Chapter 1: General Introduction

1.1 Introduction

In the last few decades, the human race has made tremendous progress in technological developments that primarily caters bodily comfort and to some extent improved bodily health. So far, mental health remains an unmet need considering the fact that prevalence of psychiatric disorders are on the rise in an alarming rate [1-3]. Amongst all psychiatric disorders, the clinical depression indiscriminately affects people both in developing and developed countries. Depression is the chronic, common, and serious mental disorder which causes disability and morbidity worldwide [4]. Considering the heterogenous disease characteristics, the researchers, clinicians, and health professionals have developed Diagnostic and Statistical Manual of Mental Disorders (DSM-IV, published in 1992) and DSM-V (published in 2013) criteria to facilitate a reliable diagnosis of depressive disorder [5, 6].

The major depressive episode can be diagnosed based on the DSM-V diagnostic criteria [7]:

a) During 2 weeks, ≥ 5 symptoms should exist, almost every day, some days, and at least loss of pleasure or depressed mood is mandatory for depressive disorder.

b) The symptoms include depressed mood, loss of pleasure, weight variations, appetite changes, sleep disturbance, fatigue, guiltiness, loss of energy, denial, discounting, feeling of worthlessness, diminished ability to think and concentrate, thoughts of death, suicide attempt or suicide.

c) The mood disturbance is sufficiently severe to cause marked impairment in social functionalities necessitating hospitalization to prevent harm to self or others.

d) The symptoms with increased energy activity must cause impaired functioning with clinically significant distress.

e) The episode is not attributable to direct physiological effects of drug, medication, or treatments.

Cardiovascular diseases (CVDs) are another major leading cause of disability and death globally [8]. Air pollution, unhealthy diet, tobacco use, obesity, alcohol use, physical inactivity, social deprivation, and stress are major contributing factors behind increased cardiac-related diseases [9]. CVDs are the disorders of blood vessels and the heart which includes coronary heart disease, myocardial infarction, peripheral artery ailment, rheumatic heart ailment, deep vein thrombosis, congenital heart ailment, and pulmonary intercalation [10, 11]. Amongst all, a heart attack or myocardial infarction (MI) is an acute event and a most common cause of death which occurs due to a blockage of an artery that prevents blood flowing to the heart [12]. MI comprised of serious symptoms such as sweating, lethargy, discomfort or pain in the centre of the chest, arms, left shoulder, jaw, and elbow, difficulty in breathing, faintness, cold sweat, sometime unconsciousness, and even death [13].

Further, there is a marked increase in patients with comorbid conditions due to unhealthy lifestyle, unhealthy diet, and pollution. The presence of a comorbid condition disproportionately increases morbidity and mortality among patients with CVDs [14]. A comorbid condition like diabetes mellitus independently increases the occurrence of a cardiac event in patients with CVDs [15]. The impact of depression as a comorbid

condition in cardiac conditions has not been studied extensively. This thesis incorporates the experiments that focuses on preventing cardiovascular abnormalities in rats caused due to comorbid depression induced by chronic unpredictable stress and maternal separation.

In our research work we have used two validated models of depression namely chronic unpredictable stress (CUS) and maternal separation. CUS model work in a naturalistic way and used as an animal model consisting of several socio-environmental stressors including food and water deprivation, isolation, restrain, light exposure, and cage tilt all mimics depression [16]. Similarly, in maternal separation subjects suffer from maltreatment, thermoregulation, nutritional dysfunctions lead isolation, distress, exhaust, and fear which translate into later in life abnormality in brain that can manifest as trouble sleep, abnormal relations, low self-esteem, educational difficulties, suicidal thoughts, lastly depression [17]. Clinically, during childhood abuse and neglect affect several parts of the brain regions including amygdala (key processing emotions), hippocampus (memory and learning), orbitofrontal cortex (emotion regulation and decision making), cerebellum (executive functioning and motor behavior), and corpus callosum (arousal and emotion) [18]. CUS and MS model qualifies all the validation criteria (predictive, face, and construct) and found suitable for screening of synthetic antidepressant drugs [19]. Therefore, in our research we used CUS and MS model to develop depressive-like behavior in animals and estimated the cardiac abnormalities followed by depression.

As a part of the literature review, PubMed, NCBI, Google Scholar, and ScienceDirect databases were searched for English-language peer-reviewed published studies with keywords relating to depression and cardiac abnormalities. Additional

references we identified using cross-references on various types of depression and cardiac diseases, preclinical studies, and clinical trials for the writing of this literature.

1.2 Epidemiology

1.2.1 Local prevalence

Recent reports indicate that almost 3.3% patients in India are diagnosed with depression than other diseases [20, 21]. In India, around 46 million people are suffering from depression which majorly contributes to disability-adjusted-life-years (DALYs) [22]. According to report, India is one of the major depressed countries in the world [23]. Around 36% Indians at some point of time in their lives suffer from major depression [24].

Similarly for cardiac disease in India, global burden of disease study stated CVDs death rate of 272 in 100000 India residents than global average of 235 in 100000 residents [25]. Out of several type of heart disease, coronary heart disease (CAD) is one of the major causes of death in India [26]. According to report, 16% of CAD disease and 24% of acute MI patients are attributable to hypertension [25]. There is an increasing trend of years of life lost (YLLs) and DALYs due to substantial increase in heart disease patients in India [2]. Interestingly, prevalence of heart disease is more in urban (9-10%) population compared to rural (4-6%) Indian population [27, 28].

Myocardial infarction is another higher leading cause of death and disability in India [29, 30]. Indians are passing through an epidemiological transition from communicable to non-communicable diseases [30]. Among non-communicable disease country faces the double burden of MI (ST elevation MI and non-ST elevation MI) [31] [32]. Risk of MI among Indians is 20 times higher than Japanese, 6 times higher than

Chinese, and 3-4 times higher than Americans [33]. In the last decade, it has been observed that youngsters are 4 to 6% more prone to MI than elder Indians [33]. Incidence of MI in younger people not only increased the mortality rate but also contribute largely to the DALYs [34]. It is considered that genetic predisposition is the major reason behind MI in young Indian population, but unhealthy lifestyle choices such as stress, obesity, dyslipidemia, dietary habits, alcohol consumption, smoking, and sedentary lifestyle regulate the gene expression paving a way for early life MI in younger generation [35].

1.2.2 Worldwide prevalence

According to recent report, approximately 280 million people have depression worldwide [36, 37]. Due to the lack of healthcare resources and stigma linked to mental disorders, most of the patients are deprived of effective treatment in low-and middle-income countries (>75% of population) [38]. According to global burden of disease (GBD), number of patients with CVDs have nearly doubled from 271 million in 1990 to 523 million in 2019 [39]. Further, WHO report states 17.9 million people died due to CVDs representing 32% of all global deaths, in which 82% were due to mainly heart attack or MI [40]. According to European society of cardiology, 45% of deaths are in females and 39% of deaths are in males due to ischemic heart disease [41].

Statistical reports state that around 45-46% patients with CVDs suffer from depressive disorders [42, 43]. Further, the prevalence of depression associated CVDs is about 18.4% (95% CI, 15% to 22.3%) [44]. Other countries with high rates of depression and cardiac disease include Japan, Poland, Czech Republic, Israel, Thailand, Nepal, and Bangladesh [45].

1.2.3 Survival

An early prognosis and diagnosis of MI and depression significantly reduces morbidity and increases survival. However, some factors such as gender, age, and severity of the disease may affect survival rate during the prognosis [46]. The survivor of the first heart anomalies and depression, face substantial risk of further morbidities and mortalities.

1.3 Etiology and pathophysiology

Depression is a complex and non-homogenous disorder having more than one etiology [47]. Depression majorly occurs due to a functional deficiency of monoaminergic transmitters: norepinephrine (NE), serotonin (5-hydroxytryptamine; 5-HT), and dopamine (DA) [48]. A thorough study of the origin and projections of the monoaminergic neurons into different brain areas suggests that these neurons are responsible for many behavioral symptoms of depression such as mood, motivation, vigilance, fatigue, and retardation or psychomotor agitation [49]. Alterations in monoaminergic syntheses, storage, and release as well as dysfunction in receptor sensitivity are the major causes of depressive states in humans [49].

On the other hand, the risk factors of heart disease are physical inactivity, dyslipidaemia, tobacco use, unhealthy diet, stressful life, and alcohol use [50]. Raised blood pressure, blood glucose, blood lipids, and obesity are the secondary risk factors of MI [51]. In primary care facilities, abovementioned factors can be measured and suitable interventions may help in preventing the increased risk of stroke, heart failure, and heart attack (*Figure 1*).

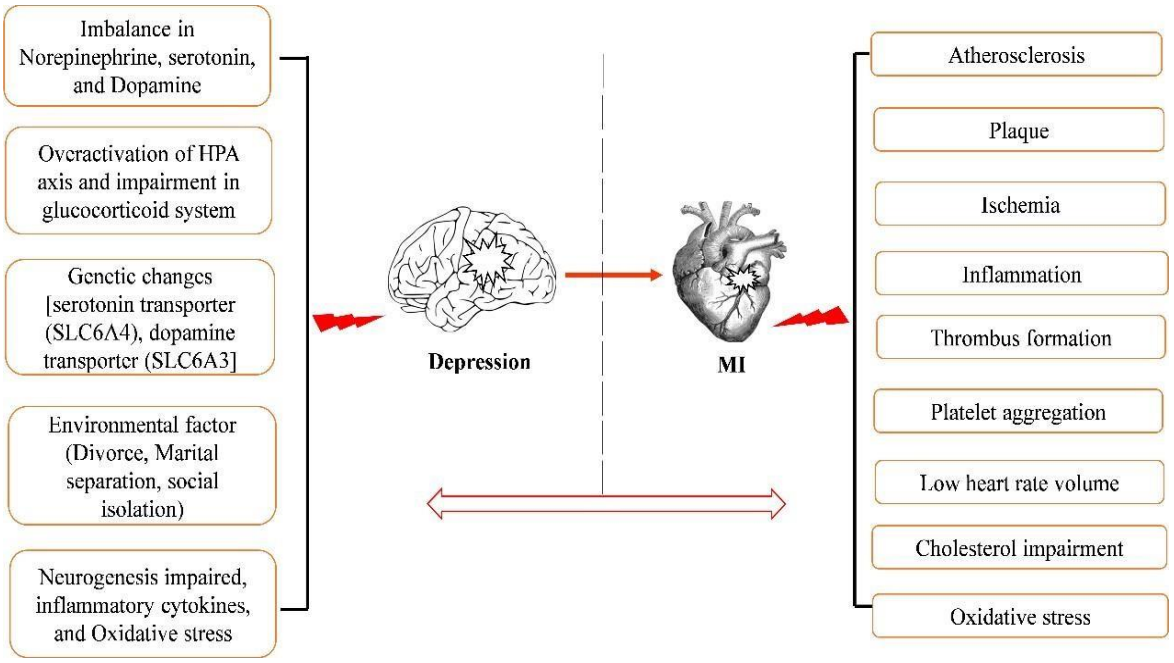

Figure 1: Schematic representation of possible factors of Depression and Myocardial Infarction

In the pathophysiology of depression, long-term stress badly affects body homeostasis *via* dysfunction of the neuroendocrine system [Hypothalamic Pituitary Adrenal axis (HPA axis)] and the sympathetic system [52]. Hippocampus, prefrontal cortex, septum, and amygdala (several forebrain structures) are the major stress influencing region of the brain [53]. The final integrator stress response is in hypothalamus (paraventricular nucleus, PVN) which is the last synaptic input that converge from several brain regions [54]. The hypothalamus produces corticotrophin-releasing hormone (CRH) which lead to modulation of behavioral functions and activation of anterior pituitary gland that secrets adrenocorticotropin (ACTH) hormone [55]. Further, ACTH stimulates the adrenal cortex to release cortisol that inhibits the negative feedback response [55]. Furthermore, researchers demonstrated that chronically stress-heightened glucocorticoid levels may lead to an abnormal HPA axis system [56]. This abnormality further reduces glucocorticoid receptors and cellular

density in the hippocampus causing further impairment in the negative feedback system [57] (*Figure 2*).

Pathophysiologically, MI is defined as cardiomyocyte death occurs due to imbalance between oxygen supply and demand (Antman et al., 2000). Prolonged ischemia and hypoxia are the major cause of irreversible injury of myocardium [58]. In MI, the rupture of atherosclerotic plaque and occlusion of coronary artery are factors of thrombus formation which leads to induction of ischemia [59]. Under this ischemic condition, cardiomyocytes are unable to produce enough energy (ATP) and halts the aerobic metabolism leads to necrosis [60]. During necrosis of myocardium, generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) induces oxidative stress led to dysfunction of membrane permeability and subsequent cell death) [61]. The final outcomes of MI are changes in cardiomyocytes, disruption of myocardial membranes, aggregation of platelets, DNA damage, and ultimately cell death [62]. Chronic stress may also aggravate the condition of MI by activation of sympathetic system [63]. During the overactivation of sympathetic system, blood vessels are constricted and cardiac stimulation increases myocardial oxygen demand that leads to hypoxia, infarction, arrhythmia, and cardiac dysfunction [64]. Other pathophysiology are HPA-axis dysfunction, environmental issues, neurogenesis impairment (brain derived neurotrophic factor), inflammatory cytokines (such as higher release in TNF-α, interleukin-6, and lower in interleukin-10 level), and oxidative stress [65-70]. Oxidative stress occurs due to an imbalance between the capacity of ROS/RNS species production and their neutralization, or removal, by numerous enzymatic and non-enzymatic antioxidant approaches [71, 72]. Further, the imbalance in ROS/RNS stimulate proinflammatory responses and aggravate the cardiovascular abnormalities [73, 74]. The involvement of psychological stress and oxidative stress in the

development depression and cardiac diseases is well established and documented [75]. Some clinical studies state that there is a clear role of low levels of antioxidant system in depression and cardiac diseases [76-80]. Taken together, the major pathophysiologic characteristics of MI are ischemia, inflammation , thrombus formation, aggregation of platelets, genetically, cell rupture, mitochondrial dysfunction, and oxidative stress [81] (*Figure 2*).

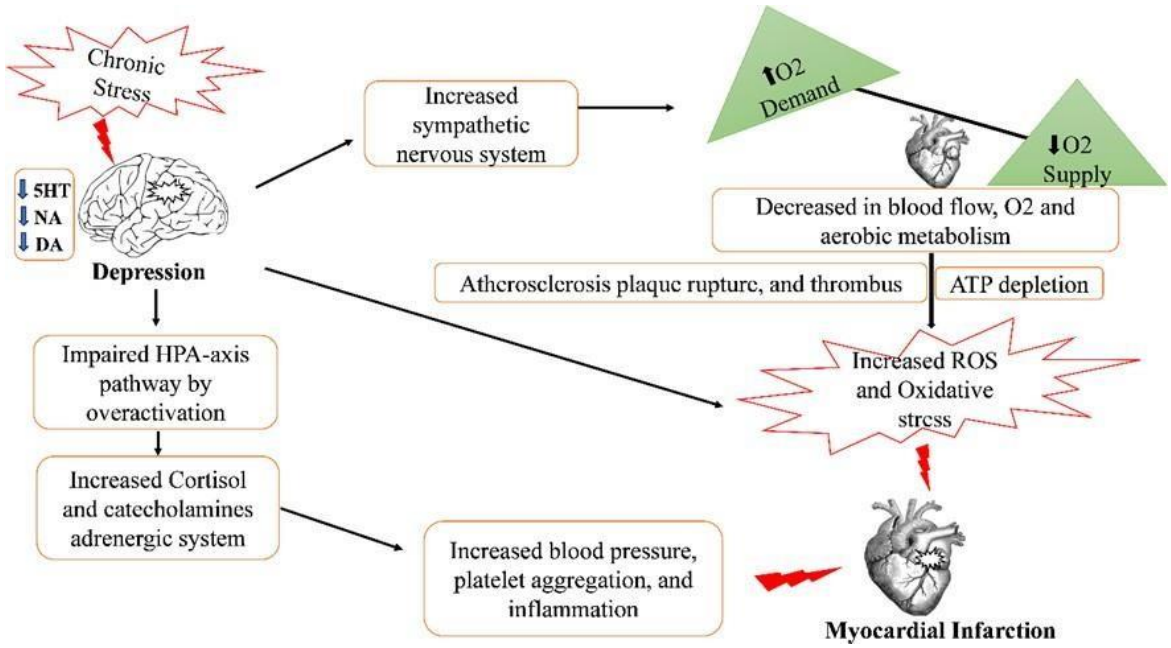

Figure 2: Pathophysiology of depression associated MI

1.4 Biological markers of depression and MI

Depression and MI can be diagnosed using different biomarkers related to neuroendocrine system, sleep (polysomnographic), and the brain (decreased size of hippocampus, basal ganglia, frontal lobe and increased white matter subcortical hyperintensity) [82]. In the psychobiology of depression, HPA-axis has been well documented for its role in the severity of depressive disorders [83, 84]. During severe depression, dexamethasone suppression test and CRH test consistently used to measure clinical depression [85].

Some biomarkers are used to assess the severity of depression and MI in this thesis are discussed further.

1.4.1 Cortisol/corticosterone

Cortisol/corticosterone is a major steroid hormone involved in metabolism and gene expressions affecting both the CNS and peripheral tissues [86]. During the stress exposure, cortisol secretion is always fluctuated, whereas normalization of cortisol corelates with the upgrading in patient health [87, 88]. Cortisol is a useful biological marker that help in determining the mental illness and severity of diseases which is an essential face in the prevalence of depressive disorder [89]. Pathologically, Stress leads to the over secretion of CRH and arginine vasopressin hormone [90]. Further the activation of pituitary gland secretes ACTH hormone that goes to the adrenal cortex (adrenal gland) through blood. ACTH stimulates the secretion and synthesis of glucocorticoids primarily cortisol in humans and corticosterone in rats [91]. Then, this glucocorticoid hormone regulate their own secretion through negative feedback mechanism *via* their receptors at the level of hypothalamus and pituitary gland [92]. Amygdala also involved in the facilitatory input to the hypothalamus and plays an important role in the regulation of HPA-axis response [93, 94]. Chronic antidepressant treatment modulate the impaired negative feedback HPA axis *via* normalization of glucocorticoid function and restoration of normal cortisol levels [95]. Therefore, researcher measure cortisol/corticosterone as a core biomarker in the depressive disorder [86]. Saliva, plasma, and serum are the main biological sample of cortisol/corticosterone test [96].

1.4.2 Serotonin

In the last decades, clinician indicated depression might be caused by reduction in serotonin levels and provided an important justification for antidepressant activity of SSRIs [97]. In 1960s, researcher suggested about role of lowered serotonin in depression [98]. But, last year a nature report mentioned about wrong hypothesis of serotonin in depression and author clearly states that “no support for the hypothesis that depression is caused by lowered level of serotonin”[97]. However, current narrative study states about more abnormality in serotonin level during the unmedicated depressive subjects [99]. Further report justified the connection between serotonin and depression *via*, antidepressant as therapeutic option through modification of selective serotonin in the brain [100, 101]. During the brain development, serotonin and its receptors play an important role as regulation of mood, cognition, and anxiety [102]. Researcher report that serotonin and its receptor are highly expressed in the prefrontal cortex part of the brain for regulating mood and behavior [103]. For regulation of emotions and stress, modulation of prefrontal (PFC) serotonin is an essential mechanism [104]. Recent report suggested about lowered level of serotonin in PFC relates with clinical depression and suicide [105]. Similarly in the present study we measured serotonin, in the PFC part of the brain, as a biomarker of depressive disorder in stressor exposed animals.

1.4.3 Brain derived neurotrophic factor (BDNF)

As a potential biomarker of clinical depression and response of antidepressants, BDNF has received considerable attention [106]. BDNF is a neurotrophic extracellular signalling protein that is involved in the survival and development of neurons [107]. It is also one of the most essential neurotrophins biomarker in CNS and highly expressed

in the crucial parts of the brain (hypothalamus, hippocampus, and amygdala) which are involved in the regulation of mood, stress, and anxiety [108]. Physiologically, BDNF exerts its effect *via* binding with tropomyosin related kinase B (TrkB) receptor located in pre and postsynaptic membranes [109]. BDNF involve in the key neural mechanisms such as synaptic plasticity, neuronal growth, and provides resistance to neuronal stress suggesting the role of BDNF in mental health [107, 110]. In the last two decades, primary studies investigated BDNF as one of the essential biomarkers in depressive patient and treatment response [111, 112]. Blood and hippocampal tissue are the major part for estimation of BDNF levels which indicates parallel changes in levels during the depressive disorders [113]. Moreover, plasma levels of BDNF correlates with the suicidal behavior and severity of depression [114]. As per above studies, we also measured BDNF level as a biomarker for depressive disorder and found significant changes depending on the stressor.

Similarly for MI, the biological markers such as cardiac troponin-I (cTn-I,), cardiac troponin-T (cTn-T), myoglobin, creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), and aspartate/alanine aminotransferase (AST/ALT) enzyme are estimated to ascertain diagnosis and severity [115]. They play an important role in the timely prognosing, diagnosing, and accurately management of MI. Electrocardiography is one of the usual platforms where we record the ongoing electrical activity of the heart in the form of electrocardiogram (ECG). A careful analysis of the ECG provides cardiac activity parameters such as T wave inversion, Q wave dysfunction, and ST-segment elevation which are the mainstay of MI diagnosis [116-118]. ST-segment elevation indicates an occlusive thrombus at ruptured coronary plaque [119, 120]. The prognosis of STEMI basically dependent on left ventricular, lifestyle changes, and preventive therapeutic section [121]. Physiologically, heart muscle has excitability property

showed by electrical potential (*via* positive and negative ions across the membrane) which triggered by action potential [122, 123]. This action potential further cause step wise depolarisation as well as repolarisation *via* transfer of ions across the cell membrane through the voltage gated channels leading to contraction and relaxation [124, 125]. For opening and closing of this voltage gated ion channels, ATP is required as energy which is obtained from the cellular metabolism process involving inhaled oxygen [126]. In MI patients, the cardiac muscles are infarcted due to deprived of oxygen leading to reduction in ATP [127]. The lack of ATP further leads to dysfunction of voltage gated ion channels causing accumulation of ions are at the gates of surrounding healthy muscle [128]. The differential cell membrane potential between the healthy muscles and ischemic cause an accumulation of negative charges on the surfaces of membrane [129]. This further leads to generation of a vector towards healthy cardiac cells (have positive charge on the surface of membrane) [130, 131]. However, if vector points towards a myocardial lesion caused a ST segment elevation in transmural (affect entire part of the tissue) or epicardial lesions and ST segment depression if only endocardial part is affected [132].

In addition, dome shaped action potential containing ST segment starts with the J point at the onset of the plateau phase 2. During the several cardiac cells gone through phase 0 rapid depolarization and phase 1 early repolarization. It ends with the T wave when phase 3 repolarisation returns the cardiac cells back into the negative charge resting phase 4 [132]. Generally, ST segment is reflecting as isoelectric horizontal line at the baseline because all cells have the alike potential during phase 2 and no net voltage gradient occurs in the myocardium [133, 134]. But, delayed activation in duration, shape, hight of the action potential cause a voltage gradient between healthy cells and injured cardiac cells that influences the ST segment. The ST segment is

elevated or depressed with many morphologies depending upon the timing and location of lesions in myocardial cells [132].

The difference in electrical potential is another reason of ST segment elevation in that phase of cardiac cycle between the normal parts of heart and dying cardiac muscle and this vector direction of that potential difference as manifest on the ECG. However, under normal condition the ST segment have same charge across the myocardium or isoelectric point [132, 135]. A decreased O_2 perfusion will decrease the ATP required to operate the Na+/K+ channels [136].

Several cardiac biomarkers other than ECG parameters involved in the diagnosis and severity of MI are described below.

1.4.4 Cardiac troponin-I &T

These are the cardiac regulatory proteins which facilitates the action of actin and myosin for controlling the calcium mediated interactions [137]. Specifically, these markers are highly preferred for the non-invasive diagnosis of myocardium necrosis due to their extreme sensitivity and unique expression in the myocardial injury [138]. Compared to cTn-T, cTn-I is more specific biomarker for MI because of its sensitivity and detect MI much earlier (< 4 h) after onset of ischemia [139]. In addition, with rise in the cardiac muscle enzyme, ECG is also another parameter which used as a marker for diagnosing of MI *via* chest pain or ST segment elevation and T wave abnormalities [140]. Researchers reported that the presence of cTn-I in serum is the indication of myocardial damage [141, 142]. Similarly, in our study we also estimated the levels of cTn-I in serum for diagnosing MI in rats.

1.4.5 Creatine kinase-MB and lactate dehydrogenase

For the diagnosis and management of MI, CK-MB an LDH are another rapid testing parameters [143]. These biomarkers are best alternative if troponins are not detected. These markers have two advantages than troponins first, they are released instantaneously after necrosis therefore, we can detect CK-MB and LDH earlier than troponin and secondly, the released CK-MB an LDH are normalized faster in the body which makes them useful in identification of further infarctions [144]. During the tissue necrosis, these enzymes are released into the blood [143]. In earlier studies, plasma CK-MB and plasma LDH were significantly increased in the ischemic model indicating the severity of MI in rats [145, 146]. Similarly, in our study we estimated the plasma level of CK-MB and LDH enzyme after induction of MI in animals.

1.4.6 Aspartate aminotransferase (AST) and alanine aminotransferase (ALT)

During MI, patient showed significantly increased levels of AST and ALT enzyme suggesting the association of enzymes with myocardial damage [147]. According to earlier reports, increased AST and ALT enzyme in STEMI myocardial damage is common and both enzymes are similarly correlates with CK-MB but independently showed severity of MI [148, 149]. Study also found that a greater activity of ALT in plasma during myocardial damage (due to ischemia) [150]. Similarly, another report mentioned that chronic stress model altered the activity of liver enzymes (AST and ALT) during depression and reversed by antidepressants [151]. Therefore, liver enzymes are another biomarker for interpreting the severity of depressive disorder [152, 153]. Accordingly, our study used AST and ALT as a biomarker and found increased activity of AST and ALT enzyme in depression associated MI in rats.

1.4.7 Matrix Metalloproteinase 2 (MMP2)

Matrix metalloproteinase 2 is a protein implicated in the development of heart failure *via* structural changes due to myocardial injury [154]. MMP2 decreases the mechanical properties of heart tissue *via* degradation of extracellular matrix and cTn-I leads to decreased thickness of heart tissue and cardiac dysfunction [155-158]. However, for improving cardiac function, its required to decrease the degradation of extracellular matrix at early stage of MI and preserve the tissue characteristics [159]. Study reports that higher level of MMP2 in heart tissue is also another biomarker to diagnose the myocardial damage and by inhibiting MMP2 one can prevent the cardiac dysfunctions [160]. We have used MMP2 as a biomarker in our study and measured in the heart tissue to identify the myocardium injury in rats.

Furthermore, in our research we have used rosmarinic acid as a therapeutic approach to control these biomarkers while targeting depression associated MI.

1.5 Management approaches

All the depressive patients can be categorized into mild, moderate, or severe based on the score obtained from Patient Health Questionnaire (PHQ-9) and Hamilton Depression Rating Scale (HDRS) [161, 162]. The PHQ-9 is a depression module in which physician scores nine diagnostic criteria and indicates the severity of depression as ‘0’ means (not at all) to ‘3’ (nearly every day) [163, 164]. Whereas, HDRS is also one of the scales to measure the depression in clinical settings. In this scale 21 items are used for indicating about depression and guide to evaluate the recovery. In this scale, depression score measured as 0-7 are being normal, 8-16 mild depression, 17-23, moderate, and more than 24 indicates severe depression [165].

Antidepressant drugs are the mainstay of depression management but a combination strategy including antidepressant drugs (tricyclic antidepressants, selective-serotonin reuptake inhibitor, and MAO inhibitor) and psychotherapy (cognitive behavioral and interpersonal) is used in most patients according to their course of illness [166, 167]. The primary goals of the therapies are to increase neurogenesis and neuronal plasticity in depressed subjects [82, 168]. For depression management, India has National Mental Health Programme (NMHP) that provides life skills training in several educational institutions, stress management approaches, service for suicide prevention [169, 170].

1.5.1 Selective serotonin reuptake inhibitors (SSRIs)

SSRIs classes of medications are the most common treatment prescribed by physicians to depressive patients. Due to their efficacy, safety, and tolerability, SSRIs are often used as first-line treatment for psychiatric disorders like depression [171]. Current SSRIs are fluoxetine, sertraline, paroxetine, citalopram, vilazodone, and fluvoxamine used in major depressive disorder, anxiety, bipolar depression, panic disorder, post-traumatic disorder, and resistant depressive disorder [172]. These SSRIs act by inhibiting serotonin reuptake leading to increase in serotonin activity (*Figure 3*). Apart from this, SSRIs have effects on other neurotransmitters such as dopamine and norepinephrine. At the presynaptic axon terminal, SSRIs inhibit the serotonin transporter leading to an increase in the level of serotonin in the synaptic left and further postsynaptic receptors are stimulated for an extended period [173, 174].

For major depressive illness, fluoxetine is the food and drug administration (FDA) approved selective serotonin reuptake inhibitors (SSRI) medicine prescribed as Prozac brand name [175]. This drug prescribed also to adolescents and child (over 8

age) for acute management and maintenance of depressive patients [176]. In depression, serotonin and norepinephrine both amines are equally involved in the maintenance of mood.

Pathologically, depressive patients show the reduced serotonin levels in the cerebrospinal fluid. In addition, depressive patient also has lower number of serotonin uptake sites. In the dorsal raphe nucleus, presynaptic serotonin (5HT1A) is present and projects to prefrontal cortex. Interestingly, it also has mild activity on 5HT1B and 5HT1C receptors, while it having very low activity on noradrenergic reuptake [177]. The initial antidepressant effect of fluoxetine emerges within 2 to 4 weeks of continuous administration. The active metabolite of fluoxetine is norfluoxetine work during the action of cytochrome P450 (CYP2D6) enzyme. The half-life of fluoxetine is 2 to 4 days while norfluoxetine half-life is 7 to 9 days [178, 179]. Though efficacious, Prozac shows severe side effects such as bleeding risk, serotonin syndrome, sexual dysfunction, suicidal behavior (< 25 old patients), mania, congenital heart defects (during administration of pregnancy), cardiac risk (QT prolongation), and seizures [180, 181]. Furthermore, a study showed two blinded control trial for fluoxetine in child and adolescent that did not reported about their efficacies [182].

1.5.2 Tricyclic antidepressants (TCAs)

Since the introduction of TCAs in the 1950s, pharmacotherapy of TCAs played an essential role in major depression [183]. Researchers reported over SSRI and SNRI, TCAs have therapeutic plasma concentration with optimum efficacies [184, 185]. TCAs are imipramine, desipramine, clomipramine, amitriptyline, nortriptyline, and protriptyline used as a therapeutic option for major depressive disorders (*Figure 3*).

However, the overdose of these TCAs is associated with cardiac issues such as arrhythmia/heart block [186, 187].

1.5.3 Selective norepinephrine reuptake inhibitors (SNRIs)

There are evidence that SNRIs (venlafaxine, duloxetine, milnacipran, and venlafaxine) have superior therapeutic activity against severe depressive disorders [188] (*Figure 3*). The superior activity of SNRIs is credited to increased noradrenaline level in the synaptic cleft and restoration of the synapse.

1.5.4 Monoamine oxidase inhibitors (MAOIs)

There are irreversible non-selective MAO inhibitors (tranylcypromine, phenelzine, isocarboxazid), selective reversible MAO-A inhibitors (moclobemide), and MAO-B inhibitors (selegiline) available to manage depressive disorder [189] (*Figure 3*). Non-selective MAO inhibitors are more suitable for bipolar depression, resistant depression, and atypical depressive disorder [190]. However, with the dietary restrictions, MAOIs are infrequently used due to safety and tolerability concerns. Therefore, current guidelines categorized them as 3rd to 5th line treatment options due to all these concerns [191]. Physiologically, MAO enzymes are found in the membrane of mitochondria majorly in brain, liver, gut and other tissues [192]. Two types of MAO are found with different affinities: MAO-A and MAO-B [193]. In the brain, the metabolism of serotonin, epinephrine, and dopamine occurred due to presence of MAO-A enzyme. Whereas, MAO-B enzyme is mainly involved degradation of dopaminergic neurons [194].

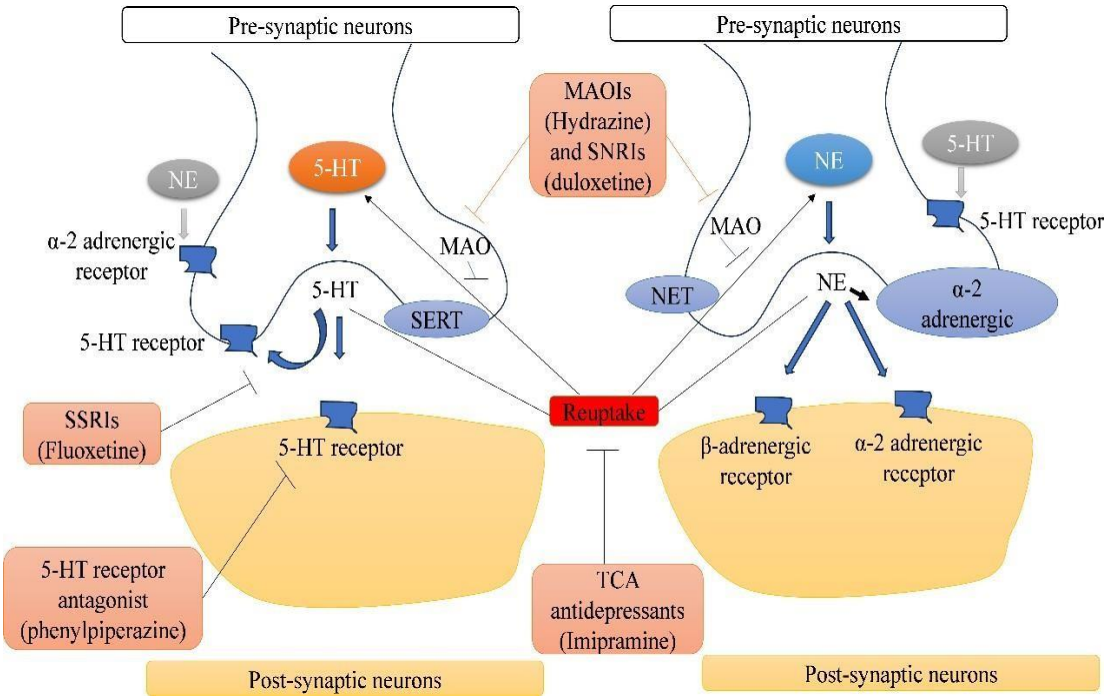

Figure 3: Mechanism of marketed antidepressant drugs

For cardiac disease, drug treatments such as beta-blockers, aspirin, angiotensin-converting enzyme (ACE) inhibitors, and statins for diabetes, hypertension, and high blood lipids are important to reduce CVDs risk [195]. Surgical operations (bypass, balloon angioplasty, valve repair, heart transplantation, and artificial heart transplantation) and medical devices (prosthetic valves, pacemakers, and patches) are also available to manage heart diseases [196, 197]. Symptoms of a heart attack include discomfort or pain in the centre of the chest, arms, left shoulder, jaw, elbow, or back. In addition, shortness of breath, vomiting, faintness, and cold sweat are evident [198]. Some common drugs used in the management and treatment of cardiac abnormalities are β-blockers, ACE inhibitors, diuretics, angiotensin receptor blockers, renin inhibitors, sympatholytic, and antiplatelet agents [199, 200]. Some α and β blockers (prazosin, phentolamine, phenoxybenzamine, propranolol, atenolol), ACE inhibitors (captopril, lisinopril, fosinopril), receptor antagonist (losartan), renin inhibitor

(aliskiren), diuretics (thiazides), and antiplatelet (aspirin) are current drugs to alleviate the cardiac disease [201-203].

1.6 Rosmarinic acid

Overtime, phytochemicals have been considered as alternative therapies to treat several pathological conditions including psychiatric disorder as well as cardiac anomalies. Phenolic compounds are studied extensively against depression and cardiac diseases. Characteristic mechanisms of phenolic phytochemicals make them promising therapeutic option.

Some recent preclinical studies demonstrated about phenolic compound such as rosmarinic acid is effective against several disorder such as depression, anxiety, cardiovascular diseases [204]. In addition, rosmarinic acid (*Figure 4*) possess various pharmacological activities such as hepatoprotective [205], neuroprotective [206], antioxidative [207], lung protective [208], anti-inflammatory activities [209]. Rosmarinic acid (ester of caffeic acid and 3,4 dihydroxy phenyl lactic acid) was first isolated from *Rosmarinus officinalis* L. family- Lamiaceae. Rosmarinic acid was also identified in several plants which belongs to Boraginaceae and Nepetoideae (Labiatae) subfamily including rosemary (*Rosmarinus officinalis*), lemon balm (*Melissa officinalis*), oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), sage (*Salvia officinalis*), and mint (*Mentha piperita*) [210, 211].

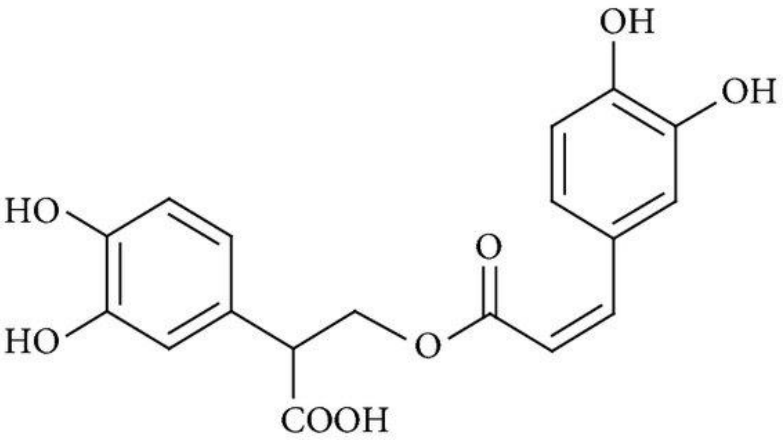

Figure 4: Chemical structure of Rosmarinic acid (3, 4 dihydroxy phenyl lactic acid)

$C_{18}H_{16}O_8$

1.7 Aims

The overall aims of the research conducted in this thesis were to evaluate the cardioprotective effect of rosmarinic acid against myocardial infarction comorbid depression using stressor models. Specifically, the aims were to:

1) Evaluate the prophylactic activity of rosmarinic acid against cardiac abnormalities comorbid depression using chronic unpredictable stress model in Wistar rats (Chapter 2)

2) Evaluate the protective effect of rosmarinic acid against MI comorbid depression using maternal separation model in Wistar rats (Chapter 3)

3) Evaluate the protective effect of rosmarinic acid against myocardial infarction comorbid depression using dual stress model in Wistar rats (Chapter 4)

1.8 Hypothesis

Chronic stress has become an established model of depression and as well as cardiac anomalies. Unlike standard depression and cardiovascular disease treatments

such as antidepressants (SSRI, TCAs, MAO inhibitors) and cardiac protective (Diuretics, nitrates, β-blockers, anticoagulants, antihypertensive drugs) that modulates individual abnormalities *via* established mechanisms. However, using single therapeutic option for comorbid disease is still not established therapeutic option.

One of the homeostasis pathways involved in the regulation of body stress response and heart action is known as HPA axis pathway *Figure 5*. During overactivation of HPA axis due to chronic stress, it leads to the production of cortisol and catecholamines hormone which further activates the aggravation of sympathetic system, decrease heart volume, pro-inflammatory cytokines (TNF-α, IL-10) and reduction of anti-proinflammatory cytokine (IL-10), platelet aggregation causing heart abnormalities like myocardial infarction (MI) *Figure 6*. Similarly, there is numerous evidence suggesting a bidirectional link between depression and cardiac anomalies and both share a common pathophysiology. Apart from HPA axis pathway, some hormone and neurotransmitter such as serotonin also involve in this comorbidity. In depression, hypertension, vasoconstriction, and inflammation play an important role. Furthermore, imbalance in antioxidative system and free radicals increased due to chronic stress led to oxidative stress which is another link between depression and cardiac abnormalities. These radicals further cause overactivation of matrix metalloproteinase (MMP2) which degrades the cytoskeletal proteins (cardiac troponin-I) leading to myocardial injury or myocardial infarction.

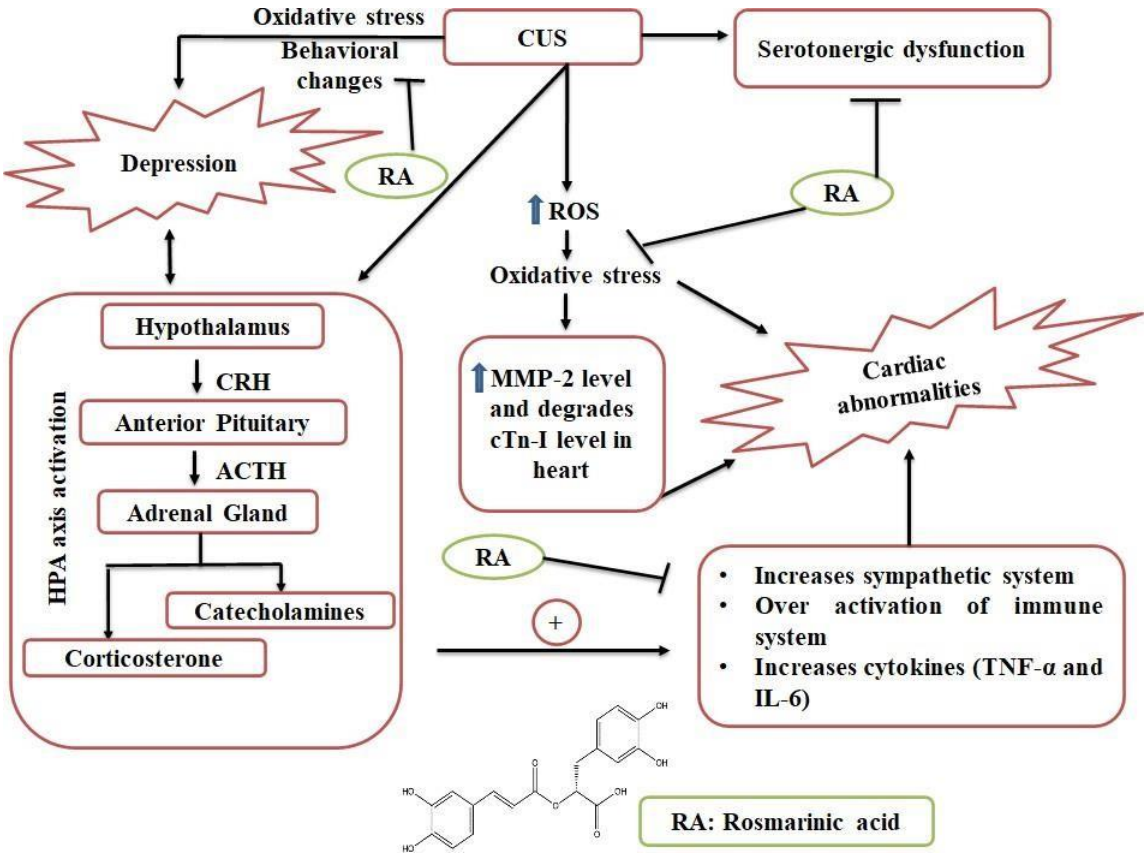

Figure 5: Mechanism of RA against chronic unpredictable stress induced cardiac abnormalities

Schematic representation of possible molecular mechanism of action of rosmarinic acid against CUS induced cardiac abnormalities. RA; rosmarinic acid, CUS; Chronic Unpredectable Stress, CRH; Corticotropin Releasing Hormone, ACTH; Adrenocorticotropic hormone, MMP-2; Mattrix Mettaloprotienase, cTn-I; Cardiac Troponin-I; IL-6; interleukin-6, TNF-α; Tumor Necrosis Factor-α.

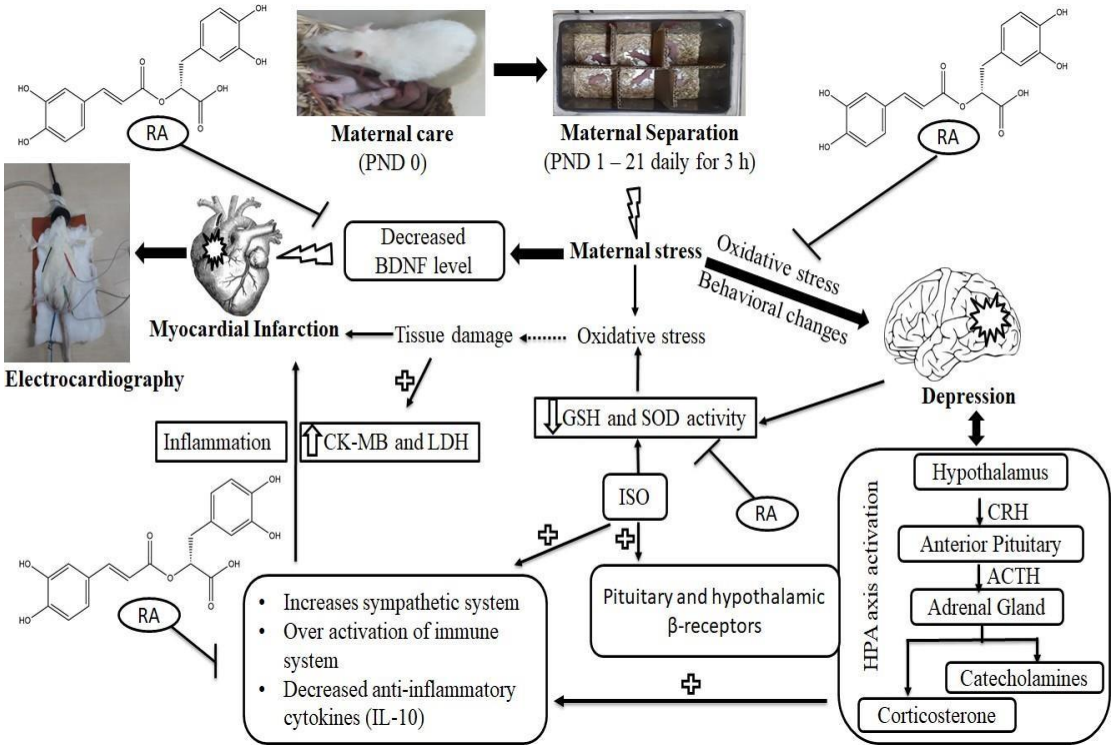

Figure 6: Mechanism of RA against maternal stress induced myocardial infarction.

Schematic representation of possible molecular mechanism of action of rosmarinic acid against MS induced myocardial infarction. RA; Rosmarinic Acid, MS; Maternal Separation, PND; Postnatal Days, ISO; Isoproterenol, BDNF; Brain Derived Neurotrophic Factor, CK-MB; Creatin Kinase-MB, LDH; Lactate Dehydrogenase, CRH; Corticotropin Releasing Hormone, ACTH; Adrenocorticotropic hormone GSH; Glutathione, SOD; Superoxide Dismutase, IL-10; Interleukin-10, MI; Myocardial Infarction.