The Potential Pharmacodynamic Interaction of Alpha-Tocopherol and Captopril on Isoproterenol-Induced Myocardial Infarction in Sprague-Dawley Rats

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Abstract: The use of vitamin supplements and other alternative medicines is increasing globally, especially in underdeveloped countries including the Philippines. It is interesting to note that a vitamin supplement is often administered in combination with known therapeutic drugs, raising the potential for pharmacodynamic interaction. Cardiovascular diseases (CVDs) are one of the major health problems globally, with myocardial infarction as the most common cause of death in many parts of the world. Vitamin E, chemically known as alpha-tocopherol, has been widely used as a supplement due to its antioxidant properties which have shown prevention of cellular damage due to free-radical oxidation during myocardial infarction. This study is designed to evaluate the pharmacodynamic interaction of alpha-tocopherol at different concentrations and captopril, an angiotensin-converting enzyme inhibitor. The animals were pretreated with 30 mg/kg captopril, and alpha-tocopherol at varying doses of 100 mg/kg, 300 mg/kg, and 400 mg/kg. Both agents were used individually and in combination for seven days, and on the eighth and ninth day, myocardial infarction was induced to the animals using 85 mg/kg isoproterenol (ISO) at an interval of 24 hours. The following parameters were determined; aspartate aminotransferase (AST), creatinine kinase-MB (CK-MB), and lactate dehydrogenase (LDH). The levels of cardiac biomarkers increased significantly ($p<0.05$) in the untreated rats intoxicated with isoproterenol whereas the rats with pretreatment have decreased cardiac enzyme levels. The combination index revealed that there was a synergistic effect using alpha-tocopherol

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(100 and 300 mg/kg), and captopril in combination. However, the highest dose of alpha-tocopherol (400 mg/kg) in combination with captopril failed to prevent the myocardial damage induced by ISO. This study demonstrated that alpha-tocopherol and captopril elicited their cardioprotective effects synergistically particularly at doses 100 and 300 mg/kg, and 30 mg/kg, respectively on isoproterenol-induced myocardial infarction in Sprague-Dawley rats.

Keywords: *alpha-tocopherol, captopril, ACE inhibitor, isoproterenol-induced myocardial infarction*

INTRODUCTION

According to the World Health Organization (WHO), cardiovascular diseases are the number one cause of death throughout the world. The disease kills 17.3 million people annually which accounts for 3 in every 10 deaths, and is expected to grow to more than 23.6 million by 2030. Cardiovascular diseases (CVDs) are disorders of the heart and blood vessels and include coronary heart disease, cerebrovascular disease, rheumatic heart disease, and other conditions. Individuals at risk of CVD may demonstrate raised blood pressure, glucose, and lipids as well as overweight and obesity (WHO, 2016).

According to the Philippine Department of Health (DOH), ischemic heart attack, stroke and cancer remain as the top causes of death among Filipinos (Althea Group, 2015). In 2008, cardiovascular deaths represented 30% of all global deaths, with 80% of those deaths taking place in low- and middle-income countries (Jambora, 2014). About 80% of deaths due to cardiovascular diseases are caused by strokes and heart attacks also known as myocardial infarction (MI) (WHO, 2016).

Myocardial infarction is pathologically defined as irreversible death of myocardial cells secondary to ischemia. A condition called myocardial ischemia occurs if blood supply to the myocardium does not meet its demand. If this imbalance persists, it causes plaque rupture and cascade of cellular, inflammatory and biochemical events, eventually leading to myocardial necrosis. Generally, initial therapy for acute MI is directed toward restoration of perfusion as soon as possible to salvage as much of the jeopardized myocardium as possible. This may be accomplished through medical or mechanical means, such as percutaneous coronary intervention (PCI), or coronary artery bypass graft (CABG) surgery (Zafari, 2017). Most people will take a

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combination of drugs including an anti-platelet drug, such as aspirin, which interferes with the blood's clotting mechanism; an angiotensin converting enzyme (ACE) inhibitor, which relaxes the arteries enabling blood to flow through them more freely; a β-blocker which slows down the heartbeat and a statin which helps to lower cholesterol and those who are likely to experience angina will be given a nitrate. Other drugs for lowering high blood pressure may also be prescribed (Dumelow, 2013).

ACE inhibitors have been used successfully for decades and effective innovative drugs are becoming available, in addition there is increased understanding of the pathophysiology and pharmacology, all of which contribute to improved management of high risk patients (Mancia, 2013). Most randomized trials have demonstrated that (ACE) inhibitor therapy with captopril, enalapril, ramipril, trandolapril, or zofenopril started within 24 hours to 16 days following an acute myocardial infarction (MI) improves the left ventricular ejection fraction (LVEF) at one month to one year. The administration of an ACE inhibitor is also associated with an important improvement in patient survival (Reeder, 2016).

ACE inhibitors differ in chemical structure and functional group (primarily the sulfhydryl [SH] group), prodrug nature, potency and duration of effect. Different structural profiles may include additional pharmacological properties which may provide significant benefits as well as different clinical pharmacokinetic profiles (Mancia, 2013). ACE inhibitors block the enzyme peptidyl dipeptidase that hydrolyzes angiotensin I to angiotensin II and inactivates bradykinin, a potent vasodilator, which works at least in part by stimulating release of nitric oxide and prostacyclin (Katzung, 2012). Angiotensin II is an essential regulator of cardiovascular function. The ability to decrease levels of angiotensin II with oral ACE inhibitors represents an advantage in the treatment of cardiovascular diseases. Captopril, the first ACE inhibitor to be marketed, is a potent ACE inhibitor. It is the only ACE inhibitor approved for use in the U.S. that contains a sulfhydryl moiety. The oral dose of captopril ranges from 6.25-150 mg two to three times daily, with 6.25 mg three times daily or 25 mg twice daily being appropriate for the initiation of therapy for heart failure or hypertension, respectively (Goodman & Gilman, 2011).

Antioxidant compounds, highly present in plants have shown protective effects against diseases without reducing their therapeutic efficacy. Moreover, there is a growing interest in the usage of natural antioxidants as a protective strategy against

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cardiovascular related problems such as ischemia reperfusion. (Beaulah, et. al., 2014). The potential myocardial implications of cellular redox imbalance are far reaching. Whereas low levels of reactive oxygen species play critical roles in cellular homeostasis, differentiation, and cell signaling, myocardial ischemia and reperfusion (IR) results in markedly accelerated oxygen radical production, trigger cellular damage. Functional changes occur after ischemia, including contractile dysfunction, insufficient coronary flow, and impaired vascular reactivity. Over the last few decades, it has become clear that myocardial reactive oxygen species (ROS) formation is accelerated after ischemia and plays a critical role in reperfusion (IR)-induced myocardial damage (Subasini & Rajamanickam, 2014). The chemotherapeutic agents, that inhibit the free radical formation and that may reduce the risk of heart diseases, have gained imperative value in the modern medicines. Herbal medicines having antioxidant properties, may therefore, have a protective role in cardiovascular diseases (Beaulah et. al., 2014).

Oxidative damage may play a role in the development of cardiovascular disease (CVD), particularly through its effect on lipid peroxidation and DNA damage. In addition, free radicals may damage arterial endothelium, encourage thrombosis, and alter vasomotor function. Antioxidants scavenge free radicals and limit the damage they can cause. Diets high in fruit and vegetable intake, and thus rich in such antioxidants, have been associated with reduced rates of coronary heart disease and stroke (Cook, et. al., 2007).

Alpha-tocopherol, the most biologically active and abundant form of vitamin E in vivo, efficiently transfers a hydrogen atom to a lipid free radical, such as peroxyl, alkoxyl, and carbon-centered radicals, giving the corresponding non-radical product of the lipid and an alpha-tocopheroxyl radical. The alpha-tocopheroxyl radicals, once formed, react with a second free radical or each other to form a non-radical product. Each molecule of alpha-tocopherol consumes thus two lipid free radicals and terminates the free-radical chain reaction. Tocopherol isomers are chain-breaking antioxidants. The antioxidative activity of the tocopherols is related to scavenging the free radicals of unsaturated lipids (Yamauchi R., 1997). The antioxidant effect of vitamin E is not limited to a role of lipid phase ROS scavenger, as it can increase glutathione peroxidase activity and diminish ROS production via downregulating NADPH oxidase (Rodrigo, et. al., 2013).

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Alpha-tocopherol or vitamin E has a strong antioxidant effect and is also used for the primary and secondary prevention of cardiovascular (CV) disease. Diets rich in vitamin E decreased the incidence rate of cardiovascular disease. The observation catches the interest of other researchers to conduct clinical trials to prove the antioxidant effect of alpha-tocopherol in cardiovascular disease (Hu, et. al., 2015).

The possible role of antioxidant of alpha-tocopherol in cardiovascular disease prevention is based on the biological rationale rendered by the "oxidative modification hypothesis," which suggests an essential role for the oxidation of lipoproteins in the origins and progression of atherosclerotic lesions, and by observational epidemiologic studies, that have shown associations between the dietary and supplemental intake of antioxidants and lower risk of coronary heart disease (CHD) (Lonn, 2001).

Isoproterenol-induced myocardial infarction

The experimental rat model of isoproterenol-induced myocardial infarction or necrosis serves as a standardized model to evaluate numerous cardiac dysfunctions and to study the effectiveness of various natural and synthetic cardioprotective agents. It is characterized by a unique technical simplicity, an exceptional reproducibility, as well as a satisfactory low mortality.

As per the research of Upaganlawar, Gandhi and Balaraman (2011), isoproterenol-induced myocardial infarction has been reported to demonstrate many metabolic and morphologic aberrations in the heart tissue of the experimental rats similar to those observed in human myocardial infarction.

MATERIALS AND PROCEDURES

Preparation of drugs and chemicals

Alpha-tocopherol, and isoproterenol of analytical grade were purchased from Sigma-Aldrich, Singapore through Belman Laboratories. Captopril was purchased from a local pharmacy [Ritemed (FDA, 2017)]. Olive oil was purchased from a local supermarket and was used as a vehicle for alpha-tocopherol. Normal saline was used to dissolve the isoproterenol and captopril.

Biological Test

Experimental animals

Fifty-four (54) eight-week old male Sprague-Dawley rats with body weight ranging from 150 - 250 grams were purchased from the University of the Philippines-Manila (UP-M) and were

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used in the experiment. The rats were housed in Lyceum of the Philippines University-Batangas (LPU-B) Animal House in rectangular plastic cages with rice husks as beddings. The LPU-B Animal House is an air-conditioned room and the animals will be placed under 12:12-hour light/dark cycles. The rats were acclimatized for one week prior to the start of the biological test. The rats were given commercially available rat feeds and water ad libitum throughout the course of the study (Beaulah, et. al., 2014; Momin, et. al., 2011).

Experimental protocol

The rats were divided into 9 groups consisting of six rats per group as follows:

Group I: Normal control (vehicle only)

Group II: Negative control – Normal saline for 7 days + isoproterenol (85 mg/kg subcutaneously for the 8th and 9th day at a 24-hour interval)

Group III: Pretreatment with 30 mg/kg captopril orally for 7 days + isoproterenol (85 mg/kg subcutaneously on the 8th and 9th day at a 24-hour interval)

Group IV: Pretreatment with 100 mg/kg alpha-tocopherol for 7 days + isoproterenol (85 mg/kg subcutaneously on the 8th and 9th day at a 24-hour interval)

Group V: Pretreatment with 300 mg/kg alpha-tocopherol orally for 7 days + isoproterenol (85 mg/kg subcutaneously on the 8th and 9th day at a 24-hour interval)

Group VI: Pretreatment with 400 mg/kg alpha-tocopherol orally for 7 days + isoproterenol (85 mg/kg subcutaneously on the 8th and 9th day at a 24-hour interval)

Group VII: Pretreatment with the combination of 100 mg/kg alpha-tocopherol and 30 mg/kg captopril (30 mg/kg) for 7 days + isoproterenol (85 mg/kg subcutaneously on the 8th and 9th day at a 24-hour interval)

Group VIII: Pretreatment with the combination of 300 mg/kg alpha-tocopherol and 30 mg/kg captopril for 7 days + isoproterenol (85 mg/kg subcutaneously on the 8th and 9th day at a 24-hour interval)

Group IX: Pretreatment with the combination of 400 mg/kg alpha-tocopherol and 30 mg/kg captopril for 7 days + isoproterenol (85 mg/kg subcutaneously on the 8th and 9th day at a 24-hour interval)

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Biochemical studies

Upon accomplishment of the experimental protocol (after 24 hours of 2nd ISO injection), about 3 mL of blood sample per animal was collected by retro-orbital plexus puncture method under mild ether anesthesia. The serum was separated through centrifugation and was used for the analysis of diagnostic marker enzymes such as creatinine kinase-MB (CK-MB), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH). These enzymes were assayed at Batangas Medical Center and Shalom Medical Diagnostic Laboratory (Kuppusamy, et. al., 2010; Ittagi, Merugumol, and Siddamsetty, 2014).

Statistical treatment

Each value was expressed as mean ± SEM. The one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test using statistical package for social sciences (SPSS) ver. 21 were used to determine whether there are any significant differences between the means of the diseased and treated group. The results were considered significant, if p-value <0.05 (Kuppusamy, et. al., 2010; Ittagi, et. al, 2014).

RESULTS AND DISCUSSION

Isoproterenol is a synthetic catecholamine and beta-adrenergic agonist that induces severe stress in the cardiac muscle leading to development of myocardial infarction (MI). MI is produced due to its action on the cardiac β1-receptors. ISO-induced myocardial necrosis showed membrane permeability alterations, which bring about the loss of function and integrity of the myocardial membrane (Karthikeyan et al., 2007). Isoproterenol produces numerous biochemical alterations which precede the histological changes in the heart. The major instabilities of isoproterenol-induced myocardial infarction have been described to enhance adenyl cyclase activity, resulting in increased cAMP formation, which in turn would lead to higher lipid accumulation in the myocardium. Biochemical alterations observed in isoproterenol induced cardiomyopathy characterize a complex pattern of modifications in cardiac marker enzymes, lipid profile, lipid metabolizing enzymes, non-enzymatic and enzymatic antioxidant levels, glycoprotein levels, decrease in ATP store and changes in electrolyte levels in the blood as well as in the myocardial tissue (Upaganlawar, 2011).

Different studies suggest that antioxidant properties of drugs play an important role in the change in pathogenesis of ISO-

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induced myocardial damage. The pathophysiological changes following ISO administration are comparable to those taking place in human myocardial alterations (Karthikeyan et al., 2007). Hence ISO-induced myocardial infarction model was used in this study. Animals were pretreated with varying doses of alpha-tocopherol (100, 300 and 400 mg/kg) alone and in combination with captopril (30 mg/kg), a known ACE inhibitor, found to have good prophylactic effects on myocardial infarction. (Ithayarasi & Devi, 1997).

A. Effect of captopril, alpha-tocopherol, and their combination on serum AST

Free radicals generated by ISO, initiates lipid peroxidation of the membrane-bound polyunsaturated fatty acids, leading to impairment of membrane structural and functional integrity. The metabolic damage of myocardium results in leakage of the marker enzymes like AST. Transaminases like AST is present in heart muscle which when injured, the concentration of AST is liberated into the serum after myocardial tissue injury, thus increasing the concentration of AST when tested (Swamy et. al., 2012). As seen on Figure 1, there was a significant increase in AST. The AST concentration in all the groups were reduced after the induction of myocardial infarction on 8th and 9th day when compared to Group II

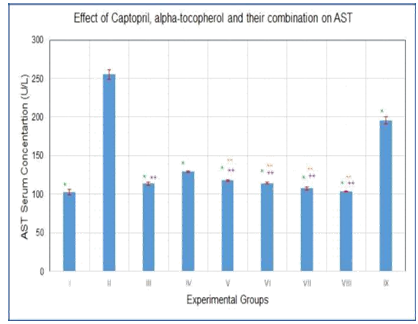

Figure 1. Effect of the different treatments on AST serum concentration after induction of ISO.

Experimental Groups: I (Normal Group); II (Isoproterenol Group); III (Captopril 30 mg/kg); IV, V, VI (Alpha-tocopherol 100, 300 and 400 mg/kg respectively); and VII, VIII and IX (Combination of captopril and alpha-tocopherol 100, 300 and 400 mg/kg respectively)

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Group II (ISO) vs. All groups * (p-value<0.05) Group I (Normal) vs. All groups ++ (p-value>0.05) Group III (Captopril) vs. All groups ^^ (p-value>0.05) (ISO only) (p-value <0.05). Also, captopril, alpha-tocopherol (300 and 400 mg/kg) and combination of captopril and alpha-tocopherol (100 and 300 mg/kg) showed no significant difference (p-value >0.05) when compared to the normal group, it connotes that the said groups were able to normalize the AST concentration by decreasing its serum concentration. However, the alpha tocopherol (100 mg/kg) and combination of captopril and the highest dose of alpha tocopherol (400 mg/kg) did not mark a significant decrease in the AST serum concentration as compared to the normal group. When compared to the standard drug captopril, only the alpha-tocopherol (300 and 400 mg/kg) and the combinations of captopril and alpha-tocopherol 100 and 300 mg/kg) showed no significant difference (p-value>0.05). Concomitant administration of captopril with alpha-tocopherol (400 mg/kg) did not make a significant decrease in the serum concentration of AST.

B. Effect of captopril, alpha-tocopherol and their combination on serum LDH

LDH, specifically LDH-1 is the predominant cardiac isoenzyme. Serum LDH increases after 12-24 hours following myocardial infarction which is evident after the biochemical test for LDH, the untreated group intoxicated with ISO have shown a significant increase in the LDH serum levels (Group II). As seen on Figure 2, all groups, (normal, captopril, alpha-tocopherol and combination of captopril and alpha-tocopherol) showed a significant decrease in the LDH when compared with Group II (p-value <0.05). Also, captopril, three doses of alpha-tocopherol and their combination showed no significant difference when compared to the LDH concentration of the normal group (p-value <0.05). It is also notable that alpha tocopherol alone and in combination with captopril showed no significant difference with the standard drug, captopril in lowering the serum LDH level (p-value>0.05).

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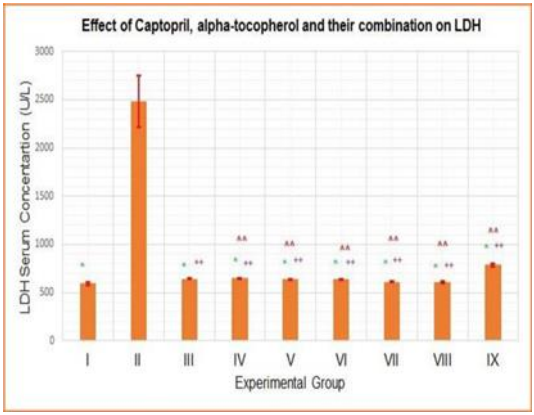

Figure 2. Effect of the different treatments on LDH serum concentration after induction of ISO.

Experimental Groups: I (Normal Group); II (Isoproterenol Group); III (Captopril 30 mg/kg); IV, V, VI (Alpha-tocopherol 100, 300 and 400 mg/kg respectively); and VII, VIII and IX (Combination of captopril and alpha-tocopherol 100, 300 and 400 mg/kg respectively). Group II (ISO) vs. All groups * (p-value<0.05) Group I (Normal) vs. All groups ++ (p-value>0.05) Group III (Captopril) vs. All groups ^^ (p-value>0.05)

C. Effect of captopril, alpha-tocopherol and their combination on serum CK-MB

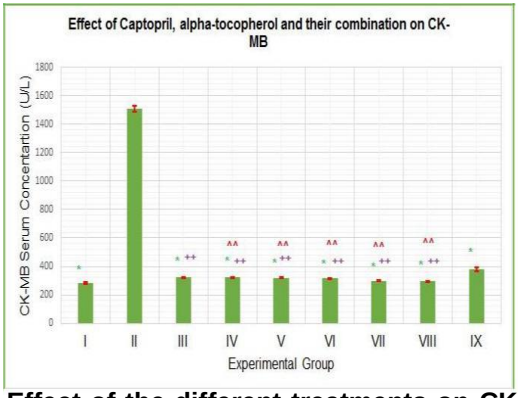

Figure 3. Effect of the different treatments on CK-MB serum concentration after induction of ISO.

Experimental Groups: I (Normal Group); II (Isoproterenol Group); III (Captopril 30 mg/kg); IV, V, VI (Alpha-tocopherol 100, 300 and 400 mg/kg respectively); and VII, VIII and IX (Combination of captopril and alpha-tocopherol 100, 300 and 400 mg/kg respectively)

Group II (ISO) vs. All groups * (p-value<0.05) Group I (Normal) vs. All groups ++ (p-value>0.05) Group III (Captopril) vs. All groups ^^ (p-value>0.05)

CK-MB is used as a biomarker for myocardial infarction. This enzyme is specific for cardiac muscle and the serum level concentration increases dramatically after 2-8 hours following myocardial infarction. Figure 3 shows that there is a significant increase in CK-MB of Group II (ISO group) when compared to the normal group, captopril, three doses of alpha-tocopherol and their combination with a p-value of <0.05. Furthermore, captopril, alpha-tocopherol alone (100, 300 and 400 mg/kg) and their combination except for the combination captopril and alpha-tocopherol at a dose of 400 mg/kg showed no significant difference when compared to the serum concentration of the normal groups with a p-value of >0.05. it means that those groups were able to decrease the serum concentration of CK-MB following myocardial infarction. Also, it was noted that only 100, 300 and 400 mg/kg alpha-tocopherol and the combination of captopril and 100 and 300 mg/kg alpha-tocopherol showed no significant difference in CK-MB levels as compared to captopril, standard drug (p-value >0.05). The combination of captopril with a higher dose of alpha-tocopherol showed significant increase in the serum concentration of CK-MB when compared to the normal group and captopril (p-value <0.05).

D. Pharmacodynamic interaction of captopril and alpha-tocopherol

It can be noted that the combination of captopril with 100 and 300 mg/kg of alpha tocopherol showed synergistic activity by decreasing the serum concentration of AST, LDH and CK-MB. Based on the dose-effect curve of the combination of captopril and alpha-tocopherol it can be noted that the combination of captopril and alpha-tocopherol 400 mg/kg showed antagonistic effect which can be observed by an increased AST serum concentration (Figure 4). Also, same observation was noted from the biochemical result for CK-MB, (Figure 6) Combination of the said drugs leads to antagonism due to increased CK-MB serum concentration. However, the result for LDH for the combination of alpha-

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tocopherol 400 mg/kg and captopril showed additive effect only because the individual effect of each drug is comparable to the effect of its combination (Figure 5).

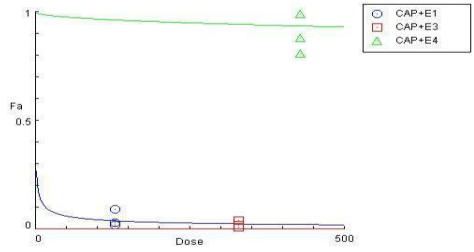

Figure 4. Effect of drug combinations on AST

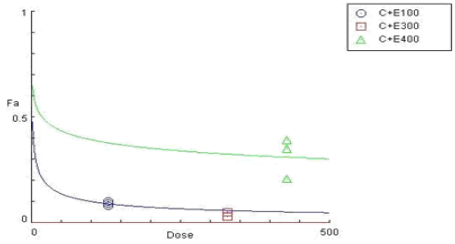

Figure 5. Effect of drug combinations on LDH

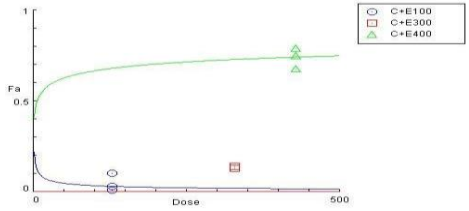

Figure 6. Effect of drug combinations on CK-MB

The result of this study showed that the combination of captopril with a high dose of alpha-tocopherol (400 mg/kg) did not show a significant effect on lowering the AST, LDH and CK-MB levels after myocardial infarction. This result is similar with the study of Lonn, 2005, 10,000 people with heart disease showed high risk of heart attack when given 400 mg/kg of alpha-tocopherol, 13% higher risk for heart failure and 21% increase in hospitalization.

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CONCLUSION

Based on the data obtained, the combination of alpha-tocopherol at increasing concentrations and captopril elicited their cardioprotective effects synergistically particularly at doses 100 and 300 mg/kg, and 30 mg/kg, respectively. Therefore, these two agents combined at these doses are of great benefit in the prevention and management of myocardial infarction. While, the combination of alpha-tocopherol at 400 mg/kg, and captopril at 30 mg/kg was the least effective in improving the condition of the myocardium. Thus, the combination attenuates the cardioprotective effects of alpha-tocopherol, and captopril.

RECOMMENDATION

The researchers recommend performing histopathological examination of the hearts for further assessment of the myocardial damage caused by isoproterenol-induced myocardial infarction in Sprague-Dawley rats and the degree of cardioprotection the agents have exerted. Moreover, a comparative testing of other agents used in the management of myocardial infarction is recommended to evaluate and further explore their cardioprotective activities.

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