

Combined Antioxidant Formulation of Ascorbic Acid with Resveratrol Ameliorates Isoproterenol-Induced Myocardial Infarction in Rats

(Gabungan Formulasi Antioksidan Asid Askorbik dengan Resveratrol yang Ditambah Baik dengan Isoproterenol-Penginfarkan Miokardium Teraruh dalam Tikus)

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ABSTRACT

The current investigation was proposed to assess the effectiveness of the combination of resveratrol and ascorbic acid against Isoproterenol (ISO)-induced myocardial infarction in rats. The experimental model was divided into six groups (n = 6 in each group). Group 1: control, Group 2: isoproterenol (ISO)-100 mg/kg b.wt, Group 3: ISO+Resveratrol (RES) (20 mg/kg b.wt) treated, Group 4: ISO+ Ascorbic acid (AA) (80 mg/kg b.wt) treated, Group 5: ISO+RES (20 mg/kg b.wt)+AA (80 mg/kg b.wt) treated and Group 6: RES (20 mg/kg b.wt)+ AA (80 mg/kg b.wt) alone treated. The study showed an increase in lipid peroxides and cardiac markers in the serum samples of experimental animals administered with ISO. Treatment with RES and AA individually and a combinational formulation brought a significant decrease in lipid peroxides and cardiac markers. They increased the level of enzymatic antioxidants and non-enzymatic antioxidant levels. Histopathological results showed the distortion of heart architecture among the experimental groups administered with ISO and significant recovery when treated with RES and AA individually and in their combination. The study presents the effective combination of RES and AA in combating ISO-induced myocardial infarction and protection against ROS-mediated oxidative stress.

Keywords: Ascorbic acid; isoproterenol; myocardial infarction; resveratrol

ABSTRAK

Penyelidikan ini dicadangkan untuk menilai keberkesanan gabungan resveratrol dan asid askorbik terhadap penginfarkan miokardium yang disebabkan oleh Isoproterenol (ISO) pada tikus. Model uji kaji dibahagikan kepada enam kumpulan (n = 6 dalam setiap kumpulan). Kumpulan 1: kawalan, Kumpulan 2: isoproterenol (ISO)-100 mg/kg b.wt, Kumpulan 3: ISO+Resveratrol (RES) (20 mg/kg b.wt) terawat, Kumpulan 4: ISO+ Askorbik asid (AA) (80 mg/kg b.wt) terawat, Kumpulan 5: ISO+RES (20 mg/kg b.wt)+AA (80 mg/kg b.wt) terawat dan Kumpulan 6: RES (20 mg/kg b.wt)+ AA (80 mg/kg b.wt) sahaja terawat. Kajian menunjukkan peningkatan dalam peroksida lipid dan penanda jantung dalam sampel serum haiwan uji kaji yang telah diberi ISO. Rawatan dengan RES dan AA secara individu dan gabungan formulasi membawa penurunan yang ketara dalam peroksida lipid dan penanda jantung. Ia meningkatkan tahap antioksidan berenzim dan tahap antioksidan bukan berenzim. Keputusan histopatologi mendedahkan herotan arkitektur jantung dalam kalangan kumpulan uji kaji yang diadministrasi dengan ISO dan pemulihan yang ketara apabila dirawat dengan RES dan AA secara individu dan secara gabungan. Kajian itu membentangkan gabungan RES dan AA yang berkesan dalam memerangi penginfarkan miokardium yang disebabkan oleh ISO dan perlindungan terhadap tekanan oksidatif pengantaraan ROS.

Kata kunci: Asid askorbik; isoproterenol; penginfarkan miokardium; resveratrol

INTRODUCTION

Myocardial infarction is defined as an injury to heart muscles caused either due to blockage in the coronary

arteries, which supply blood to the heart or lack of oxygen supply. The cause also includes oxygen-derived free radicals generated as a by-product of cellular metabolic

processes, which is also responsible for the myocardial injury (Simpson & Lucchesi 1987). These oxygen-derived free radicals cause the membranes' integrity by lipid peroxidation, leading to a higher rate of fluidity and high permeability (Ferrari et al. 1991). The production of free radicals is inevitable for cellular processes. But every cell maintains a balance between the production of oxidants and neutralization with an antioxidant defence system, both enzymatic and non-enzymatic. When the balance favours the production of oxidants and inefficiency of the antioxidant system, then the condition leads to 'oxidative stress' that contributes as a potential factor to cause many pathological conditions, including myocardial infarction (Birben et al. 2012). It is well known that acute myocardial infarction stands as the leading cause of death worldwide; there are continuous attempts of intense research hoping for progress in new treatment methods (Kim et al. 2019).

This plant-based natural polyphenol is known to exert antioxidant, cardioprotective, antitumor, anti-ageing, and neuroprotective properties. RES has great attention in the field of pharmacology due to its nutraceutical with pharmacological properties (Kanamori et al. 2013). It is a promising therapeutic agent shown to provide various resveratrol-mediated cardioprotection like reduced infarct size and improved vascular and cardiac function (de Lucia et al. 2018). Along with plant-based phytochemical and its derivatives, antioxidants have been claimed to effectively combat oxidative stress and related pathological conditions (Nasri et al. 2014). Phytochemical enhances immune response, induces apoptosis in tumour cells, and increases the efficiency of mitochondria in preventing stroke and myocardial infarction (Dasgupta & Milbrandt 2007; Rimbaud et al. 2011). One of its outstanding features is the ability to protect the heart against cardiovascular diseases, which is widely spoken as the 'French Paradox' where despite high consumption of fat in their diet, the French people could remain protected from cardiovascular diseases as they also consume red wine rich in RES (Cheng et al. 2015). Studies on experimental models serve as evidence for their high potential in protecting heart tissues against injury (Zhu et al. 2018). Many investigations are underway to determine the molecular mechanism behind its miracle power of protecting the cardiac system from injury. The studies on RES are also favoured as it imparts little or no side effects when administered as a drug molecule for the therapeutic interventions treating any disease when compared to chemical compounds (Bonnefont-Rousselot 2016).

AA, predominantly found in citrus fruits, is well exploited for its antioxidant property (Jideani et al. 2021). The deficiency of this water-soluble vitamin showed an imbalance in redox homeostasis leading to necrosis and cell death. Ascorbic acid has been shown to prevent oxidative stress-mediated deleterious effects over the cell and its components (Kaźmierczak-Barańska et al. 2020). The cardioprotective role of AA is exhibited by relating the concentration of AA inversely with cardiovascular mortality. Ventricular functions were aided to be recovered after chronic administration of vitamin C in an investigation of heart failure in aged patients (Witte et al. 2005). Experiments done with cardiovascular complication models suggest that AA effectively protects myocardial tissues from apoptosis and prevents further advancement in the dysfunction of myocardial cells (Shite et al. 2001). The current study is an in-depth attempt to develop a new method for treating ISO-induced myocardial infarction by combining the therapeutic effects of resveratrol and ascorbic acid in a dose-dependent manner. The experimental parameters of biochemical and histological studies were expected to show the combined activity of ascorbic acid and resveratrol in treating myocardial infarction in experimental rats.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

Ascorbic acid was purchased from CSPC Pharmaceutical Group Ltd, Shijiazhuang, China. Resveratrol, Isoproterenol hydrochloride, heparin, trichloroacetic acid (TCA), thiobarbituric acid (TBA), 5,5'-dithiobis 2-nitro benzoic acid (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB), Ellman's reagent, sodium dodecyl sulphate (SDS), m-phosphoric acid, 2,4-dinitro phenylhydrazine (DNPH), phenazine methosulphate (PMS) and nitroblue tetrazolium (NBT) were purchased from Sigma Aldrich (St. Louis, MO, USA).

ANIMALS

Adult male Wistar rats weighing 175-200 g were obtained from the Experimental Animal Center of Chengde Medical College. They were provided *ad libitum* water and commercial pellet rat food after being acclimatized to animal house settings. The study follows the Animal Welfare Guidelines issued by The Affiliated Hospital of Chengde Medical University for the

Care and Use of Laboratory Animals. The experimental design was approved by the medical ethics committee of Affiliated Hospital of Chengde Medical College, Chengde.

MYOCARDIAL INFARCTION INDUCTION IN AN ANIMAL MODEL

Isoproterenol (100 mg/kg) was dissolved in normal saline and given to Wister rats subcutaneously for 24 h for two days. ISO-induced myocardial infarction was indicated by increased serum creatine phosphokinase (CK) and lactate dehydrogenase (LDH) in rats.

EXPERIMENTAL DESIGN

Thirty six healthy male Wister rats were categorized into six groups consisting of 6 animals each. The experimental animals were categorized as follows. Group 1: Control; Group 2: Isoproterenol (ISO) -100 mg/kg b. wt; Group 3: ISO+ Resveratrol (RES) (20 mg/kg b. wt) treated; Group 4: ISO+ Ascorbic acid (AA) (80 mg/kg b. wt) treated; Group 5: ISO+RES (20 mg/kg b. wt) +AA (80 mg/kg b. wt) treated; Group 6 : RES (20 mg/kg b.wt)+ AA (80 mg/kg b.wt) alone treated.

The rats in Group 1 were given 100 μ L of distilled water every day. This group was considered the untreated control group. The Group 2 animals were treated with 100 μ L of distilled water for 14 days. Then 100 mg/kg b.wt of ISO was administered intraperitoneally for 2 days to induce the myocardial infarction (15th and 16th day) (Ohkawa et al. 1979). Group 3 animals were treated with 20 mg/kg b. wt of RES and 80 mg/kg b. wt of AA. Groups 4, 5, 6 animal were treated with RES (20 mg/kg b. wt), AA (80 mg/kg b. wt), and RES (20 mg/kg b. wt) +AA (80 mg/kg b. wt), respectively, for 14 days and then the myocardial injury was established in 15th and 16th day.

BIOCHEMICAL ANALYSIS

After the experimental period (on the 17th Day), overnight-fasted mice were sacrificed by cervical decapitation. Blood and cardiac tissues were separated to study cardiac markers and to perform biochemical calculations. Lipid peroxides were estimated as malondialdehyde (MDA), also referred to as thiobarbituric acid-reactive substance (TBARS) in tissue homogenates by the method of Ohkawa et al. (1979). Glutamate oxaloacetate transaminase and glutamate pyruvate transaminase activities were estimated using the method Bergmeyer and Gawehn (1974) described.

The lactate dehydrogenase activity was determined by King's standard protocol (King 1965) and expressed as μ moles of pyruvate liberated/h/mg protein. Creatine phosphokinase (CPK) assay was done by the Rosalki method (Okinaka et al. 1961). The hearts of the experimental rats were excised at once after the sacrifice, washed with ice-cold isotonic saline solution, and homogenized. The homogenate served as the source for the antioxidant activity assay of enzymes such as superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione-S-transferase (GST), and glutathione peroxidase (GPx). Superoxide dismutase activity was determined by the method of Misra and Fridovich (1972). Takahara method (1960) was adopted for catalase (CAT) assay. Reduced glutathione was estimated by the method of Ellman (Beutler & Kelly 1963). The method of Rotruck et al. (1973) was adopted to estimate GPx and the Habig et al. (1974) method was adopted for the assay of GST. The activity was expressed as moles of 1-chloro-2,4-dinitrobenzene (CDNB) conjugate formed/min/mg protein. The levels of non-enzymatic antioxidants enzymes such as vitamin C and vitamin E levels were estimated by Desai (1984) and Omaye et al. (1979), respectively.

HISTOPATHOLOGICAL STUDIES

A part of the heart tissues were preserved in a 10% buffered neutral formalin solution for histological examinations. The tissues were fixed, embedded in paraffin wax, cut into 5 μ m solid sections, stained with hematoxylin and eosin, and examined under a light microscope for histological alterations.

STATISTICAL ANALYSIS

One-way analysis of variance (ANOVA) was used to assess the statistical significance of the data, and Duncan's Multiple Range Test was used to assess differences between treatment groups (DMRT). The results were considered significant at $p < 0.05$. IBMSPSS software was used to perform all statistical calculations (IBMSPSS, Tokyo, Japan).

RESULTS AND DISCUSSION

Myocardial infarction is caused by injury to the myocardial membrane leading to infarct-like necrosis of the heart muscles. This happens in conditions where there is the insufficient blood supply to the heart, which renders a retarded oxygen and nutrient supply, build-up of free radicals and oxidative stress,

distortion in the myocardial membrane architecture, abnormal cardiomyocyte function, and finally, death of cardiomyocytes (Upaganlawar et al. 2009). This

deleterious effect in humans is successfully mimicked in rodents or experimental animal models when induced with Isoproterenol (Patel et al. 2010).

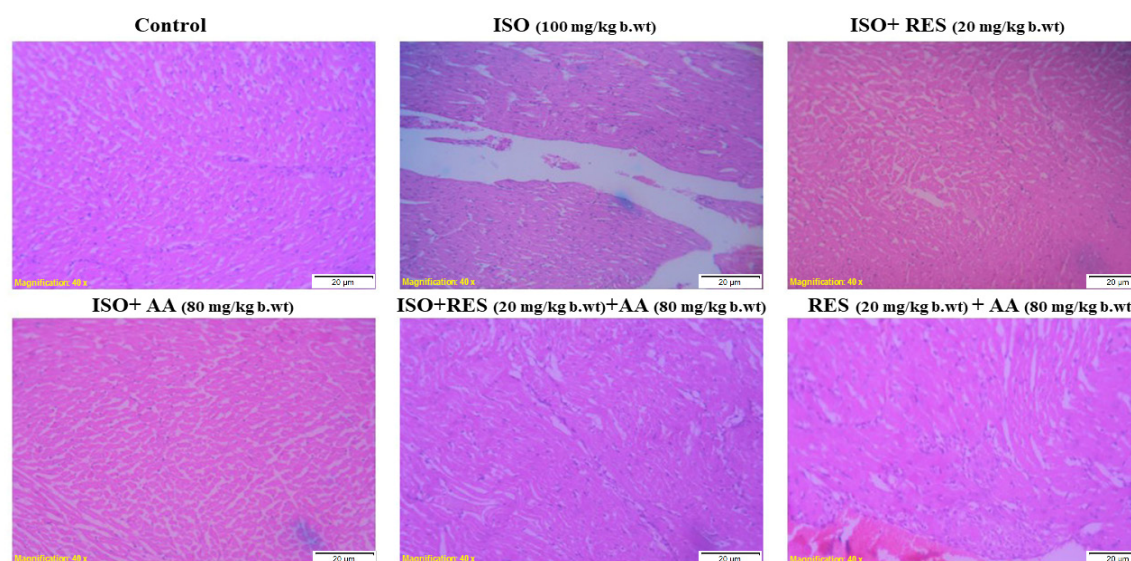


FIGURE 1. Histopathological changes in ISO, RES, and AA treated heart tissue (40× magnification)

Control and AA+RES alone treated rats showed typical cardiac tissues with no infarction or inflammatory cell infiltration. ISO treated rats showed a significant myocardial structural abnormality, as well as subendocardial necrosis, capillary dilatation, and leukocyte infiltration, were observed. ISO+ RES and ISO+AA treated rats showed mild and moderate edema with marked infiltration of inflammatory cells with degeneration and necrosis of myofibers also observed. ISO-induced histological changes such as vacuolar changes, capillary dilatation, edema, and leukocyte infiltration were significantly improved by AA+RES treatment.

The current study induced myocardial infarction in experimental rats using Isoproterenol. Isoproterenol is widely used to study the therapeutic possibilities of various plant-based therapeutically molecules over myocardial infarction (Kumar et al. 2017; Nirmala & Puvanakrishnan 1996). It is known to cause autoxidation of catecholamines resulting in the generation of free radicals capable of damaging the membrane lipids, producing lipid peroxides and hydroperoxides, compromising the permeability of the myocardial membrane, and finally leading to myocardial infarction

(Karthick & Prince 2006). Myocardial infarction caused due to ISO results in various metabolic and morphological alterations in cardiac tissues of the experimental models as seen in human myocardial infarction.

Histopathological results showed typical myocardial architecture in control groups. In contrast, the heart's anatomy was found to be distorted in groups administered with ISO, which was characterized by myocarditis, i.e., the inflammation of myofibers, edema, and necrosis and can be refer from Figure 1. The study also displayed significant recovery in the architecture and membrane lipid organization in groups treated with RES and AA individually and combined with both formulations. In comparison to the normal control group, the RES and AA alone treated rats displayed no changes in the morphology of heart tissue showing that the treatment does not have any negative consequences.

The antioxidant properties of RES and AA might have prevented the ISO-autoxidation mediated ROS invasion, thereby, preventing the harmful effect of ISO in distorting the structure of heart tissues. The current study shows the level of heart damage caused by Isoproterenol by showing elevated levels of cardiac

marker enzymes like serum glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, creatine phosphokinase, and lactate dehydrogenase. However, the rats pre-treated with the combined formulation of

RES and AA significantly ($p < 0.05$) reduced the cardiac marker enzymes. Nevertheless, no significant difference in the above parameter was observed in RES and AA alone treated rats compared to the control group and can be refer from Table 1.

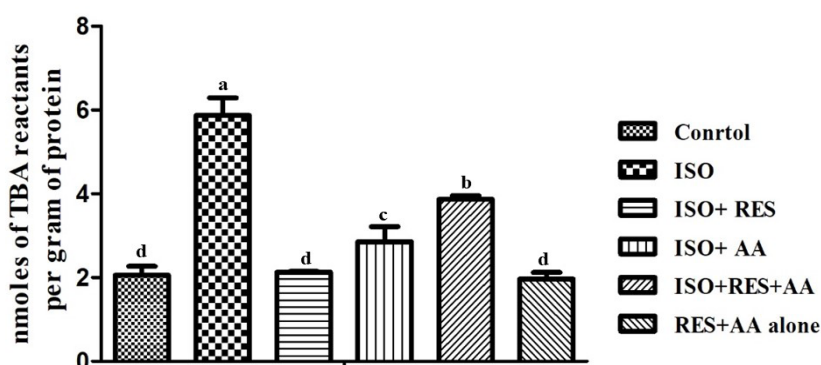
TABLE 1. The effects of resveratrol and ascorbic acid on the activity of cardiac marker enzymes in the serum of rats in both the control and experimental groups

Group	Glutamate oxaloacetate transaminase (IU/L)	Glutamate pyruvate transaminase (IU/L)	Creatine phosphokinase (IU/L)	Lactate dehydrogenase (IU/L)
Control	71.56±4.28 ^d	20.46±2.54 ^d	86.27±4.63 ^d	884.16±74.52 ^d
ISO (100 mg/kg b.wt)	146.85±10.63 ^a	35.79±2.96 ^a	195.51±11.47 ^a	1594.37±96.67 ^a
ISO+ RES (20 mg/kg b.wt)	100.78±9.37 ^b	25.06±1.94 ^b	134.93±9.69 ^b	1105.69±96.64 ^b
ISO+ AA (80 mg/kg b.wt)	106.25±10.59 ^b	27.57±2.41 ^b	145.21±10.24 ^b	1217.25±117.24 ^b
ISO+RES (20 mg/kg b.wt)+AA (80 mg/kg b.wt)	91.78±3.96 ^c	23.17±3.35 ^c	94.30±8.52 ^c	931.82±965.76 ^c
RES (20 mg/kg b.wt)+ AA (80 mg/kg b.wt) alone	74.69±3.21 ^d	22.53±1.08 ^d	78.92±5.28 ^d	901.34±83.36 ^d

Results are expressed as mean ± S.D. ($n = 6$). Activity is expressed as μmol of pyruvate liberated/mg of protein/h for GOT, GPT, and LDH, μmol of phosphorus liberated/mg of protein/h for CPK. Values not sharing a common marking (a,b,c,d) differ significantly at $p < 0.05$ (DMRT)

The elevated levels of marker enzymes and lipid peroxides are due to the prevalence of oxidative stress induced by ISO, which causes damage to the myocardial membrane and leakage of the marker enzymes in the blood that biochemical investigations could analyze.

Increased levels of lipid peroxides in ISO-induced rats were found to be normalized effectively when treated with RES and AA, individually. A significant decrease in the level of LPO was also observed when the rats were given the combination of RES and AA and can be refer from Figure 2.



Values not sharing a common marking (a,b,c,d) differ significantly at $p < 0.05$ (DMRT)

FIGURE 2. Protective effect of resveratrol and ascorbic acid on lipid peroxidation status in Isoproterenol induced myocardial infarcted rats

Isoproterenol successfully generates oxidative stress through excessive production of ROS, which causes abnormal alterations in lipids, proteins, and carbohydrates and results in increased production of lipid peroxides and leakage of cardiac markers (McMichael & Moore 2004; Sánchez-Hernández et al. 2020). This enhanced deleterious action of ROS and its consequences also cause a decline in the antioxidant levels in the myocardial tissues. The imbalance in the redox homeostasis indicates the necessity of discovering an effective therapeutic agent that could restore the redox status and protect the heart cells from necrosis (Abdel-Daim et al. 2016).

The effects of ISO, RES, and AA treatment on enzymatic and non-enzymatic antioxidants GSH, GST, GPx, SOD, CAT, vitamin C, and Vitamin E are shown in Tables 2 and 3. When compared to the control group, the activity of these enzymes was considerably lower in ISO-treated rats. The study investigated the combined effect of RES and AA against ISO-induced myocardial infarction, which was found to be significantly effective in increasing antioxidant and non-enzymatic antioxidants. Compared to control rats, the rats treated with RES and AA individually, no significant differences were seen.

TABLE 2. Enzymatic and non-enzymatic antioxidant enzyme activity in the hearts of rats in both the control and experimental animals

Group	GSH (nmol/mg protein)	GST (μ mol CDNB-GSH conjugate formed/ min/mg protein)	GPx (nmol of GSH oxidized/ min/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)
Control	3.81 \pm 2.96 ^c	0.49 \pm 0.05 ^d	2.51 \pm 0.04 ^d	10.06 \pm 2.18 ^d	15.61 \pm 1.03 ^d
ISO (100 mg/kg b.wt)	0.76 \pm 0.04 ^a	0.17 \pm 0.02 ^a	1.39 \pm 0.02 ^a	3.41 \pm 0.20 ^a	7.28 \pm 0.86 ^a
ISO+ RES (20 mg/kg b.wt)	2.84 \pm 0.27 ^c	0.32 \pm 0.05 ^b	1.48 \pm 0.01 ^b	7.06 \pm 1.01 ^b	11.27 \pm 1.59 ^b
ISO+AA (80 mg/kg b.wt)	2.05 \pm 0.93 ^b	0.35 \pm 0.03 ^b	1.57 \pm 0.01 ^b	7.35 \pm 0.16 ^b	11.13 \pm 2.04 ^b
ISO+RES (20 mg/kg b.wt)+ AA (80 mg/kg b.wt)	3.09 \pm 0.34 ^d	0.41 \pm 0.02 ^c	2.43 \pm 0.02 ^c	10.55 \pm 0.95 ^c	13.44 \pm 1.06 ^c
RES (20 mg/kg b.wt)+ AA (80 mg/kg b.wt) alone	3.58 \pm 2.54 ^c	0.57 \pm 0.04 ^d	2.43 \pm 0.03 ^d	9.82 \pm 0.17 ^d	14.93 \pm 3.52 ^d

Results are expressed as mean \pm S.D. ($n = 6$). Activity is expressed as nmol/g heart tissue for GSH, μ mol of GSH oxidized/min/mg of protein for GPx; units/min/mg of protein for GST; 50% inhibition of epinephrine auto-oxidation for SOD; μ mol of hydrogen peroxide decomposed/min/mg of protein for CAT. Values not sharing a common marking (a,b,c,d) differ significantly at $p < 0.05$ (DMRT)

The reactive species involved in injuring the membranes of myocardial tissues are superoxide, hydrogen peroxide (H_2O_2), hydroxyl anions, and hydroxyl radicals. These free radicals disrupt the redox homeostasis by conveniently diffusing across the plasma membranes and increasing oxidative stress (Moris et al. 2017; Venardos & Kaye 2007). Superoxide dismutase converts superoxide ions into H_2O_2 , which CAT and GPx then detoxify as they convert H_2O_2 to H_2O and O_2 . This co-operative function of antioxidant enzymes

helps the cells and tissues to overcome oxidative stress. But this systematic function of the antioxidant system is highly challenged in the case of myocardial infarction due to excessive production of ROS and inflammatory proteins that worsen the pathology severely (Bagulho et al. 2015; Bienert et al. 2007; Rhee et al. 2018). Another evidence suggests that RES treatment decreases nitric oxide (NO) level and protects the myocardial cells from NO-mediated oxidative stress (Aguilar-Alonso et al. 2018).

TABLE 3. Non-enzymatic antioxidant enzyme activity in the hearts of rats in both the control and experimental groups

Group	Vitamin-C (mmol/mg protein)	Vitamin-E (mmol/mg protein)
Control	1.24±0.03 ^d	0.92±0.04 ^d
ISO (100 mg/kg b.wt)	0.39±0.01 ^a	0.25±0.03 ^a
ISO+ RES (20 mg/kg b.wt)	0.86±0.03 ^b	0.41±0.01 ^b
ISO+AA (80 mg/kg b.wt)	1.02±0.02 ^b	0.53±0.04 ^b
ISO+RES (20 mg/kg b.wt)+AA (80 mg/kg b.wt)	1.17±0.03 ^c	0.77±0.04 ^c
RES (20 mg/kg b.wt)+AA (80 mg/kg b.wt) alone	1.27±0.01 ^d	0.90±0.08 ^d

Results are expressed as mean ± S.D. ($n = 6$). Values not sharing a common marking (a,b,c,d) differ significantly at $p < 0.05$ (DMRT)

Various studies claim that the defensive property of RES might be due to its involvement in varied mechanisms like interfering and decreasing the generation of ROS, decreasing the conditions of oxidative stress and inflammation, bringing out alterations in intracellular signaling pathways that lead to activation of antioxidant gene expression and suppression of inflammatory protein production, regulating the mitochondrial function, managing Ca^{2+} flux, which altogether provides a protective milieu for the myocardial tissues against injury or damage (Feng et al. 2019; Gu et al. 2014; Hashemzaei et al. 2017). The protective effect of RES is also studied in post infarction heart failure cases where RES administration showed an improved function of the left ventricle, a decrease in hypertrophy of myocardial tissues, and a reduction in the severity of heart failure. Resveratrol administration has been shown to alter various signaling pathways like Akt-1, ERK1/2, GSK-3 β , MKP-1, p38-MAPK, COX-2, and iNOS to its antioxidant and scavenging property (Riba et al. 2017).

There are also studies stating that RES is efficient in protecting myocardial tissues against oxidative stress and inflammatory induced damages through the Nrf2/ARE pathway (Qi et al. 2020). The pathway attenuates the unfavorable consequences of oxidative stress and inflammation through the internalization of Nrf2 into the nucleus, which triggers the antioxidant genes (Kaspar et al. 2009; Shah et al. 2007). Many scientific studies have reported the function of vitamin C (ascorbic acid) in combating oxidative stress. The activities of blood marker enzymes were significantly reduced in rats pre-treated with AA or combined formulation of AA+RES. This

result ties well with previous studies wherein the ferulic acid and ascorbic acid considerably counteracted the ISO induced oxidative stress by inhibiting lipid peroxidation, restoring antioxidant status, and increasing the levels of cardiac marker enzymes (Yogeeta et al. 2006). It has been reported that AA protects myocardial cells against the harmful effects of ROS-mediated oxidative stress through its ability to reduce lipid peroxidation and alterations in the endogenous antioxidant enzyme activities (Buttros et al. 2009; Ribeiro et al. 2009; Yogeeta et al. 2006). Among many dietary antioxidants or plant-based non-dietary antioxidants, ascorbic acid has found its importance in guarding cells and tissues against the harmful impacts of reactive oxygen species. Interestingly, Oral administration of Vitamin C (150 mg/kg b.wt) significantly reduced the ISO induced mild degenerative changes in heart tissue. Besides, the treatment favors the decrease in the iNOS expression. These findings suggest that Vitamin C administration can prevent ISO induced myocardial infarction in experimental animals (Ribeiro et al. 2009). The results suggest that the supplementation with the combined antioxidants formulation of resveratrol and ascorbic acid synergistically retard the isoproterenol-induced acute myocardial infarction. This is evident by its ability to reduce the generation of lipid peroxides, decrease cardiac makers in the serum sample, and enhance antioxidant enzymes and the availability of GSH in the cell.

CONCLUSION

The combination of RES and AA in the current study seems to protect membrane structure and integrity as a

sign of reduced ISO effect on the experimental groups. The combined effect of RES and AA has shown a significant protective role against ISO-induced myocardial infarction by showing a higher level of GSH and antioxidant enzymes and a decline in the concentration of lipid peroxides and cardiac markers compared to the experimental groups treated individually with RES and AA. The combined formulation of RES and AA seems to perform a synergistic inhibitory effect over the harmful changes induced by ISO to the heart cells. The result of the study suggests that the combination of RES and AA could serve as a better therapeutic intervention for myocardial infarction, where the future of the investigation could be extended to understand the molecular mechanism and signaling pathways and the molecules involved in the protective function.

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