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## Research Article

# Therapeutic Potential of Polyherbal Formulation Against Experimentally Induced Insulin Resistant Myocardial Infarction in Rats

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## Abstract

**Background and Objective:** The present study was aimed to investigate the effect of polyherbal formulation Rumalaya Forte on experimentally induced insulin resistant myocardial infarction in rats. **Materials and Methods:** The effect of polyherbal formulation Rumalaya Forte (160 and 320 mg kg<sup>-1</sup> b.wt., p.o.) was studied on the high fat diet induced insulin resistant myocardial infarction animal model. Vitamin E (100 mg kg<sup>-1</sup> b.wt., p.o.) was used as standard. The study was evaluated with the help of various biochemical parameters and histopathological examination. **Results:** Results showed that the Rumalaya Forte treatment prevented myocardial infarction, increase in blood glucose level, body weight and weight gain due to antioxidant property and showed a significant dose dependent decrease in the parameters like alanine aminotransferase, aspartate aminotransferase, creatinine phosphokinase, total cholesterol, total triglycerides, weight and number of adipocytes and in infarction size. The histopathological examination also reveals the significant protection in the Rumalaya Forte treated group with a minimal or no red coloration in a dose dependent manner. Rumalaya Forte 160 mg kg<sup>-1</sup> b.wt., p.o. groups showed relatively less disruption of myofibers with Rumalaya Forte 320 mg kg<sup>-1</sup> b.wt., p.o. and vitamin E showing maximum fiber integrity. Rumalaya Forte 320 mg kg<sup>-1</sup> b.wt., p.o. was found to be more effective compared to Rumalaya Forte 160 mg kg<sup>-1</sup> b.wt., p.o. **Conclusion:** The pleiotropic effect of Rumalaya Forte can be utilized as add on therapy for treatment of myocardial infarction.

**Key words:** Vitamin E, Rumalaya Forte, high fat diet induced model, myocardial infarction

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**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Myocardial Infarction (MI) has been ranked as the major factor contributing to the occurrence and severity of Coronary Heart Diseases (CHDs). Coronary heart disease is a common cardiovascular disease which is also an important reason to cause human death<sup>1-3</sup>. Myocardial infarction is a common ailment in society due to change of lifestyle and food intake. Apart from medication, diet also plays a vital role in the management of lipid and lipoprotein concentrations in blood. Earlier studies have shown that High Fat Diet (HFD) also leads to Insulin Resistance (IR) because the Saturated Fatty Acids (SFA) interfere with the action of insulin. The HFD leads to insulin resistance which enhances the probability of developing diabetes mellitus and its associated complications such as diabetic nephropathy, neuropathy, retinopathy, gastroparesis and oxidative stress etc. Oxidative stress also occurs as a result of decreased antioxidant defenses and has been related to the pathogenesis of myocardial infarction. The essential aspect in the etiology of these disorders is insulin resistance which is linked to a wide range of other complications including hyperlipidemia and myocardial infarction etc<sup>4</sup>.

Cardiovascular disorder is the principal cause of mortality among individuals with diabetes mellitus and more than 50% of patients may die from a cardiovascular event especially coronary artery disease but also stroke and peripheral vascular disease<sup>5</sup>. Several studies have demonstrated weight gain, hyperinsulinemia and insulin resistance in those receiving high fat diet<sup>6</sup>.

Isoprenaline (ISO) is a  $\beta$ -agonist and has been found to produce stress in the heart due to the production of free radicals by its auto-oxidation. Some of the mechanisms anticipated to explain ISO-induced damage to cardiac myocytes include coronary hypotension, hypoxia, energy depletion, calcium overload and excessive production of free radicals as a result of catecholamine auto-oxidation<sup>7,8</sup>. There are strong facts that adrenochrome and other oxidation metabolites of catecholamines can cause contractile failure of rat heart and cell necrosis<sup>9</sup>.

Rumalaya Forte is a herbal product that has been marketed in India and currently used in ayurvedic system for treating rheumatoid arthritis, osteoarthritis, cervical and lumbar spondylitis, traumatic inflammatory condition like fibrositis, bursitis, synovitis, capsulitis, tenosynovitis, myositis, sciatica, arthralgia, gout and frozen shoulder. Rumalaya Forte is made up of six aqueous extracts of 28.57% of *Commiphora wightii*, 34.29% of *Boswellia serata*, 7% of *Glycyrrhiza glabra*, 7% of *Alpinia galanga*, 8.6% of *Tinospora cardifolia* and 8.6% of *Tribulus terrestris*<sup>10</sup>. This polyherbal formulation has

been traditionally used by the ayurvedic practitioners in India for the treatment of various inflammatory disorders. Rumalaya Forte demonstrated a significant Nitrous Oxide (NO) free radical scavenging activity<sup>11</sup>. The present study was therefore designed to assess the effect of Rumalaya Forte (160 and 320 mg kg<sup>-1</sup> b.wt., p.o.) against experimentally induced insulin resistant myocardial infarction in rats. Vitamin E (100 mg kg<sup>-1</sup> b.wt., p.o.) was taken as a standard.

The present study was also aimed to develop an experimental model of myocardial infarction and insulin resistance using high fat diet. This study had also planned to confirm the efficacy of Rumalaya Forte using standard drug vitamin E, a known insulin sensitizer in Indian traditional systems of medicine that could be used for the treatment of heart ailments.

## MATERIALS AND METHODS

**Animals:** The experiments were carried out with male Sprague-Dawley rats, weighing 180-200 g, obtained from CSIR-Indian Institute of Toxicology Research, Lucknow, Uttar Pradesh, India. Research on experimental animals was conducted in accordance with the internationally accepted principles for laboratory animal use and care (1088/07/CPCSEA). They were kept in polyacrylic cages (22.5 × 37.5 cm) and were maintained under standard housing conditions (room temperature of 24-27°C and humidity of 60-65%) with a 12 h light/12 h dark cycle. Food and water were available *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethics Committee which follow the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and conform to the international norms of the Indian National Science Academy. Ethical norms were strictly followed during all experimental procedures [Hygia/M.Pharm./22/2014-15].

**Drugs and chemicals:** Test drug marketed polyherbal drug Rumalaya Forte was obtained from Himalaya Drug Company (Bengaluru, India). Standard drug vitamin E was obtained from Central Drug House Private Ltd. (New Delhi, India). Isoprenaline was obtained from Sigma-Aldrich (St. Louis, MO, USA).

### Experimental design and administration of Rumalaya Forte:

Animals were divided into seven groups with six rats each. One group was designated control that fed a balanced, purified diet containing protein and carbohydrate (standard laboratory diet). The second group, fourth group, fifth group, sixth group and seventh group were fed high fat diet containing 20% fat and 4% vitamin and master mineral

mixture for 90 days. Third group was treated with isoprenaline (85 mg kg<sup>-1</sup>, s.c.). The fourth group was fed with high fat diet for 90 days and after that treated with isoprenaline (85 mg kg<sup>-1</sup>, s.c.). The fifth group and sixth group were treated with test drug Rumalaya Forte 160 and 320 mg kg<sup>-1</sup> b.wt., p.o. and seventh group was treated with standard drug vitamin E (100 mg kg<sup>-1</sup> b.wt., p.o.). The standard laboratory diet and high fat diet was all fed in lumps or pellets form are provided *ad libitum* for 90 days and food intake and body weight were recorded daily. All animals were maintained on a 12 h light/12 h dark cycle for the duration of the experimental feeding period.

**Group I: Control:** All the rats were fed with Standard Laboratory Diet (SLD) upto 90 days, after that no treatment was given

**Group II: HFD control (HFD):** All the rats were fed with High Fat Diet (HFD) upto 90 days

**Group III: Isoprenaline control (ISO):** All the rats were fed with Standard Laboratory Diet (SLD) upto 90 days, after that treated with isoprenaline (85 mg kg<sup>-1</sup>, s.c.) for 2 days

**Group IV: HFD+isoprenaline control (HFD+ISO):** All the rats were fed with High Fat Diet (HFD) upto 90 days, after that treated with isoprenaline (85 mg kg<sup>-1</sup>, s.c.) for 2 days

**Group V: Rumalaya Forte treated I (RF I):** All the rats were fed with High Fat Diet (HFD) along with Rumalaya Forte (160 mg kg<sup>-1</sup>, p.o.) upto 90 days, after that treated with isoprenaline (85 mg kg<sup>-1</sup>, s.c.) for 2 days

**Group VI: Rumalaya Forte treated II (RF II):** All the rats were fed with High Fat Diet (HFD) along with Rumalaya Forte (320 mg kg<sup>-1</sup>, p.o.) upto 90 days, after that treated with isoprenaline (85 mg kg<sup>-1</sup>, s.c.) for 2 days

**Group VII: Vitamin E treated (VIT E):** All the rats were fed with High Fat Diet (HFD) along with vitamin E (100 mg kg<sup>-1</sup>, p.o.) upto 90 days, after that treated with isoprenaline (85 mg kg<sup>-1</sup>, s.c.) for 2 days

**Biochemical estimation:** At the end of the experimental period, blood was collected from retro-orbital sinus in EDTA coated vial under mild ether anesthesia. Plasma was obtained by cold centrifugation (4°C) of the vials for 10 min at 3000 rpm. Later, animals were sacrificed by cervical dislocation and epididymal fat pad and heart were excised and fixed in 10% buffered paraformaldehyde. Plasma was used to measure intraperitoneal glucose tolerance test<sup>12</sup>, intraperitoneal insulin response test<sup>12</sup>, total cholesterol level<sup>13</sup>, total triglycerides level<sup>14</sup>, alanine aminotransferase ALT<sup>15</sup>, aspartate aminotransferase AST<sup>15</sup> and creatinine phosphokinase (CK-MB)<sup>16</sup>. Histopathology of epididymal fat pad and heart were also done.

**Statistical analysis:** Data were expressed as Mean ± SEM. The statistical significance of differences between the groups was determined by one-way ANOVA followed by Bonferroni's multiple comparison tests using the software GraphPad Prism 5 (San Diego, CA, USA).

## RESULTS

**Effect of Rumalaya Forte and vitamin E on body weight:** The HFD and HFD+ISO groups showed significant increase in the body weight (p<0.05) compared to control but ISO group did not show any significant alterations in the body weight as compared to control. The RF I and II showed significant dose dependent decrease in the body weight (p<0.001) as compared to HFD and HFD+ISO groups. Standard drug vitamin E also showed significant decrease in the body weight (p<0.001) as compared to HFD and HFD+ISO groups (Fig. 1).

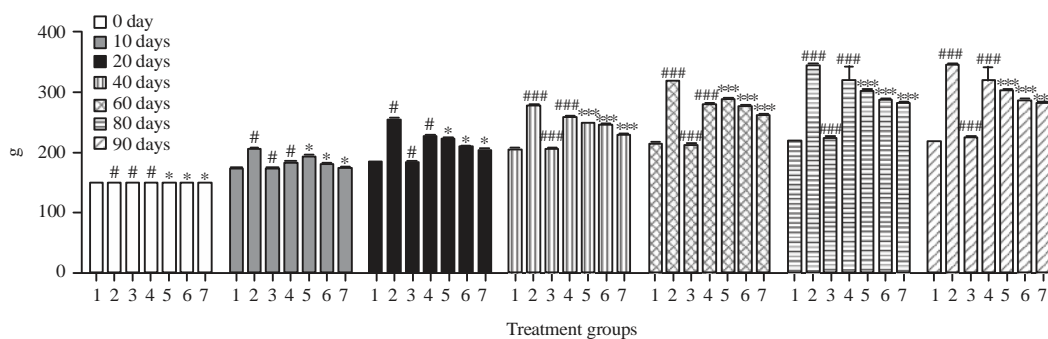


Fig. 1: Effect of Rumalaya Forte and vitamin E on body weight, data were expressed as Mean ± SEM, \*Significant difference (#p<0.05, ##p<0.01 and ###p<0.001) when control vs HFD, ISO, HFD+ISO and (\*p<0.05 and \*\*\*p<0.001) when HFD+ISO vs RF I, RF II and vitamin E

**Effect of Rumalaya Forte and vitamin E on weight gain:** The HFD and HFD+ISO groups showed significant increase in the weight gain ( $p < 0.001$ ) as compared to control but ISO group did not show any significant alterations in weight gain as compared to control. The RF I and II showed significant dose dependent decrease in the weight gain ( $p < 0.05$ ) as compared to HFD and HFD+ISO groups. Standard drug vitamin E also showed significant decrease in the weight gain ( $p < 0.05$ ) as compared to HFD and HFD+ISO groups (Fig. 2).

**Effect of Rumalaya Forte and vitamin E on food intake:** The HFD and HFD+ISO groups showed increase in food intake ( $p < 0.05$ ) as compared to control but ISO group did not show any significant alterations in food intake as compared to control. The RF I and II showed significant dose dependent decrease in food intake ( $p < 0.05$ ) as compared to HFD and HFD+ISO groups. Standard drug vitamin E also showed

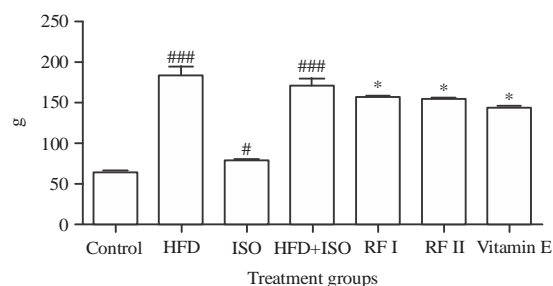


Fig. 2: Effect of Rumalaya Forte and vitamin E on weight gain, data were expressed as Mean  $\pm$  SEM, \*Significant difference ( $\#p < 0.05$  and  $###p < 0.001$ ) when control vs HFD, ISO, HFD+ISO and ( $*p < 0.05$ ) when HFD+ISO vs RF I, RF II and vitamin E

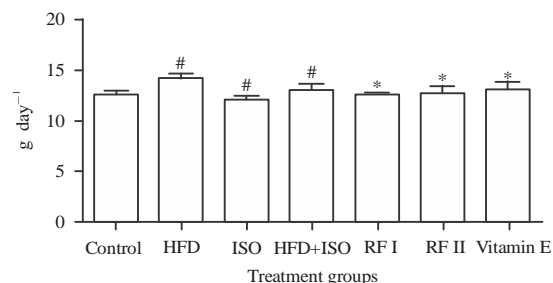


Fig. 3: Effect of Rumalaya Forte and vitamin E on food intake, data were expressed as Mean  $\pm$  SEM, \*Significant difference ( $\#p < 0.05$ ) when control vs HFD, ISO, HFD+ISO and ( $*p < 0.05$ ) when HFD+ISO vs RF I, RF II and vitamin E

significant decrease in food intake ( $p < 0.05$ ) as compared to HFD and HFD+ISO groups (Fig. 3).

**Effect of Rumalaya Forte and vitamin E on food efficiency ratio:** The HFD and HFD+ISO groups showed significant increase in food efficiency ratio ( $p < 0.001$ ) as compared to control but ISO group did not show any significant alterations in food efficiency ratio as compared to control. The RF I and II showed significant dose dependent decrease in food efficiency ratio ( $p < 0.05$ ) as compared to HFD and HFD+ISO groups. Standard drug vitamin E also showed significant decrease in food efficiency ratio ( $p < 0.05$ ) as compared to HFD and HFD+ISO groups (Fig. 4).

**Effect of Rumalaya Forte and vitamin E on intraperitoneal glucose tolerance test:** The HFD and HFD+ISO groups showed significant elevation in glucose level ( $p < 0.01$ ) at 30 min that failed to return to its normal level at 120 min. In HFD group, glucose level was significantly higher ( $p < 0.01$ ) compared to control. However, RF I and II showed dose dependent decrease in glucose level ( $p < 0.01$  and  $p < 0.05$ ) compared to that of HFD and HFD+ISO groups. Standard drug vitamin E also showed significant decrease in glucose level ( $p < 0.05$ ) compared to HFD and HFD+ISO groups. The RF II was found to be more effective compared to RF I (Fig. 5).

**Effect of Rumalaya Forte and vitamin E on intraperitoneal insulin response test:** The HFD group showed significant elevation in glucose level ( $p < 0.05$ ) compared to control. However, RF I and II showed dose dependent decrease in glucose level ( $p < 0.05$ ) compared to that of HFD and HFD+ISO groups. Standard drug vitamin E also showed significant

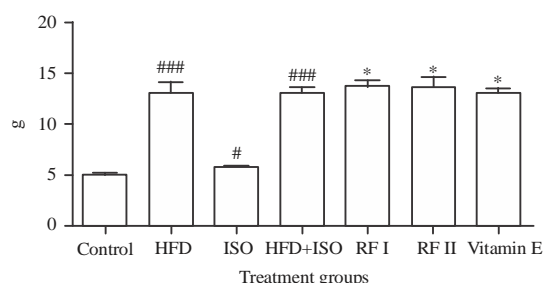


Fig. 4: Effect of Rumalaya Forte and vitamin E on food efficiency ratio, data were expressed as Mean  $\pm$  SEM, \*Significant difference ( $\#p < 0.05$  and  $###p < 0.001$ ) when control vs HFD, ISO, HFD+ISO and ( $*p < 0.05$ ) when HFD, ISO and HFD+ISO vs RF I, RF II and vitamin E

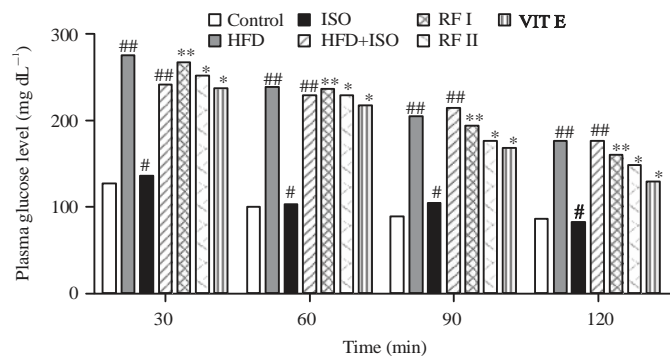


Fig. 5: Effect of Rumalaya Forte and vitamin E on intraperitoneal glucose tolerance test, data were expressed as Mean  $\pm$  SEM, \*Significant difference (# $p < 0.05$  and ## $p < 0.01$ ) when control vs HFD, ISO, HFD+ISO and (\* $p < 0.05$  and \*\* $p < 0.01$ ) when HFD+ISO vs RF I, RF II and vitamin E

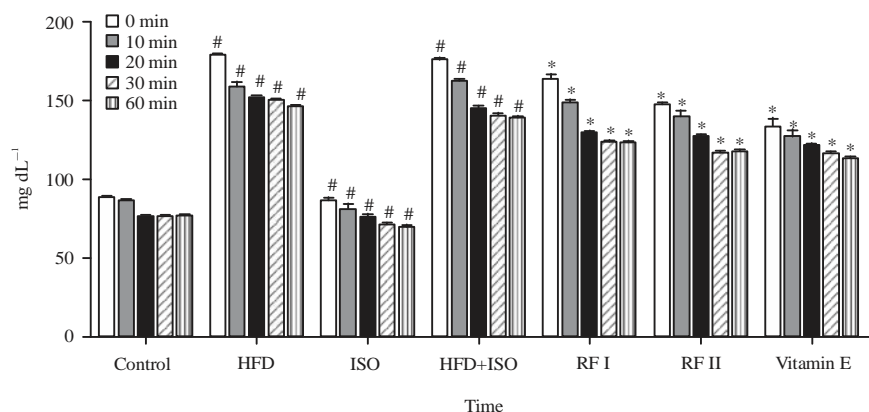


Fig. 6: Effect of Rumalaya Forte and vitamin E on intraperitoneal insulin response test, data were expressed as Mean  $\pm$  SEM, \*Significant difference (# $p < 0.05$ ) when control vs HFD, ISO, HFD+ISO and (\* $p < 0.05$ ) when HFD+ISO vs RF I, RF II and vitamin E

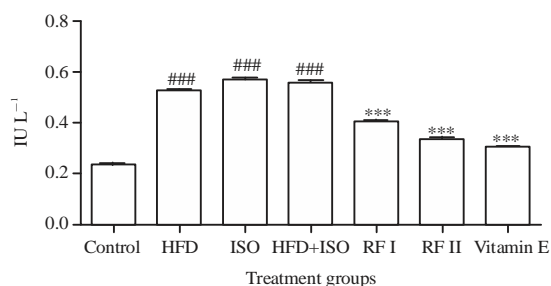


Fig. 7: Effect of Rumalaya Forte and vitamin E on creatinine phosphokinase (CK-MB) level, data were expressed as Mean  $\pm$  SEM, \*Significant difference (### $p < 0.001$ ) when control vs HFD, ISO, HFD+ISO and (\*\*\*) $p < 0.001$ ) when HFD+ISO vs RF I, RF II and vitamin E

decrease in glucose level ( $p < 0.05$ ) compared to HFD and HFD+ISO groups. The RF II was found to be more effective compared to RF I (Fig. 6).

**Effect of Rumalaya Forte and vitamin E on creatinine phosphokinase (CK-MB) level:** The HFD, ISO and HFD+ISO groups showed significant increase in plasma CK-MB level ( $p < 0.001$ ) compared to control. However, pretreatment with RF I and II prevented the increase in CK-MB level ( $p < 0.001$ ) in a dose dependent manner. Standard drug vitamin E also prevented the increase in CK-MB level ( $p < 0.001$ ) compared to HFD, ISO and HFD+ISO groups. The RF II was found to be more effective compared to RF I (Fig. 7).

**Effect of Rumalaya Forte and vitamin E on aspartate aminotransferase (AST) level:** The HFD, ISO and HFD+ISO groups showed significant increase in plasma AST level ( $p < 0.001$ ) compared to control. However, pretreatment with RF I and II prevented the increase in AST level ( $p < 0.001$ ) in a dose dependent manner. Standard drug vitamin E also prevented the increase in AST level ( $p < 0.001$ ) compared to

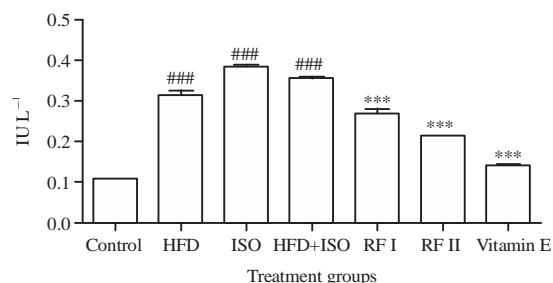


Fig. 8: Effect of Rumalaya Forte and vitamin E on aspartate aminotransferase (AST) level, data were expressed as Mean  $\pm$  SEM, \*Significant difference ( $^{\#}p < 0.01$  and  $^{\#\#}p < 0.001$ ) when control vs HFD, ISO, HFD+ISO and ( $^{**}p < 0.01$  and  $^{***}p < 0.001$ ) when HFD+ISO vs RF I, RF II and vitamin E

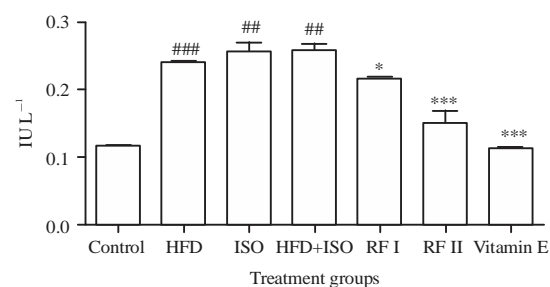


Fig. 9: Effect of Rumalaya Forte and vitamin E on alanine aminotransferase (ALT) level, data were expressed as Mean  $\pm$  SEM, \*Significant difference ( $^{\#}p < 0.05$ ,  $^{\#\#}p < 0.01$  and  $^{\#\#\#}p < 0.001$ ) when control vs HFD, ISO, HFD+ISO and ( $^{*}p < 0.05$  and  $^{***}p < 0.001$ ) when HFD+ISO vs RF I, RF II and vitamin E

HFD, ISO and HFD+ISO groups. The RF II was found to be more effective compared to RF I (Fig. 8).

**Effect of Rumalaya Forte and vitamin E on alanine aminotransferase (ALT) level:**

The HFD, ISO and HFD+ISO groups showed significant increase in plasma ALT level ( $p < 0.001$ ) compared to control. However, pretreatment with RF I and II prevented the increase in ALT level ( $p < 0.001$ ) in a dose dependent manner. Standard drug vitamin E also prevented the increase in ALT level ( $p < 0.001$ ) compared to HFD, ISO and HFD+ISO groups. The RF II was found to be more effective compared to RF I (Fig. 9).

**Effect of Rumalaya Forte and vitamin E on cholesterol level:**

The HFD, ISO and HFD+ISO groups showed significant increase in cholesterol level ( $p < 0.001$ ) compared to control.

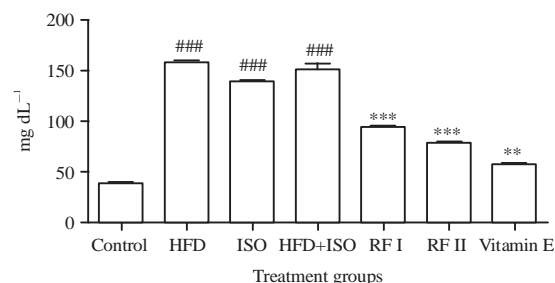


Fig. 10: Effect of Rumalaya Forte and vitamin E on cholesterol level, data were expressed as Mean  $\pm$  SEM, \*Significant difference ( $^{\#\#\#}p < 0.001$ ) when control vs. HFD, ISO, HFD+ISO and ( $^{**}p < 0.01$  and  $^{***}p < 0.001$ ) when HFD+ISO vs RF I, RF II and vitamin E

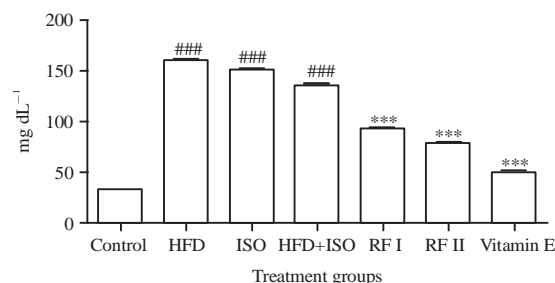


Fig. 11: Effect of Rumalaya Forte and vitamin E on triglyceride level, data were expressed as Mean  $\pm$  SEM, \*Significant difference ( $^{\#\#\#}p < 0.001$ ) when control vs HFD, ISO, HFD+ISO and ( $^{***}p < 0.001$ ) when HFD+ISO vs RF I, RF II and vitamin E

However, RF I and RF II showed dose dependent significant decrease in cholesterol level ( $p < 0.001$ ) and standard drug vitamin E also showed significant decrease in cholesterol level ( $p < 0.01$ ) compared to HFD, ISO and HFD+ISO groups. The RF II was found to be more effective compared to RF I (Fig. 10).

**Effect of Rumalaya Forte and vitamin E on triglyceride level:**

The HFD, ISO and HFD+ISO groups showed significant increase in triglyceride level ( $p < 0.001$ ) compared to control. However, RF I and RF II showed dose dependent significant decrease in triglyceride level ( $p < 0.001$ ) and standard drug vitamin E also showed significant decrease in triglyceride level ( $p < 0.001$ ) compared to HFD, ISO and HFD+ISO groups. The RF II was found to be more effective compared to RF I (Fig. 11).

**Effect of Rumalaya Forte and vitamin E on weight and number of epididymal fat pad:**

Microscopic examination of epididymal fat pad of HFD+ISO group showed a significant



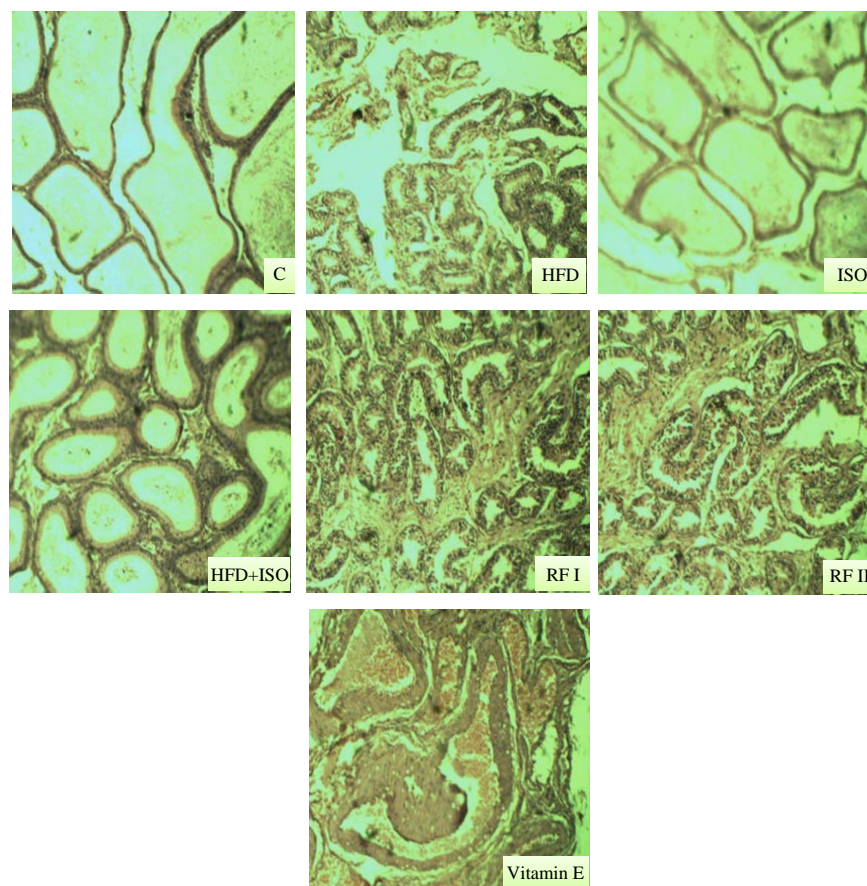


Fig. 12: Photomicrograph of epididymal fat pad showing the effect of Rumalaya Forte and vitamin E on adipocyte morphology, C: Control, HFD: High fat diet control, ISO: Isoprenaline control, HFD+ISO: High fat diet+isoprenaline control, RF I: Rumalaya Forte treated I, RF II: Rumalaya Forte treated II and magnification 400X

increase in weight and number of adipocytes compared to the adipocytes of control group. The RF I and II groups showed adipocytes with mixed dimensions. However, the overall score of measurements of weight and number of adipocytes recorded in RF I and II were significantly lower than that of HFD+ISO group. Vitamin E also showed moderate decrease in weight and number of adipocytes compared to HFD+ISO group (Fig. 12).

**Histopathology of heart:** The TTC staining of heart of control rats showed more number of viable cells whereas, ISO, HFD and HFD+ISO groups showed large area of red coloration, indicated myocardial necrosis areas. However, RF showed a protective effect with a minimal or no red coloration in a dose dependent manner. Hematoxylin-eosin staining of cardiac tissues from control rats showed histoarchitecture of myofibers that were characteristically multinucleated and intact. The ISO treatment resulted in focal

myocardial necrosis (red color) and disrupted myofibers. However, RF I groups showed relatively less disruption of myofibers to RF II and vitamin E showing maximum fiber integrity. The RF II was found to be more effective compared to RF I (Fig. 13).

## DISCUSSION

Metabolic syndrome encompasses cluster of risk factors for cardiovascular diseases which includes abdominal obesity, dyslipidemia, hypertension and hyperglycemia<sup>17</sup>.

Insulin resistance impaired biological response to insulin. In general insulin resistance can be due to a pre-receptor or post-receptor abnormality. One signaling pathway for insulin and IGF-I is the phosphatidylinositol 3-phosphate system. Upon binding of the receptor autophosphorylation of  $\beta$  subunit which mediate stable interaction between receptor and cellular protein<sup>18</sup>.



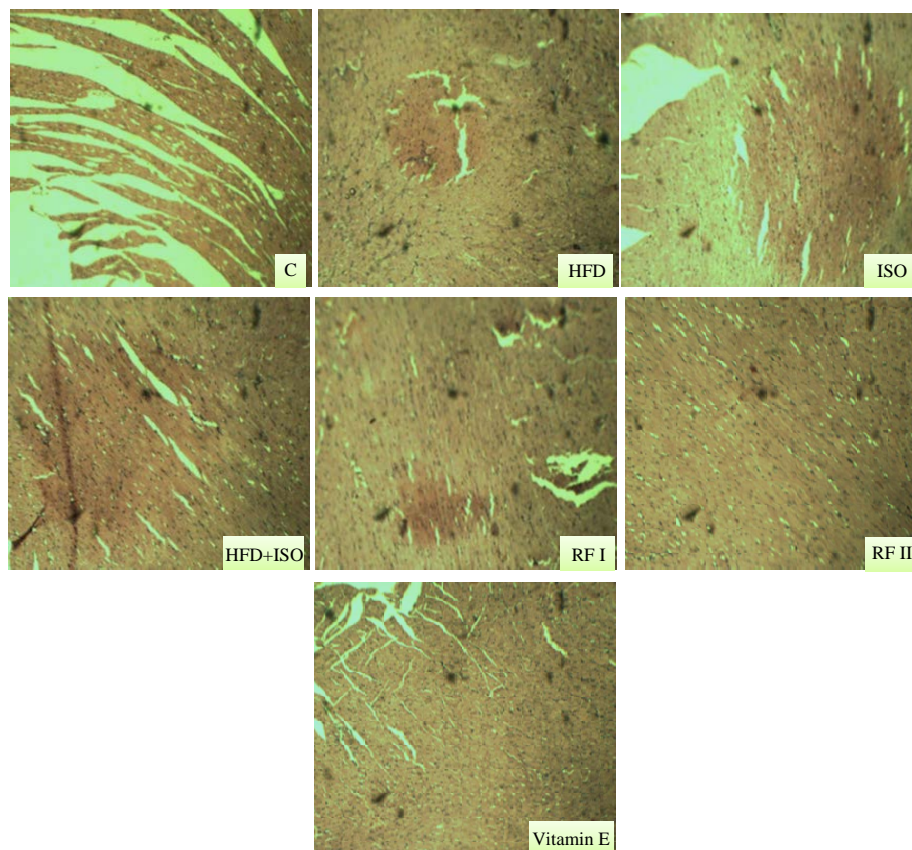


Fig. 13: Effect of Rumalaya Forte on cardiac histopathology of cardiac tissue, tissue sections (7  $\mu$ M) are stained with hematoxylin-eosin (400X), red spotted area indicates myocardial necrosis areas whereas, arrows indicate healthy myofibers, C: Control, HFD: High fat diet control, ISO: Isoprenaline control, HFD+ISO: High fat diet+Isoprenaline control, RF I: Rumalaya Forte treated I, RF II: Rumalaya Forte treated II and magnification 400X

Over nutrition is a major cause of insulin resistance. Insulin resistance due to over nutrition has been best characterized in the liver. More food intake impairs fatty acid oxidation with redirection of long-chain acyl-coenzyme A (CoAs) to pathway that supports the production of diacylglycerol and triglycerides. This is mediated by increase in the concentration of malonyl-CoA by insulin. In addition, insulin inhibits the expression of  $\beta$ -oxidation enzymes in the hepatocyte. Accumulation of fat leads to liver steatosis, imbalance in free fatty acid availability and impairment in the oxidative capacity of mitochondria in the end causing mitochondrial dysfunction and further buildup of fats in the cell. Multiple observations have identified an increase in the risk of insulin resistance with heart diseases<sup>19</sup>.

Adverse cardiac remodeling after myocardial infarction leads to progressive heart failure. Obese insulin resistance increases risk of myocardial infarction and heart failure<sup>20</sup>.

Insulin resistance is a specific aspect of type 2 diabetes mellitus and obesity and impacts the heart in several ways.

Impaired insulin-mediated glucose uptake is a consistently observed characteristic of the heart. Although, insulin signaling may directly regulate cardiac metabolism, its chief role is likely the regulation of substrate delivery from the periphery to the heart. In addition to promoting glucose uptake, insulin regulates long-chain fatty acid uptake, protein production and vascular function in the normal cardiovascular system. Recent advances in understanding the role of metabolic, signaling and inflammatory pathways in obesity have provided opportunities to better understand the pathophysiology of insulin resistance in the heart<sup>21-23</sup>. Myocardial Infarction (MI) is the acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand. Inflammation is a key process involved in mediating myocardial tissue damage after an ischemic event. Myocardial infarction is one of the most common manifestations of cardiovascular disease. The morbidity and mortality due to myocardial infarction is now reaching epidemic proportion throughout the world.

It has been reported that myocardial infarction occurs due to insulin resistance which can be induced by high fat diet<sup>24</sup>. Hyperglycemia, hyperlipidemia, hypertension and inflammation induced oxidative stress are major risk factor for the development of microvascular pathogenesis in diabetic myocardium which results in abnormal gene expression, altered signal transduction and the activation of pathways leading to programmed myocardial cell deaths<sup>25</sup>. Sasikumar and Shyamala Devi<sup>26</sup> have also studied the effect of Abana, a polyherbal formulation on isoproterenol-induced myocardial infarction in rats. In this study, they have stated that isoproterenol treatment results in significant decrease in the activities of enzymes like CK, LDH, AST and ALT in heart with subsequent increase in the activities in serum when compared to normal and Abana treatment minimised the changes. The result indicates that Abana pretreatment offers significant protection to myocardium against the damage caused by isoproterenol induced lipid peroxidation. Manjula *et al.*<sup>27</sup> have also studied the effect of aspirin on isoproterenol-induced myocardial infarction in rats. An increase in the activities of marker enzymes in serum could be due to the leakage of enzymes from heart as a result of isoproterenol induced necrosis and the amount of enzymes appear in serum in proportion to the number of necrotic cells.

At present, the mechanism of myocardial infarction is not completely clear yet. A lot of epidemiological evidences have proven that the incidence of cardiovascular diseases is closely related to metabolic disorder like diabetes and also due to high-fat diet that can establish myocardial infarction in rat model<sup>28-31</sup>.

To check the effect of Rumalaya Forte in animals, high fat diet was given for 90 days along with Rumalaya Forte and last 2 days treated with isoprenaline and vitamin E used as standard drug. Rumalaya Forte treatment groups prevented myocardial infarction due to antioxidant property and showed a significant decrease in the parameters like blood glucose level, weight gain, food efficiency ratio, intraperitoneal glucose tolerance test, intraperitoneal insulin response test, alanine aminotransferase, aspartate aminotransferase, creatinine phosphokinase, total cholesterol, total triglycerides, weight and number of adipocytes. The histopathological examination also reveals the significant protection in the Rumalaya Forte treatment and standard groups on comparing with toxic and standard groups, respectively. There was significant increase in weight gain, food intake and feed efficiency ratio in HFD+ISO group as compared to control group. The RF I and II showed significant dose dependent decrease in weight gain,

food intake and feed efficiency ratio as compared to HFD+ISO fed groups. Vitamin E also showed decrease of the said parameters.

There was significant elevation in glucose level at 30 min that failed to return to its normal level at 120 min. However, intraperitoneal glucose tolerance test for RF showed dose dependent decrease in glucose level compared to that of HFD+ISO group. Vitamin E also showed a decrease in glucose level compared to HFD+ISO group. In intraperitoneal insulin response test, RF showed significant improvement in the intraperitoneal insulin response test.

There was significantly increase in alanine aminotransferase, aspartate aminotransferase and creatinine phosphokinase level in HFD+ISO group as compare to control. The increased aminotransferase, aspartate aminotransferase and creatinine phosphokinase level was significantly decreased by Rumalaya Forte treated group as compare to HFD+ISO group. Rumalaya Forte (320 mg kg<sup>-1</sup>, p.o.) (RF II) was found to be more effective than Rumalaya Forte (160 mg kg<sup>-1</sup>, p.o.) (RF I).

There was significant increase in triglycerides and cholesterol level in HFD+ISO group as compare to control. Rumalaya Forte (320 mg kg<sup>-1</sup>, p.o.) (RF II) was found to be more effective than Rumalaya Forte (160 mg kg<sup>-1</sup>, p.o.) (RF I) in decreasing both triglycerides and cholesterol level.

There was significant increase in number and weight of epididymal fat pad in HFD+ISO group as compare to control. The increase in epididymal fat pad weight and number was significantly decreased by Rumalaya Forte as compare to HFD+ISO group. Rumalaya Forte (320 mg kg<sup>-1</sup>, p.o.) (RF II) was found to be more effective in decreasing the number and weight of the epididymal fat pad.

Microscopic examination of heart of control group showed more number of viable cells whereas, HFD+ISO group showed large area of red coloration. However, RF showed a protective effect with a minimal or no red coloration in a dose dependent manner. Hematoxylin-eosin staining of cardiac tissue from control group showed histoarchitecture of myofibers that were characteristically multinucleated and intact. Rumalaya Forte 160 mg kg<sup>-1</sup>, p.o. (RF I) treated groups showed relatively less disruption of myofibers but Rumalaya Forte 320 mg kg<sup>-1</sup>, p.o. (RF II) showing maximum fiber integrity.

## **CONCLUSION**

It can be concluded from the present study that myocardial infarction is caused by high fat diet. Basically, high

fat diet induces insulin resistance which causes the oxidative stress that leads to myocardial infarction. Pretreatment with Rumalaya Forte at selected dosage regimen in dose dependent manner significantly prevented myocardial infarction. The pleiotropic effect of Rumalaya Forte can be utilized as add on therapy for treatment of myocardial infarction. Thus, polyherbal formulation Rumalaya Forte can be added as a new drug for treatment of myocardial infarction which has no side effect with antioxidant property.

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