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Research Article

CARDIOPROTECTIVE EFFECT OF VITAMIN E AGAINST MYOCARDIAL INFARCTION INDUCED BY ISOPRENALINE IN ALBINO RATS

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ABSTRACT

Objective: Vitamin E is an antioxidant which can help in the prevention of cardiovascular disease. The objective of this study was to estimate the effects of Vitamin E on cardiac marker enzymes in isoprenaline (ISO)-induced myocardial infracted rats.

Methods: Adult male albino rats were divided into three groups. The first group was the control negative group. The second group was the control positive group that was subcutaneously injected with ISO (100 mg/kg). The third group was pretreated with Vitamin E (100 mg/kg) once daily for 30 days, then subcutaneously injected with ISO at an interval of 24 h for 2 days. Aspartate aminotransferase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), cardiac troponin-I (CTn-I), glutathione (GSH), superoxide dismutase (SOD), malondialdehyde (MDA), and histopathological examinations were measured. Comparison between groups was achieved by one-way analysis of variance (ANOVA) test through SPSS software.

Results: The levels of AST, ALT, LDH, creatine kinase (CK), and CTn-I significantly decreased in pretreated group with Vitamin E compared to its increasing in the control positive group. The level of GSH and SOD markedly increased in pretreated group with Vitamin E compared to its decreasing in the control positive group, while the level of MDA significantly decreased in pretreated group with Vitamin E compared to its increasing in the control positive group. ISO + Vitamin E rats group reflected a cardioprotective role of Vitamin E in myocardial infarcted rats.

Conclusion: Pretreatment with Vitamin E can protect the myocardial membranes against ISO-induced oxidative stress in rats and can be used for routine clinical and epidemiological purposes.

Keywords: Vitamin E, Cardioprotective, Isoprenaline, Myocardial infraction.

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INTRODUCTION

Myocardial infarction is acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand [1]. Several mechanisms of isoprenaline-induced myocardial infarction have been suggested. These include oxidative stress, functional hypoxia and ischemia, alterations in metabolism, coronary insufficiency, decreased level of high-energy phosphate stores, changes in electrolyte contents, and intracellular Ca^{2+} overload [2]. Spontaneous oxidation of catecholamines results leads to the formation of catecholamines O-quinones that can be oxidized to several compounds such as adrenolutin that generate free radicals. The oxidized products interact with sulfhydryl groups of the protein structures and generate superoxide anions and hydrogen peroxide which lead to change in microsomal permeability, due to mitochondrial Ca^{2+} uptake that resulted in decrease in ATP production [2,3].

Myocardial infarction is a common presentation of ischemic heart disease. The World Health Organization estimated in 2004 that 12.2% of worldwide deaths were from ischemic heart disease with it being the leading cause of death in high- or middle-income countries and the second only to lower respiratory infections in lower-income countries [4].

Ischemic heart disease is becoming a more common cause of death in the developing world. For example, in India, ischemic heart disease had become the main cause of death by 2004 accounting for 1.46 million deaths (14% of total deaths) and deaths due to ischemic heart disease were expected to double during 2005–2015 [5]. Globally, it is predicted that disability-adjusted life years (DALYs) lost to ischemic heart disease will account for 5.5% of total DALYs in 2030, making it the second most important cause of disability (after unipolar depressive disorder), as well as the leading cause of death by this date [5]. It is widely agreed that increased consumption of fruits, grains, and vegetables, decreased intake of saturated fats and moderate degree of exercise would improve the cardiovascular health of the populations in most developed and "near-developed" countries [6].

Fruits, grains, teas, and vegetables are rich in antioxidants (ascorbate, tocopherols, tocotrienols, flavonoids, other phenols, and carotenoids) are among the antioxidants found in various plants consumed by humans [7] and so it is widely thought that antioxidants make an important contribution to this cardiovascular protective effect [8]. In the biology system, the free radicals are often derived from oxygen, nitrogen, and sulfur molecules. These free radicals are parts of groups of molecules called reactive oxygen species (ROS) and reactive nitrogen species [9]. ROS can attack bases in nucleic acids, amino acid side chains in proteins and double bonds in unsaturated fatty acids, in which OH is the strongest oxidant. ROS attacking macromolecules is often termed oxidative stress [10]. Thus, oxidative stress significantly contributes to the pathogenesis of inflammatory disease, cardiovascular disease, cancer, diabetes, Alzheimer's disease, cataracts, autism, and aging [10].

Antioxidants may be molecules that can neutralize free radicals by accepting or donating an electron(s) to eliminate the unpaired condition of the radical [11]. Many antioxidants may directly react with ROS and/or free radical intermediates induced by ROS and terminate the chain reaction, thereby stopping the ROS-induced damage [12]. Small molecules such as Vitamin C, Vitamin E, uric acid, and glutathione (GSH) play an important role as cellular antioxidants [13]. As an antioxidant, Vitamin E prevents cell damage by inhibiting the oxidation of lipids and the formation of free radicals [14]. Vitamin E may provide additional benefits in protecting against heart disease and strokes by its ability to reduce low-density lipoprotein (LDL) cholesterol peroxidation and increase plasma LDL breakdown inhibit excessive platelet aggregation, increase high-density lipoprotein cholesterol levels and increase fibrinolytic activity [15]. In the year 2000, all RDA values were in the process of being replaced by dietary reference intakes (DRIs). The DRI has been established at 15 IU of alpha-tocopherol. The revised DRI levels are the same for both men and women [16]. The aim of the present study was to evaluate the presence of cardioprotective effects of Vitamin E on cardiac marker enzymes, troponin-I (CTn-I), creatine kinase, lipid peroxidation, and antioxidant system in isoprenaline (ISO)-induced myocardial infracted rats.

METHODS

Adult male albino rats were chosen as an animal model for this study. Rats were brought from animal house, Faculty of Medicine, Assiut University, Assiut, Egypt, and were maintained on a balanced diet with water supply freely in clean containers. They were kept for 2 weeks to adapt to the laboratory conditions before the start with the experiment. 40 age-matched male albino rats with initial body weights ranging from 150 to 200 g were used. The rats were divided into three groups (10 rats each). Eight animals were used for biochemical estimations and two animals for histopathological study. The first group was the control negative group. The second group was the control positive group that was subcutaneously injected with ISO (100 mg/kg). The third group was pretreated with Vitamin E (100 mg/kg) once daily for 30 days, then subcutaneously injected with ISO at an interval of 24 h for 2 days.

Drugs and chemicals used were Vitamin E and ISO hydrochloride. Biochemical assessments such as aspartate aminotransferase (AST), alanine transaminase (ALT), and lactate dehydrogenase (LDH), CTn-I, GSH, superoxide dismutase (SOD), and malondialdehyde (MDA) were done, and histopathological examination of the heart tissue was done.

Statistical analysis was done using the computer program (SPSS). The quantitative data were presented in the form of mean±standard error (SE). Statistical analysis of the difference between groups was performed using one-way ANOVA followed by Tukey-Kramer test for differences between means. A value of $p<0.05$ was used as the limit for statistical significance.

RESULTS

Table 1 summarized a significant difference by one-way ANOVA test between the serum level of different enzymes, antioxidant and oxidation product in the negative control, positive control (ISO injected group), and pretreated groups. The results showed an increase in the activity of AST, ALT, LDH, CK, CTn-I, and MDA in the serum of positive control group that injected with isoprenaline when compared with negative control group. Prior treatment with Vitamin E (100 mg/kg) daily for 30 days significantly (*$p<0.05$) decrease the activity of AST, ALT, LDH, CK, CTn-I, and MDA; however, their levels did not decrease to the normal level as in negative control group. Prior treatment with Vitamin E (100 mg/kg) daily for 30 days significantly (**$p<0.05$) increase the level of GSH and SOD.

Fig. 1 showed histopathological examination of the hearts of normal rats showing normal striation of the myocardium. Fig. 2 showed histopathological examination of H and E-stained sections of the hearts of the rats injected with ISO. Histopathologic changes were expressed as focal area of coagulative necrosis of the myocardium (Fig. 2a). The myofibers show karyopyknosis, hypereosinophilia, and loss of striation (Fig. 2b) infiltration of macrophage in the interstitium (Fig. 2c). Thrombosis of blood vessels can be observed (Fig. 2d). Fig. 3 showed histopathological examination of the hearts of the rats treated with ISO +Vitamin E. It showed thrombosis of blood vessels with more or less normal myocardial fibers (Fig. 3a and b).

DISCUSSION

ISO in large doses induces morphological and functional alterations in the heart leading to myocardial necrosis [17,18]. Serum CK, AST, ALT, and LDH are well-known markers of myocardial infarction. These enzymes enter into the bloodstream, thus increasing their concentration in the serum [19]. The present study reveals that ISO treatment results in marked elevation in the levels of cardiac serum marker enzymes such as AST, ALT, LDH, and CK. Elevated (CTn-I) levels predict the risk of both

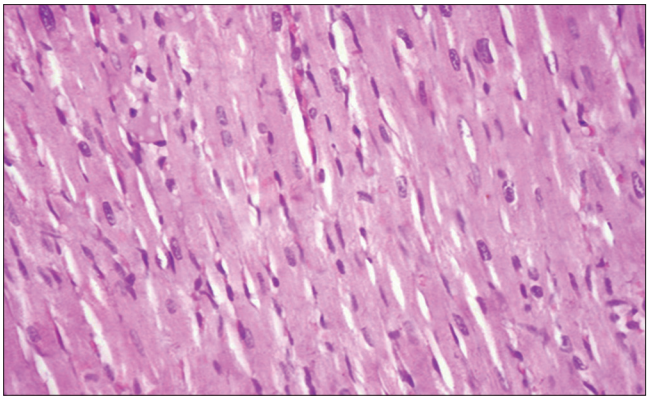

Fig. 1: Histopathological examination of the hearts of the negative control group

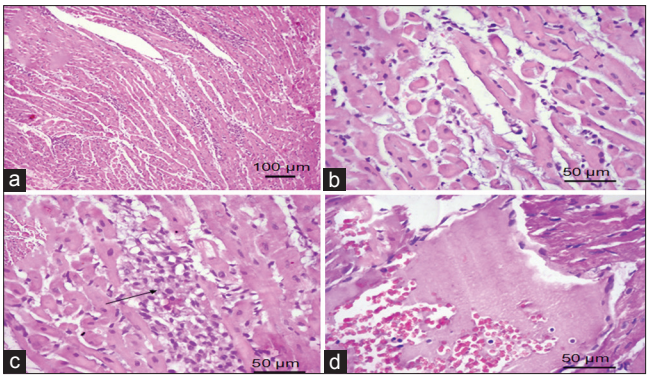

Fig. 2: (a) Focal area of coagulative necrosis of the myocardium. (b) Pyknosis and eosinophilia of the myocytes, loss of striation. (c) Infiltration of macrophage in the interstitium (arrow). (d) Thrombosis of blood vessels

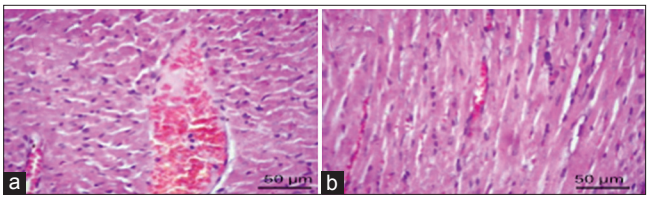

Fig. 3: (a and b) Histopathological examination of the hearts of the rats treated with ISO +Vitamin E showing thrombosis of blood vessels with more or less normal myocardial fibers

Table 1: Descriptive and one-way ANOVA tests to estimate the protective effect of pretreatment with Vitamin E on the activity of AST, ALT, LDH, CK, GSH, SOD, and MDA in serum of ISO-induced myocardial infarcted rats.

Biochemical Measurements	Negative control group (1st group)	Positive control ISO injected group (2nd group)	Pretreated group with Vitamin E	p value between 1st and 2nd groups	p value between 2nd and 3rd groups	p value between 1st and 3rd groups
AST (IU/l)	34.02±02.24	60.57±2.81	47.23±2.48	p<0.05	p<0.05	p<0.05
ALT (IU/l)	24.48±2.62	53.31±2.28	37.88±2.36	p<0.05	p<0.05	p<0.05
LDH (IU/l)	80.98±3.63	163.03±2.2	95.13±3.26	p<0.05	p<0.05	p<0.05
CK (IU/l)	165.76±4.56	282.47±8.57	192.6±3.88	p<0.05	p<0.05	p<0.05
CTn-I (ng/ml)	0.158±0.027	2.35±0.015	0.96±0.048	p<0.05	p<0.05	p<0.05
GSH (mmol/g tissue)	12.62±0.83	3.91±0.39	9.05±0.5	p<0.05	p<0.05	p<0.05
SOD (U/g tissue)	17.47±1.34	6.03±0.65	11.96±0.94	p<0.05	p<0.05	p<0.05
MDA (nmol/g tissue)	1.04±0.16	2.65±0.18	1.68±0.1	p<0.05	p<0.05	p<0.05

Each value represents the mean±SE (standard error) of 10 animals, AST: Aspartate aminotransferase, ALT: Alanine transaminase, LDH: Lactate dehydrogenase, CK: Creatine kinase, CTn-I: Cardiac troponin-I, GSH: Glutathione, SOD: Superoxide dismutase, MDA: Malondialdehyde

cardiac death and subsequent infarction. In the present study, increased levels of CTn-I were observed in serum of ISO-treated rats.

Results of the present study are in line with those reported by Eman *et al*. and Chikku and Rajamohan. They found that ISO-treated rats showed statistically significant (*p<0.05) increased activity of LDH, CK, AST, and ALT and increased the concentration of cardiac troponins in the serum compared to normal control rats. They also reported that ROS is formed at an accelerated rate in the myocardium due to ISO administration [20,21].

Kumaran and Mainzen reported that the activity of serum CK was considerably (*p<0.05) increased in ISO-induced rats compared to normal control rats. They also reported that rats treated with ISO showed considerable (*p<0.05) elevation in the levels of serum CTn-I compared to normal control rats. The observed increased levels of CTn-I might be due to ISO-induced cardiac damage [22].

In the present study, the results showed that pretreatment with Vitamin E significantly decreased the activity of AST, ALT, LDH, CK, and the levels of CTn-I in serum of ISO-induced rats. These results were in agreement with the results obtained by other researchers; Upaganlawar *et al*. reported that treatment with Vitamin E for 30 days showed a statistically significant (**p<0.01) reduction in the activities of all serum cardiac marker enzymes such as AST, ALT, LDH, and CK as compared to the ISO-treated group [23].

The results of the present study showed that ISO treatment showed an increase in the levels of lipid peroxidation products MDA in the heart. Increased lipid peroxidation appears to be the initial stage to the tissue making it more susceptible to oxidative damage. Results of the present study are in line with those reported by Chikku and Rajamohan 2012. They found that ISO administration is associated with increased levels of lipid peroxidation as evidenced by increased levels of MDA in the heart. Activated lipid peroxidation is an important pathogenic event in MI, and the levels of lipid peroxide reflect the major stages of disease and its complications [21].

The results of the present study showed that pretreatment with Vitamin E (100 mg/kg) daily for 30 days significantly (**p<0.01) decreased the level of MDA compared with ISO alone induced rats this finding is in agreement with the results obtained by Upaganlawar *et al*., 2010 who reported that maximum induction of lipid peroxides was observed in ISO intoxicated rats. The change in lipid peroxides was significantly decreased in rats treated with Vitamin E compared to ISO intoxicated rats [22].

Antioxidants constitute the foremost defense system that limits the toxicity associated with free radicals. Free radical scavenging enzymes such as SOD against oxidative injury. These enzymes are lowered due to enhanced lipid peroxidation. Superoxide radicals generated at the site of damage in myocardial infarction modulate (SOD) resulting in the lowered activities of the enzyme and accumulation of superoxide anion, which also damages the myocardium [23].

The results of the present study showed that rats induced with ISO showed a considerable (*p<0.01) decrease in the levels of reduced GSH and SOD in the heart compared to normal control rats. This finding is in agreement with the results obtained by Kumaran and Mainzen [24].

Chikku and Rajamohan found that decrease in the activities of SOD and GSH were observed in the heart of ISO alone treated rats. The decreased activities of GSH-dependent enzymes such as GSH peroxidase (GPx) in the heart of MI-induced rats may be due to decreased GSH concentration. Filho *et al*., 2011 reported that the induction of myocardial infarction led to a reduction of SOD activity in myocardial tissue. Free radical scavenger enzymes such as SOD are the first line of defense against oxidative injury [25].

Priscilla and Prince 2009 reported that decreased concentration of Vitamin E in plasma, and heart was observed in ISO-induced rats. The lowered level of this non-enzymatic antioxidant might be due to increased lipid peroxidation [23]. These results are in disagreement with the results obtained by the heart outcomes prevention evaluation study investigators 2000. They reported that Vitamin E did not reduce myocardial infarction, stroke, or death in adults at high risk for cardiovascular events [15]. The results of the present study show that pretreatment with Vitamin E (100 mg/kg) daily for 30 days to ISO-induced rats significantly (*p<0.01) increased the levels of GSH and SOD compared with ISO alone induced rats. These results are in agreement with the results obtained by Upaganlawar *et al*., 2010 [26]. Thus, Vitamin E is a powerful antioxidant found in different tissues that protect against oxidative damage [27].

CONCLUSION

Histopathological findings of Vitamin E showed a normal morphology of cardiac muscle by an absence of necrosis and inflammatory cells which is in accordance with biochemical changes. Vitamin E appears to be the most effective lipid-soluble antioxidant in the biological system. It involves in the inhibition of lipid peroxidation and regenerates GSH. Thus, Vitamin E protects the heart against ISO-induced oxidative stress in rats.

CONFLICT OF INTEREST

None.

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