Abstract

Depression is a common, chronic, and reoccurring disorder characterized by depressed mood, anhedonia, insomnia, loss of appetite, low energy, and loss of interest in activities. By 2030, depression is expected as a leading cause of disability worldwide. Cardiovascular diseases (CVDs) are another leading cause of disability worldwide. CVDs are the group of disorders (congenital heart disease, coronary heart disease, cerebrovascular disease, and rheumatic heart disease) of the blood vessels and heart. In a recent study, death from all heart-related abnormalities was reported to be 18.5 million globally.

A recent report revealed that depression is a common comorbid condition frequently observed in cardiac patients, one out of four patients experience depressive disorders. There is a bidirectional relationship between depression and CVDs. A great body of literature supports the fact that both depression and CVDs share several common pathophysiological mechanisms. The pathophysiology of depression in CVD patient is multifactorial includes inflammation, oxidative stress, and decreased activity of serotonin (5-HT) and brain-derived neurotrophic factor (BDNF). Inflammation and immune activation have been implicated in the pathogenesis of both CVD and depression. It has been suggested that proinflammatory cytokines such as TNF-α and IL-6 have the potential to increase in the heart causing CVDs. Depression is associated with overactivation of hypothalamic-pituitary-adrenal (HPA) axis and overproduction of glucocorticoids leading to negative feedback inhibition. Furthermore, stressful events or exposure contribute in the development of depressive-like behavior. Despite vast improvement in our understanding on MI comorbid depression, there is an unmet need to develop therapeutic strategies to treat both MI and comorbid depression.

Rosmarinic acid (RA) supplementation has been shown to produce cardioprotective and antidepressant activity. On the other hand, fluoxetine is a potent antidepressant recommended as the first-line therapy with serious adverse effects. However, RA has been shown to elicit marked antioxidant activity, anti-inflammatory activity, and antidepressant-like activity with an acceptable adverse effect profile. The pleiotropic pharmacological activities of RA may make it suitable for the treatment of MI comorbid depression, which involves a myriad of pathophysiological characteristics. However, till date, no studies have been conducted to assess the therapeutic potential of RA against MI comorbid depression using Chronic Unpredictable Stress (CUS) and Maternal Separation (MS) model in rats.

Considering all the pathophysiological factors, it can be **hypothesized** that RA treatment strategy which can abrogate the CUS-induced overactivation of HPA axis, inflammation, oxidative stress, and imbalance in neurotransmitter levels would be a possible therapeutic option for treating myocardial infarction and comorbid depression. In the present study, we explored the potential benefits of rosmarinic acid in a rat model of MI comorbid depression that primarily focuses on a clinical situation where occurrence of depression leads to myocardial infarction. Psychiatric disorders and associated cardiac comorbidities have increased the risk of mortality worldwide. Researchers reported that depression increases the possibility of future cardiac abnormalities by approximately 30%. Therefore, there is an unmet need to develop therapeutic interventions to treat depression and associated cardiac abnormalities. The aim of the **first study** was to evaluate the prophylactic effect of rosmarinic acid against **chronic unpredictable stress (CUS)-**induced depression associated cardiac abnormalities in Wistar rats. The CUS paradigm, which comprised several stressors, was employed for 40 days to induce depressive-like behavior and associated cardiac

abnormalities in rats. Along with CUS, rosmarinic acid at a dose of 25 and 50 mg/kg was administered orally to two groups of animals for 40 days. Behavioral tests (forced swim test and sucrose consumption test) and molecular biomarkers (corticosterone and serotonin) were performed. Electrocardiography was performed before CUS at day 0, day 20, and day 40 to study electrocardiogram parameters. Furthermore, changes in body weight, organ weight, tissue lipid peroxidation, glutathione, catalase, cTn-I, MMP-2, and pro-inflammatory cytokines (TNF-α and IL-6) were estimated. Our results showed that rosmarinic acid treatment caused reduction in immobility period, adrenal hyperplasia, corticosterone level, tissue lipid peroxidation, cTn-I, MMP-2, pro-inflammatory cytokines, and QRS complex duration, while an increase in sucrose consumption, brain serotonin level, T-wave width, glutathione, and catalase activity as compared to CUS-control group. The results of our study proved that rosmarinic acid administration ameliorates CUS-induced depression associated cardiac abnormalities in rats *via* serotonergic, oxidative, and inflammatory pathways.

Work stress, childhood abuse, discrimination, death of somebody close, poverty, trauma, isolation, neglect, and maternal separation (MS) are other significant factors that chronically lead to depression. In this context, MS is a considerable risk factor that leads to depression *via* hyperactivation of the HPA axis and oxidative stress. Neglectful parenting is a major worldwide issue for humans. The foremost reasons for neglecting a child or MS are employed women, abandonment, death of the mother, divorce, mother in prison, and boarding schools. MS is characterized by the loss of a mother in childhood, causing depression and cardiovascular diseases due to adverse effects on the HPA axis later in life. A recent study reported MS-induced alterations in behavioral, cardiac, and hippocampal functions.

The **second study** evaluated the effectiveness of RA against MI in comorbid depression induced by **maternal separation** in rats. Maternal stress is one of the childhood crises that may be a potential risk factor for coronary heart disease in later in the life. As per protocol, 70–80 % of pups were separated daily for 3 h between postnatal day 1 (PND1) and postnatal day 21 (PND21). Forced-swim test, sucrose preference test, and electrocardiography were performed during the experiment. Body weight was measured on PND0, PND35, and PND55. Oral administration of rosmarinic acid (25 mg/kg and 50 mg/kg) and fluoxetine (10 mg/kg) was performed from PND35 to PND55. On PND53 and PND54, isoproterenol (100 mg/kg, SC) was administered to induce myocardial infarction. On PND55, blood was collected and animals sacrificed, and plasma corticosterone, brain-derived neurotrophic factor (BDNF), cardiac biomarkers, interleukine-10, and anti-oxidant parameters were measured. Rosmarinic acid and fluoxetine ameliorated the maternal separation induced increase in immobility period, anhedonia, body weight, ST-elevation, corticosterone, creatine kinase-MB (CK-MB), and lactate dehydrogenase (LDH). At the same time, both drugs elevated the tissue levels of BDNF, IL-10, glutathione, and superoxide dismutase. This study provides the first experimental evidence that maternal stress is an independent risk factor of cardiac abnormalities in rats. Moreover, maternal stress synergistically increases the severity of cardiac abnormalities induced by isoproterenol. Interestingly, the reference drug (fluoxetine) and rosmarinic acid effectively ameliorated behavioral anomalies and myocardial infarction in maternally separated rats.

Results from the first and second studies suggest that the existence of both childhood maltreatment (child neglect and abuse) and unpredictable stress in daily living may badly aggravate depression and cardiac abnormalities. To this predicament, both MS and CUS were applied to mimic a condition of a maternally separated

individual confronting unpredictable stress in daily living. In our study, we used MS model by separating pups from mother cages for 15 minutes to 180 minutes and chronic unpredictable stress (CUS) model (isolation, tilt cages, restraint, light exposure, and food and water deprivation for weeks) to induce depressive-like behavior in rodents as previously described. In the third study, we evaluated the cardioprotective effect of rosmarinic acid in **dual stress model** namely: **maternal stress (MS) and chronic unpredictable stress** (CUS) associated cardiac abnormalities. To fulfil this objective, network pharmacology approach, gene ontology, docking (Auto dock), behavioral studies, biochemical studies, molecular studies, and histopathology of heart were performed. Rosmarinic acid (25 and 50 mg/kg) and fluoxetine (10 mg/kg) were administered orally to different treatment groups of rats for 40 days (PND35 to PND75). Isoproterenol (100 mg/kg) was administered on PND 73 and PND 74 to induce cardiac abnormalities in rats. Electrocardiography was performed on PND35, PND55, and PND75, to study changes in electrocardiogram parameters. The **MS combined CUS rats** showed adrenal hyperplasia, ST-segment elevation and an increase in immobility period, corticosterone level, lipid peroxidation, expressions of anti-proinflammatory cytokine, creatine kinase-MB (CKMB) level, lactate dehydrogenase (LDH) level, aspartate transferase (AST) level, alanine transaminase (ALT) level, cardiac troponin (cTn-I) level, and platelet aggregation, while a decrease in sucrose consumption, serotonin level, and superoxide dismutase activity, pro-inflammatory processes (increased levels of proinflammatory and decreased levels of anti-inflammatory cytokines) than individuals stress (MS or CUS) exposure. Interestingly, drug treatment provided protection against stress models (MS and CUS) and isoproterenol induced cardiac abnormalities *via* serotonergic, oxidative, and inflammatory pathways in Wistar rats.

Rosmarinic acid monotherapy was highly efficacious and found to be a potential therapeutic option against MI comorbid depression *via* reducing oxidative stress, inflammation, BDNF modulation, serotonergic modulation, and HPA axis pathway. Restoration of monoamine levels by RA treatment is possibly through alterations in BDNF modulation and behavioral improvement. *In-silico* studies identified the possible pathways and target receptors through which RA is showing therapeutic efficacy in MI comorbid depression.