 2021).

GLs and ITCs are plant-derived phytochemicals which have been shown to be useful in disease prevention (Lopez-Rodriguez et al., 2020). The health-promoting effects of MG from *M. oleifera* Lam. tree are mainly related to the activation of various detoxification enzymes and the reduction of certain inflammatory markers. To date, most studies have focused on certain inflammatory diseases. Therefore, research using different models and clinical trials that could confirm the effectiveness of these bioactive compounds is indispensable (Lopez-Rodriguez et al., 2020; Peng et al., 2019). The cardio- and neuroprotective effects of the ITC from *M. oleifera* Lam. seeds are yet to be explored in MI.

GMG is a GL from *M. oleifera* Lam. seeds with very limited effect, but its bioactive ITC MG has been reported to be responsible for most of the bioactivities. MG is derived from myrosinase hydrolysis of GMG both *in vivo* and *in vitro* at neutral pH. Following bioactivation to MG, high performance liquid chromatographic (HPLC) and nuclear magnetic resonance spectroscopic (NMR) studies were used for confirmation, chemical characterization, and structural elucidation. Because MG has poor solubility and stability in aqueous medium, NMR, and

mass spectroscopic techniques – based complexation of MG with alpha cyclodextrin led to the formulation of a more pharmacokinetically bioactive compound (alpha cyclodextrin moringin, α-CD/MG) before being used in this study. The method used was similar to the complexation of beta cyclodextrin with benzyl isothiocyanate as previously reported.

The effect of an extract, compound or drug on MI models is determined among other things by their effect on hemodynamic parameters, serum cardiac markers, myocardial oxidative stress markers, and histopathological alterations of the myocardium(Filho et al., 2011). Hemodynamic parameters can be assessed with invasive and non-invasive techniques. However, non-invasive CODA apparatus is a simple method that accurately measures hemodynamic parameters and effectively measures diastolic blood pressure in contrast to other blood pressure systems that rely on calculated value (CODA® High Throughput System, 2023). Similarly, drug effect on the brain can be determined by its effect on behavioral symptoms, brain neurotransmitters, oxidative stress, and histopathological alterations (Tkachenko et al., 2018; R. Zhang & Miao, 2015). One of the sensitive methods of determining the effect on the behavior of experimental animals is Open Field test as the generated data can be used to deduce alterations in both locomotor and exploratory activities of experimental animals that point to anxiety or depression. The OFT was employed to achieve elucidation of behavioral parameters in rats. Levels of biomarkers in serum and tissues and the activity of enzymes were measured using colorimetric and enzyme-linked immunosorbent assays (ELISA) which are indispensable tools in biochemical studies. Hematoxylin and eosin (H&E)-stained preparations were used to analyze histopathological alterations in the myocardium and hippocampus. Histopathological analysis is the gold standard for tissue examination for both research and diagnostic purposes used to assess inflammation and/or healing stage (Paramitha et al., 2017).

This study focused on determining the cardio- and neuroprotective effects of α-CD/MG on rats with isoproterenol-induced myocardial infarction. The study covered the bioactivation of pure GMG (from French collaborators) to MG using myrosinase and subsequent quantification using HPLC and structural characterization using one- and two-dimensional NMR. α-CD/MG was synthesized by collaborators (UPM/France Hibiscus collaboration) but was subjected to one- (proton) and two-dimensional (total correlation spectroscopy) NMR to confirm complexation. The cardioprotective effects of α-CD/MG were assessed based on the hemodynamic variables (heart rate and blood pressure and mean arterial pressure), serum cardiac injury markers (cardiac troponin I and creatine kinase MB), oxidative stress markers (catalase, superoxide dismutase, malondialdehyde) as well as histopathological alterations of the myocardium (using light microscopy of H&E-stained sections). The neuroprotective effects of α-CD/MG were evaluated on behavioral parameters (with the aid of the Open-Field test (OFT)), hippocampal oxidative stress and monoamines as well as histopathological alterations of the hippocampus (using light microscopy of H&E-stained sections).

1.4 Hypotheses

1.4.1 Null hypothesis (H_0)

α-CD/MG does not show cardio- and neuroprotective effects in rats with isoproterenol-induced myocardial infarction.

1.4.2 Alternate hypothesis (H_A)

α-CD/MG shows cardio- and neuroprotective effects in rats with isoproterenol-induced myocardial infarction.

1.5 Research objectives

1.5.1 General objective

The general objective of this study was to assess the cardio- and neuroprotective effects of α-CD/MG in rats with isoproterenol-induced myocardial infarction.

1.5.2 Specific objectives

1. To bioactivate glucomoringin to moringin, develop and verify complexation of moringin with alpha cyclodextrin.

2. To assess the cardio- and neuroprotective effects of α-CD/MG on hemodynamic parameters and behavioral parameters of rats with isoproterenol-induced myocardial infarction.

3. To evaluate the cardio- and neuroprotective effects of α-CD/MG on serum cardiac injury markers, heart and brain oxidative stress, hippocampal monoamines, and histological alterations of myocardium and hippocampus.

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