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CARDIOPROTECTIVE EFFECT OF CYCLOALLIIN IN ISOPROTERENOL-INDUCED MYOCARDIAL INFARCTION IN ALBINO RATS: A MECHANISTIC STUDY

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Abstract: Necrosis of the myocardium due to imbalance between coronary blood supply and oxygen demand of the myocardial tissue promotes the evolution of acute myocardial infarction (MI). Onions are considered a rich source of medicinal compounds, of which cycloalliin (CYC), a sulfur containing imino compound with a cyclic structure, has been been extensively reported to exhibit various pharmacological activities. The present study aimed to elucidate the protective effect of cycloalliin in isoproternol (ISO)-induced myocardial infarction in male albino Wistar rats. Results of our investigation showed that treatment with CYC (10 mg/kg) significantly elevated both (+) and (−) left ventricular delta pressure/delta time (LVdP/dt) and decreased left ventricular end diastolic pressure LVEDP compared to the isoproterenol-treated group. The level of TBARS was significantly increased in the ISO-treated group. This was accompanied by diminished myocardial creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) activities when compared with the control group ($p<0.001$). In CYC-treated groups, glutathione (GSH) activity was improved significantly ($p<0.05$) in comparison to the ISO-treated group. Treatment with CYC showed no sign of alteration in myocardial fibers.

Keywords: cardioprotective effect; cycloalliin; isoproterenol-induced myocardial infarction; antioxidants; hemodynamic functions

INTRODUCTION

Necrosis of the myocardium resulting from a disparity between coronary blood supply and oxygen demand of myocardial tissue leads to the evolution of acute MI [1], which is a severe life-threatening condition responsible for the majority of morbidity and fatality among ischemic heart diseases [2]. During this phase, myocardial tissue experiences severe injury resulting from free radical damage, thrombosis, hyperlipidemia and lipid peroxidation [3]. The animal model of isoproterenol-induced myocardial ischemia has been used by researchers to evaluate the protective effect of investigational drugs against MI [4]. Isoproterenol is an adrenergic agonist that induces severe stress in the myocardium after administration [5] as a result of reactive oxygen species (ROS) generation and lipid peroxidation, which is believed to exert permanent injury to myocardial membranes [6] and intensify ischemic injury. The deleterious effects can be controlled by antioxidants [7].

Many plant products have been shown to be effective in the management of numerous diseases [8]. Cragg and Newman have highlighted the importance of various natural products as medicinal agents [9]. The bioactivity of plants has been attributed to the presence of diverse compounds, from complex alkaloids to simple terpenes. Many plants or their constituents show beneficial effects in myocardial infarction by limiting hyperlipidemia and scavenging free radicals [10]. Cycloalliin (CYC), a sulfur-containing imino compound with a cyclic structure isolated from onions and garlic has not been extensively studied for its pharmacological actions [11]. Therefore, the present study aimed to elucidate the protective effect of cycloalliin in isoproternol (ISO)-induced myocardial infarction in albino rats.

MATERIALS AND METHODS

Animals

The experiments were performed on male albino rats (150-175 g) obtained from the central animal house facility of the institute and were approved by the In-

stitutional Animal Ethics Committee. The rats were housed under controlled temperature and humidity in polypropylene cages with a 12-h light/dark cycle. The animals were fed with standard laboratory diet and water *ad libitum*.

Chemicals

The MI inducing agent isoproterenol (ISO) hemisulfate was obtained from Sigma Chemicals Co. (St. Louis, MO, USA). A 20% solution of ISO was prepared in 0.9% sterile saline before injection. Cycloalliin was obtained from Nippon Shinyaku (Kyoto, Japan).

Treatment protocol

The rats were randomly divided into five groups, with ten rats per group. These were the control, ISO and CYC (10, 20 and 30 mg/kg) treatment groups. CYC was administered orally at doses of 10, 20 and 30 mg/kg for 30 days, whereas the control and ISO group received distilled water for the same duration. On the 28th and 29th days, the ISO- and CYC-treated groups received isoproterenol (85 mg/kg, SC) at a 24-h interval. The animals in the control group received saline subcutaneously (SC). On the 30th and last day, the hemodynamic parameters were recorded. The rats animals were killed with an excess of anesthesia, and their hearts were excised and immediately processed for histopathological and ultrastructural examinations. For biochemical estimation, six hearts were taken and kept in liquid nitrogen until use.

Evaluation of hemodynamics and left ventricular functions (surgical procedure)

Rats were anesthetized with pentobarbitone sodium (60 mg/kg; IP); atropine (0.6 mg/kg, IP) was administered to preserve heart velocity during the surgical process and to lessen tracheobronchial discharges. The body temperature was monitored and continuously maintained at 37 C. Tracheostomy was performed by opening the neck with the help of a ventral midline incision. The rats were well-ventilated with a positive pressure ventilator at appropriate parameter settings. The left jugular vein was cannulated with a polyethylene tube for continuous infusion of 0.9% saline. For assessment of blood pressure and heart rate, the right carotid artery was cannulated with a cannula filled with heparinized saline and connected via a pressure transducer to CARDIOSYSCO-10l (Experimentia, Hungary). The heart was exposed via left thoracotomy through the fifth intercostal space. A wide-bore sterile metal cannula connected to a pressure transducer (Gould Statham P23ID, USA) was inserted into the cavity of the left ventricle from the posterior apical region of the heart for recording left ventricular pressure dynamics on a polygraph (Grass 7D, USA). After completion of the surgical procedures, a saline-soaked gauze was placed in the thoracic cavity to prevent drying of the heart. The animals were then allowed to stabilize for 10 min before recording the basal hemodynamic variables.

Measurement of biochemical parameters

Frozen heart samples were removed from the liquid nitrogen and brought to room temperature. A 10% homogenate of myocardial tissue was prepared in 50 mM of phosphate buffer (pH 7.4); an aliquot was used for the thiobarbituric acid reactive substances (TBARS) assay that was performed as described [12]. For the estimation of reduced glutathione (GSH), a protein-free supernatant was obtained by the addition of an equal volume of 10% trichloroacetic acid to the tissue homogenate and centrifuged for 10 min [13]. The tissue homogenate was centrifuged at 6000xg for 30 min at 4°C and the obtained supernatant was used for the estimation of lactate dehydrogenase (LDH) [14], superoxide dismutase (SOD) [15] and catalase (CAT) [16]. Creatine kinase-MB (CK-MB) was estimated spectrophotometrically using a kit from Spinreact, Spain.

Histopathological and ultrastructural studies

Light microscopy was performed to assess the ultrastructural changes in the myocardial tissue. At the end of the study, all rats were killed by cervical decapitation and the hearts were dissected out and washed in ice cold saline. Myocardial tissue was immediately fixed in 10% buffered neutral formalin solution. After fixation, the tissue was embedded in paraffin, serial sections were cut and each section was stained with hematoxylin and eosin. The slides were examined under a light microscope.

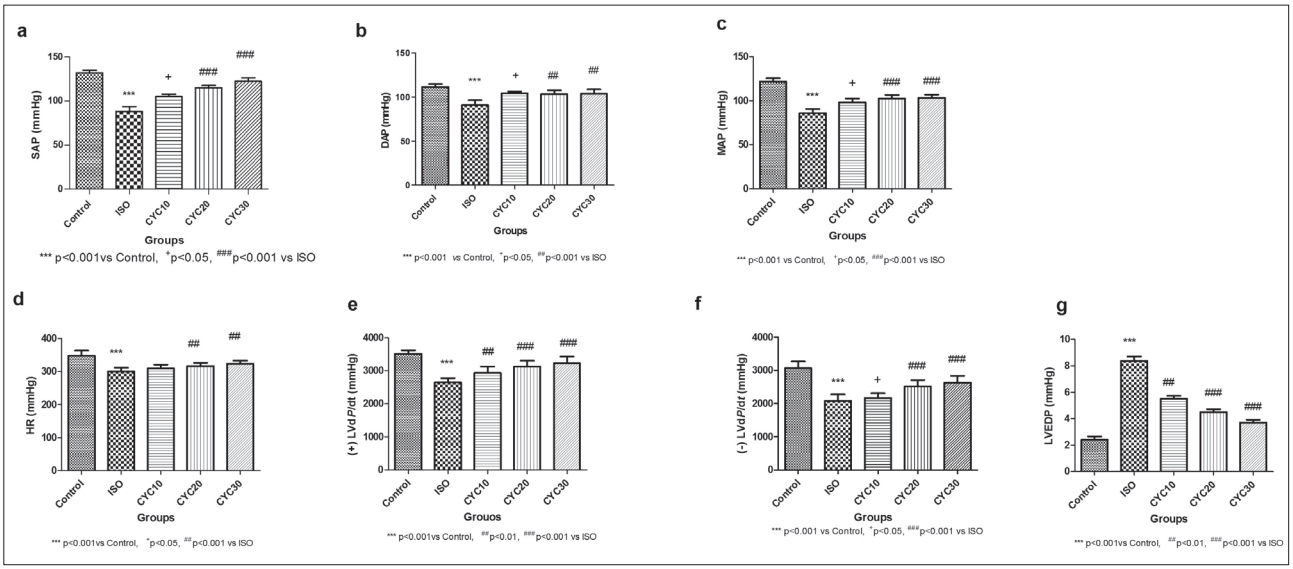

Fig. 1. The effect of CYC treatment on the major cardiac parameters in ISO-induced myocardial ischemic injury. **a** – SAP; **b** – DAP; **c** – MAP; **d** – HR; **e** – (+) LVd*P*/d*t*; **f** – (–) LVd*P*/d*t*; **g** – LVEDP. The treated group received CYC at doses of 10, 20 and 30 mg/kg. The values are expressed as the mean±SD; *n*=10 in each group.

Statistical analysis

The results are presented as the means±standard deviation (SD). One-way analysis of variance (ANOVA) with post hoc analysis (Bonferroni Multiple Range Test) were applied. A p value 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

The effect of CYC on hemodynamics and left ventricular functions

The effect of CYC on cardiac function was examined by assessing the hemodynamic parameters, such as systolic, diastolic and mean arterial pressures (SAP, DAP, MAP, respectively), the heart rate (HR) and left ventricular functions. As can be seen in Fig. 1, compared to the control, the ISO-treated group displayed a significant decrease ($p<0.001$) in the SAP, DAP, MAP, HR, and in the rate of change of pressure (+LVd*P*/d*t*) and its decline (–LVd*P*/d*t*), and elevated left ventricular end diastolic pressure (LVEDP) ($p<0.001$). Compared to the ISO-treated rats, rats from the experimental groups that were pre-exposed to CYC showed improved SAP, DAP and MAP (Fig. 1a-c). Despite this, the level of HR was not significantly restored at the initial concentration of the tested compound, however, in rats that received 30 mg CYC/kg it was significantly increased (Fig. 1d). The treatment with CYC significantly elevated the both (+) and (–) LVdP/dt and decreased LVEDP as compared to the ISO-treated group (Fig. 1e-g). The hemodynamic parameters, together with left ventricular function, were significantly improved after treatment with CYC. Previous studies conducted on animal and human lines suggest that these effects could be attributed to angiotensin II inhibiting and vasodilating effects due to the production of hydrogen sulfide [17].

Determination of the effect of CYC on lipid peroxidation and release of associated enzyme

Studies have revealed the role ROS in the damage of cell membrane integrity, which results in leakage of intracellular components. This process is mediated via the interaction of ROS with cellular component and organelles, including membrane phospholipids. Therefore, our next aim was to determine the effect of CYC on lipid peroxidation by the TBARS assay, and on specific markers of myocardial injury, CK-MB and LDH. These results are presented in Fig. 2, where it can be observed that the level of TBARS was significantly increased in the ISO-treated group; this was accompanied by diminished activity of myocardial

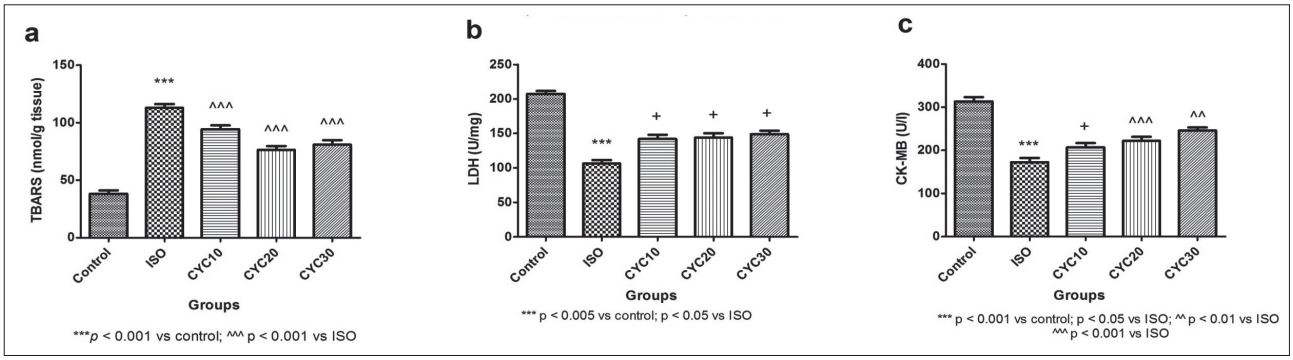

Fig. 2. The effect of CYC on lipid peroxidation and bio-marker enzymes of cardiac injury in different treatment group. **a** – TBARS; **b** – LDH; **c** – CK-MB. The treated group received CYC at doses of 10, 20 and 30 mg/kg. The values are expressed as the mean±SD; *n*=10 in each group.

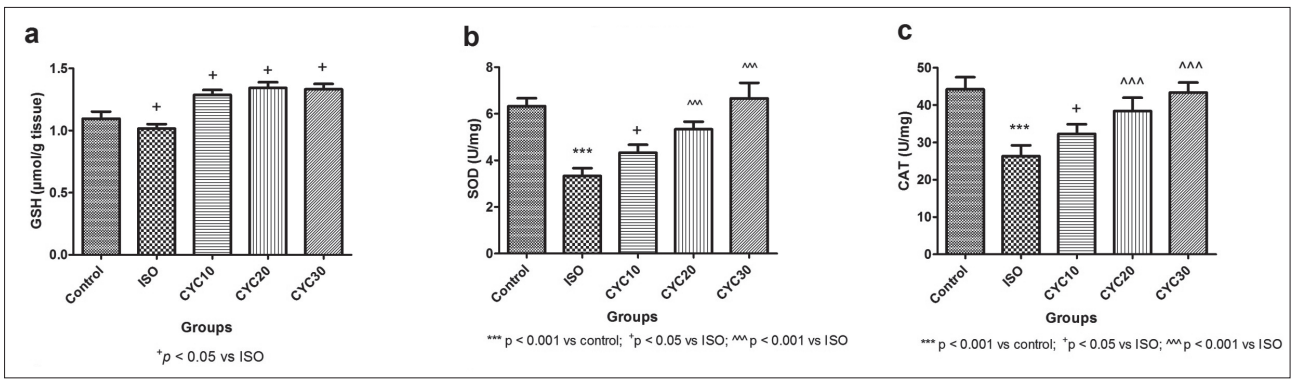

Fig. 3. The effect of CYC on endogenous components of the antioxidant system. **a** – GSH; **b** – SOD; **c** – CAT. The treated group received CYC at doses of 10, 20 and 30 mg/kg. The values are expressed as the mean±SD.; n=10 in each group.

CK-MB and LDH when compared with the control group (p<0.001). Compared to the ISO-treated group, the pretreatment with CYC reduced the increase in TBARS (p<0.001) in a dose-dependent manner.

The effect of CYC on endogenous antioxidants

To prevent injury associated with oxidative stress, the endogenous defense mechanism utilizes various enzymatic and nonenzymatic components to counteract the generation of ROS. This antioxidant system is comprised of enzymes such as SOD, CAT, GSHPx and the nonenzymatic antioxidant molecule GSH. Under normal conditions, these endogenous antioxidants can prevent ROS generation and its deleterious effects. However, if the antioxidant defense is impaired, the control of ROS formation is ineffective and various detrimental effects are facilitated. This part of the study was aimed at elucidating the effect of CYC on these critical defensive components. As can be seen in Fig. 3, compared to the control the ISO-treated group displayed a drastic decline (p<0.001) in SOD and CAT activities, with no changes in GSH.

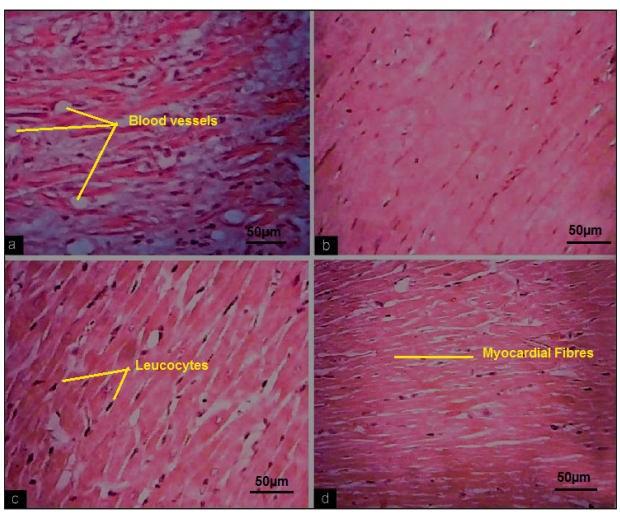

Fig. 4. The effect of CYC on cardiac tissue histology. **a** – control group with normal histology; **b** – ISO-treated group; **c** – CYC (10 mg/kg)-treated; **d** – CYC (30 mg/kg)-treated. H&E staining; magnification 100×. Necrotic tissue is indicated by colored arrows: red arrow – necrosis; yellow arrow – edema.

Compared to the ISO-treated group, CYC-treated rats showed a significant improvement in SOD and CAT activities. Further, the level of GSH was also significantly improved ($p<0.05$). These results show that the antioxidant property of CYC cancelled the ISO-induced harmful effects of ROS generation, and that this activity is cardioprotective [18].

Determination of effect of CYC on histopathological changes

To further confirm the protective effect of CYC, we performed histopathological examinations of cardiac tissues. Examination of heart sections of control rats group showed an intact myocardium without necrosis, edema and inflammation (Fig. 4a), whereas tissue sections from the ISO-treated rats exhibited degraded cardiac fibers, with extensive subendocardial necrosis, intracellular leakage with prominent myocellular edema (Fig. 4b). The treatment with the lowest dose of CYC (10 mg/kg) did not produce an improvement in the myocardial fibers (Fig. 4c); however, higher concentrations of CYC provided improvement, with the myocardial fibers returning to their original state, with no evidence of inflammatory cell infiltration, edema and necrosis (Fig. 4d).

Authors' contributions: PD designed the experiments and evaluated hemodynamic and left ventricular functions; LQ performed the histopathological and ultrastructural studies and the statistical analysis.

Conflicts of interest disclosure: The authors declare no conflict of interest.

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