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POTENTIATION OF EFFECTS OF PROPRANOLOL AND HEPARIN BY ANTIOXIDANT IN ADRENALINE INDUCED MYOCARDIAL INFARCTION IN RATS

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ABSTRACT

Background: Myocardial Infarction also known as heart attack is still a major cause of morbidity and mortality around the world. Vitamin C is a powerful antioxidant that can strengthen body's natural defenses and propranolol and heparin are the drugs which affect the cardiovascular system. So there is need to see if Propranolol, heparin, vitamin C and combinations of vitamin C with low doses of propranolol and heparin can show cardioprotection against adrenaline induced myocardial infraction (MI) in rats. **Objective:** To study cardioprotective effect of Vitamin C and its combination with low dose of propranolol and heparin against adrenaline induced myocardial infarction (MI) in rats. **Materials and Methods:** Rats were divided randomly into seven groups (6 rats each); Group I-Normal control; Group II-Toxicant control (adrenaline treated group); Group III-rats were pre-treated with propranolol (10 mg/kg p.o) for 10 days (from day 12 to day 21); Group IV-rats were pre-treated with heparin (500 units/kg) for 2 days (on 20^{th} and 21^{th} day); Group V-rats were pretreated with vitamin C (40 mg/kg i.p) for 21 days; Group VI-rats were pretreated with vitamin C (40 mg/kg) and propranolol (5 mg/kg) for 21 days and from 12 to 21 days respectively; Group VII-rats were pretreated with vitamin C (40 mg/kg) and heparin (250 units/kg) for 21 days and on 20^{th} -21^{th} day respectively. The rat model of myocardial infarction was produced by injecting adrenaline (2 mg/kg b.w.) subcutaneously into all animals (except the control) twice at a 24-hour interval on days 20^{th} and 21^{th} day of the experiment. The cardiac markers tests were done at the conclusion of the trial. **Results:** Treatment with Adrenaline showed significantly increased in biochemical parameters (CK-MB, LDH, SGOT, Troponin-I). Prior treatment with propranolol, heparin, vitamin C and its combination with low doses of propranolol and heparin showed significant

alteration in these parameters. **Conclusion:** The results of present study highlights that propranolol, heparin and vitamin C when given alone showed cardioprotection but combination of vitamin C with Propranolol and Heparin with low doses has showed better cardioprotection based on the alterations of cardiac biomarkers.

KEYWORDS: Myocardial infarction, Adrenaline, Oxidative stress, Inflammation, Propranolol, Heparin, Vitamin C, Cardiac biomarkers.

1. INTRODUCTION

Cardiovascular Diseases CVDs, often known as silent killers, are the largest cause of disease burden and mortality around the world. Annual CVD deaths in India are expected to increase from 2.26 million in 1990 to 4.77 million in 2020, accounting for 29 percent of all deaths worldwide (17.9 million deaths), and by 2030, more than 23.3 million people would die from CVDs (WHO).[1] Coronary heart disease, cerebrovascular disease, congestive heart failure, and other heart and blood vessel illnesses are all classified as CVDs. Heart attacks account for more than four out of every five CVD deaths.

Myocardial infarction, often known as a heart attack, is a life-threatening disorder in which blood flow to the heart's coronary artery slows or ceases, causing damage to the heart muscle. It is the irreversible destruction of heart muscle caused by a lack of oxygen for an extended period of time. As a result, there is a mismatch between coronary blood supply and myocardial demand. It's linked to an inflammatory response and a change in the extracellular matrix as a result of free radical release.[2] Prolonged MI causes ischemia and cardiac cell death.[3] It has been well characterized that oxidative stress and inflammation are the main pathophysiological process involved in Myocardial Infarction.[4] The severity of cardiac lesions can be influenced by the inflammatory process. Anti-inflammatory medications may help to minimise the size of ischemic lesions in myocardial ischemia. Antioxidant therapy can also have cardioprotective effects by lowering oxidative stress during myocardial ischemia and reperfusion damage. The enzymes most widely used in the detection of MI are Troponin I, Creatine kinase-MB(CK-MB), Lactate dehydrogenase(LDH).[5]

Adrenaline, also known as epinephrine, is a stress hormone produced by the adrenal glands and released into the bloodstream. It is a component of the "fight or flight" reaction. Catecholamine is a naturally occurring substance. It also has medicinal uses in the treatment of cardiac arrest, allergic responses, and asthma, among other things. However, at doses

beyond physiological levels, it has been shown to increase the generation of reactive oxygen species (ROS) and tissue damage caused by reactive nitrogen species (RNS). Adrenaline-induced MI in rats is regarded as a reliable experimental model for studying medication cardioprotective effects. Adrenaline has been discovered to cause MI by promoting lipid peroxidation, which causes cellular antioxidants to be depleted.[6]

Vitamin C is a water-soluble antioxidant and cofactor for enzymes in both plants and animals.[7] It is a potent antioxidant that helps the body's natural defences against oxidative stress mediated by reactive oxygen species (ROS) by shielding cells from free radicals, which are damaging chemicals. Consuming extra vitamin C raises your blood antioxidant level, which aids the body's natural defences in fighting inflammation, according to studies.[8] Futhermore, vitamin C intake from food or suppliments can increase the bioavailability of iron by improving the absorption of the non-heme iron.

Propranolol is a nonselective beta-adrenoreceptor antagonist. It has a number of pharmacological properties that may be useful in MI and support its usage. It exerts its response by competitively blocking beta-1 and beta-2 adrenergic stimulation in the heart, which is typically induced by adrenaline.[9] It exerts its effects primarily by blocking the action of the endogenous catecholamines, epinephrine and norepinephrine.[10] antagonizes the action and relieve stress on the muscle. It has the property to decrease the workload of heart by slowing the heart rate and force of contraction and also decrease the demand of oxygen.

Heparin is an anticoagulant which is known as blood thinner, a chemical substance that prevent or reduce coagulation of blood prolonging the clotting time. It interacts with the naturally occuring plasma protein, Antithrombin III, to induce conformational change. It inhibit Factor Xa and thrombin (Factor II a). It also prevent the formation of a stable fibrin clot by inhibiting the activation of the fibrin stabilizing factor.[11] It blocks the activity of coagulation factor and is used for reinfarction and thromboembolism and can lead to reduction in dead.

In this study, we assess the cardioprotective effects of propranolol, heparin, vitamin C and its combination with low doses of propranolol and heparin in rats with adrenaline-induced myocardial infarction. Since propranolol and heparin are used to treat MI, but the study of low doses of these drugs has not been made and if this drugs are combined with antioxidant then enhancement in immune system will take place and can show advanced effect which can

enhance immune system and improve its function. This research also sheds light on how metabolic changes affect therapy efficacy.

2. MATERIALS AND METHODS

2.1. Experimental animals

The experiments were conducted according to ethical guidelines as approved, Wistar Albino rats were used in the present study. These rats were procured from registered breeder and was acquainted in the quarantine area for one week. After acquaintation, animals were transferred to the standard laboratory conditions of 22 ± 2ºC temperature, 50 ± 15% of relative humidity, 12 hr dark/12 hr light cycle and the animals received free access to pellet diet & water provided *ad libitum*. The study protocol was presented to the IAEC and was approved.

2.2. Drugs and Chemicals

Adrenaline was purchased from Aqua fine injecta pvt. ltd. pune, Propranolol and vitamin C were purchased from Sigma-Aldrich Chemical Co. (st. Louis, USA), Heparin from Pfizer Labs division of Pfizer Inc NY, NY 10017, CK-MB, LDH, SGOT and Troponin kits was purchased from Meril Diagnostics Pvt. Ltd, Gujarat, India.

2.3. Induction of experimental MI

Adrenaline (2 mg/kg body weight) was subcutaneously injected into all groups of rats except normal control group for 2 consecutive days at an interval of 24 h to induce experimental myocardial infarction.

2.4. Experimental protocol

A total of 42 wistar albino rats were used for this study. After acclimatization, they were randomly divided into seven groups, consisting of six rats per group.

2.4.1. Group I (Normal control)

Served as untreated normal control rats and received distilled water (1 ml p.o).

2.4.2. Group II (Toxicant control)

Animals received Adrenaline (2 mg/kg body weight s.c) on 20th and 21th day.

2.4.3. Group III (Propranolol + Adrenaline)

Animals received Propranolol (10mg /kg body weight p.o) for 10 days (from 12 to 21 days) and challenged with adrenaline (2 mg/kg, s.c) on 20th and 21th day.

2.4.4. Group IV (Heparin + Adrenaline)

Animals received heparin (500 units/kg body weight s.c) on 20th and 21th day and challenged with adrenaline (2 mg/kg, s.c) on 20th and 21th day.

2.4.5. Group V (Vitamin C + Adrenaline)

Animals received vitamin C (40 mg/kg body weight i.p) for 21 days from and challenged with adrenaline (2 mg/kg, s.c) on 20th and 21th day.

2.4.6. Group VI (Vitamin C + Propranolol + Adrenaline)

Animals received vitamin C (40 mg/kg body weight i.p) for 21 days with Propranolol (5 mg/kg body weight p.o) for 10 days from 12th -21th and challenged with adrenaline (2 mg/kg, s.c) on 20th and 21th day.

2.4.7. Group VII (Vitamin C + Heparin + Adrenaline)

Animals received vitamin C (40 mg/kg body weight i.p) for 21 days with heparin (250 units/kg body weight s.c) on 20th and 21th day and challenged with adrenaline (2 mg/kg, s.c) on 20th and 21th day.

2.5. Biochemical estimation

The blood samples were taken from the retro-orbital plexus and centrifuged to separate them. The serum of numerous experimental animals was collected and utilised to perform various biochemical analyses.

The activities of serum creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), Serum glutamic oxaloacetic Transaminase (SGOT) and Troponin-I were estimated using commercially available kits as per instructions.

2.6. Statistical analysis

Data were expressed as mean $\pm$ S.E.M (six rats per group). Groups of data were compared by one-way analysis of variance (ANOVA) followed by Dunnett's t-test. Values $p < 0.05$ were consider statistically significant. Statistical analysis was carried out using Graph Pad Prism 5.0 software (Graph Pad Software, San Diego, California, USA).

3. RESULTS

3.1. Effects of Vitamin C and its combination with low doses of Propranolol and Heparin against adrenaline induced MI in rats

The effects of Propranolol, Heparin, Vitamin C and its combination with propranolol and heparin on Cardiac biomarkers are seen in the table No:1. Rats treated with adrenaline (Group II) showed a significant increase in the level of serum LDH, CK-MB, SGOT and Troponin-I (Positive) as compared to the normal control (Group I). Pre-treatment with propranolol (10 mg/kg), heparin (500 units/kg) and vitamin C (40 mg/kg) when given alone reduced the elevated level of the cardiac markers induced by adrenaline. Prominent inhibition effect on cardiac markers was observed when animals were treated by combination of vitamin C with low dose of propranolol and heparin.

Table no. 1: Effects of vitamin C and its combination with low doses of propranolol and heparin against adrenaline induced MI in rats.

Groups	CK-MB(IU/L)	LDH(U/L)	SGOT(IU/L)	Troponin-I
Control	96.44±3.05	477.22±16.35	56.83±02.33	Negative
Adrenaline	273.77±12.09	749.55±33.13	219.44±07.33	Positive
Propranolol + Adrenaline	201.36±10.33	649.41±22.63	155.13±10.33	Negative
Heparin + Adrenaline	228.41±11.88	705.31±35.33	187.35±09.50	Negative
Vitamin C + Adrenaline	264.25±12.48	731.58±11.27	201.21±12.11	Negative
Vitamin C +Propranolol + Adrenaline	129.12±05.33	537.13±16.33	126.57±06.33	Negative
Vitamin C +Heparin + Adrenaline	162.51±8.33	613.24±25.13	141.29±10.48	Negative

Data are expressed as mean ±S.E.M (n= 6 animals in each group). Statistical analysis are carried out by one-way ANOVA followed by Dunnett's t-test.

Significance difference from normal group at $p < 0.05$.

Significance difference from control (MI) group at $p < 0.05$.

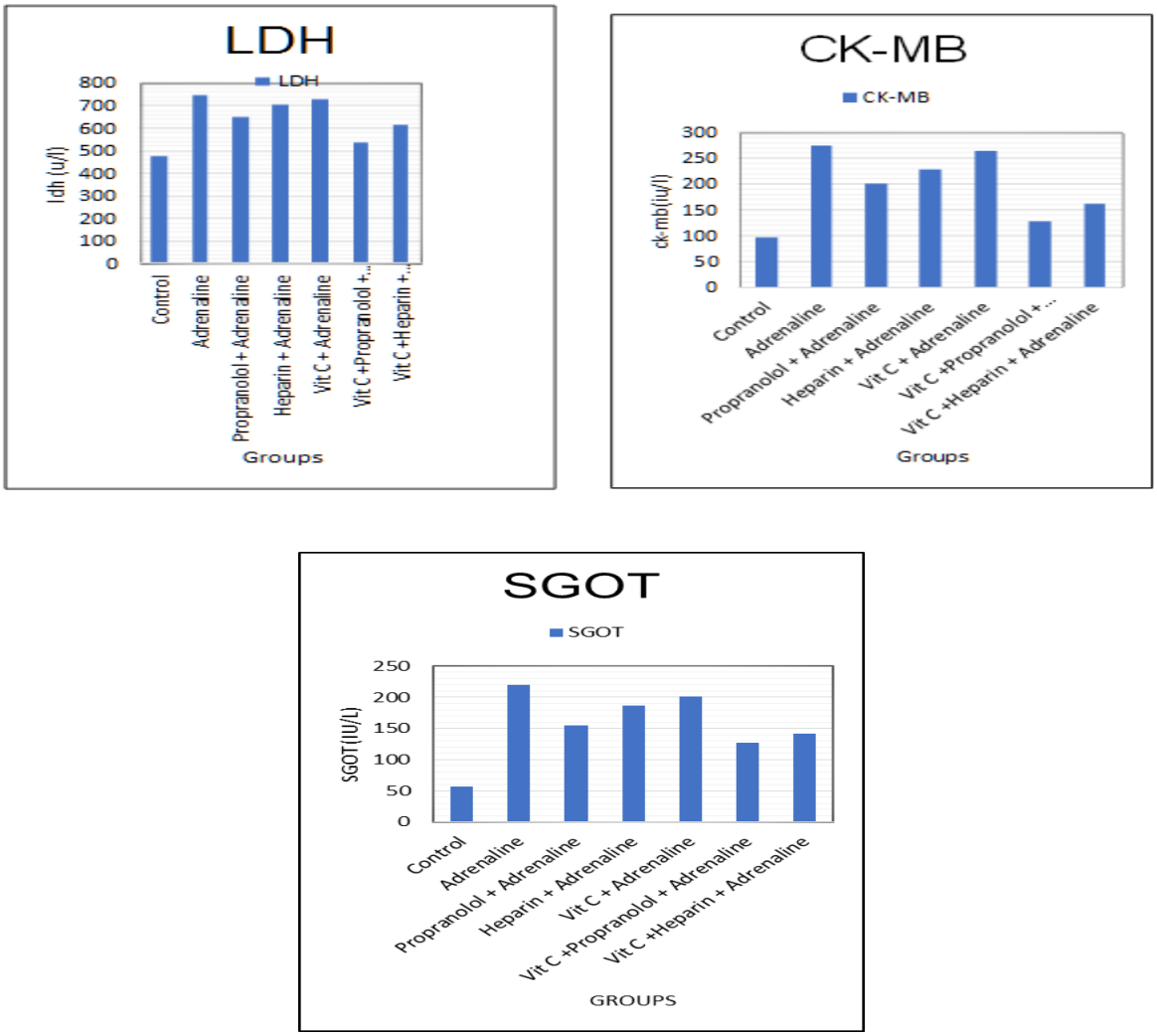

Fig. 1: Graphical representation of effects of Vitamin C and its combination with low doses of propranolol and heparin against adrenaline induced MI in rats.

4. DISCUSSION

In 2021, myocardial infarctions were on the rise, and there was no age limit. This year, young, healthy, fit persons with no medical history have suffered heart attacks, with some cases even resulting in death, contributing to an increase in mortality. The pandemic of COVID had also contributed in the increasing cases of heart attacks.

For a long time, myocardial cell protection and cell necrosis prevention have been therapeutic targets. Because present treatments have a limited influence on survival and annual expenditures, new therapeutics are needed to treat myocardial infarction.

The purpose of this investigation was to evaluate the potential cardioprotective role of propranolol, heparin, vitamin C and combination of vitamin C with low doses of propranolol and heparin in adrenaline induced MI in rats.

In this study, adrenaline was administered subcutaneously at a dose of 2 mg/kg for two days, resulting in a significant increase in blood levels of cardiac enzymes CK-MB, LDH, SGOT, and Troponin-I. This demonstrates that adrenaline injection causes myocardial injury and cardiac enzyme leakage into the circulation. This can take place due to increased in lipid peroxidation, inflammation or apoptosis.[12] Due to lipid peroxidation oxidative stress generates which can lead to the injury or damage to the cells which leads to the increase in the cardiac biomarkers. Our results supports the hypothesis that adrenaline has got the ability to produce free radicals and has caused myocardial infarctions.

Damage to the cell membranes, by production of large number of free radicals and ROS, generation of lipid peroxides, and lowering of antioxidative defense lines are major outcomes of MI.[13]

Since, ROS is important in the pathogenesis of MI, antioxidants and their combinations in the treatment of MI were the focus of research.

Pre-treatment with propranolol, heparin, vitamin C and its combination with low doses of propranolol and heparin has been observed. It was found that the dugs shows cardioprotective effects.

In recent years, long-term prevention of CVD is associated with consumption of fresh fruits, vegetables or plants rich in antioxidant. As a result there has been considerable interest in antioxidants.[14]

Vitamin C is a powerful dietary antioxidant. It is widely known vitamin in different kinds of fruits and is available as a supplement.[15] Antioxidants constitute the foremost defense system that limits the toxicity associated with free radicals.[16] It act by reducing the reactive oxygen species and leads in decrease in lipid peroxidation which is the main cause of depilation of cellular antioxidant and which leads to damage of cells and inflammation which leads to MI. Vitamin C act against it and showed mild effect by decreasing the levels of CK-MB, LDH, SGOT and Troponin-I helps in reducing the impact caused due to adrenaline.

Propranolol antagonizes the effect of adrenaline by lowering heart rate[17] and reducing the lipid peroxidation as it decreases the demand for oxygen, it also relaxes the blood vessels and improve blood flow with decrease in blood pressure therefore possesses the cardioprotective effect.

As the blood gets clots, when the tissue gets damaged due to the generation of oxidative stress the use of heparin helped in the anticoagulation process by inhibiting the enzymes due to which the clots are reduced. It also attenuates the rate of rise in blood pressure and prevents severe fibrinoid vascular lesions and thus shows cardioprotection.[18]

So, as the drugs showed positive effect there was need to see if the low doses of these drugs shows some advanced effect if combined with the antioxidant.

In this study, when the low doses of propranolol and heparin was combined with vitamin C, the combination with propranolol showed more advanced effect on oxidative stress which lead to lipid peroxidation due to its strong antioxidant property due to which the level of cardiac biomarkers were decreased as compared to that of the adrenaline treated animals (Toxicant group).

Similarly, when it was combined with heparin it showed advanced cardioprotection and lead to decreased inflammation, oxidative stress and blood clots which were observed by alteration of parameters which were caused by adrenaline induced MI in rats.

5. CONCLUSIONS

It can be concluded that propranolol, heparin and vitamin C when given alone improved adrenaline induced abnormal changes in the biomarkers and showed cardioprotective effect in rats after being exposed to adrenaline but the combined administration of vitamin C with low dose of propranolol and heparin produce a potentiation effect and showed better results possibly via reducing cardiac biomarkers associated with oxidative stress, inflammation, and apoptosis. More research is needed to determine the exact molecular pathways involved in the cardioprotective impact of medicines in MI rats.

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