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Research Article

Protective effects of 7-hydroxycoumarin on cardiac markers and non-enzymatic antioxidant in isoproterenol induced myocardial infarction

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Abstract

The present study evaluates the protective effects of 7-hydroxycoumarin on cardiac markers and non-enzymic antioxidant in isoproterenol induced myocardial infarcted rats. Male albino Wistar rats were pre- and co-treated with 7-hydroxycoumarin (16 mg/kg body weight) daily for 8 days. Isoproterenol (100 mg/kg body weight) was subcutaneously injected into rats on 7th and 8th day. Increased activity of serum creatine kinase and level of plasma thiobarbituric acid reactive substances and decreased levels of plasma vitamin-C were observed in isoproterenol induced myocardial infarcted rats. Pre- and co- treatment with 7-hydroxycoumarin (16 mg/kg body weight) decreased activity of serum creatine kinase and decreased level of plasma lipid peroxidation products and increased level of plasma vitamin-C in myocardial infarcted rats. Thus 7-hydroxycoumarin protects isoproterenol induced myocardial infarction by its antilipid peroxidation and antioxidant properties. 7-Hydroxycoumarin, study will be useful for the prevention of myocardial infarction.

Keywords: Antioxidants; Cardiac marker enzyme; Isoproterenol; Lipid peroxidation; Myocardial infarction; 7-hydroxycoumarin.

Introduction

Cardiovascular disease (CVD) will be the most important cause of mortality in India. Thus it is a valuable to mention that ischemic heart disease (IHD) continues to be the major cause of this CVD. Myocardial infarction (MI) affects a high proportion of the population. MI, a major public health problem, not only in western countries but also rising in developing countries and makes major contribution to the mortality statistics (Gilski and Borkenhagen, 2005). It is a clinical syndrome arising from sudden and persistent curtailment of myocardial blood supply resulting in necrosis of the myocardium (Anversa and Sonnenblick, 1990).

Oxidative stress produced by free radicals or reactive oxygen species (ROS), as confirmed by a marked increase in the production of lipid peroxidative products and momentary inhibition of endogenous antioxidant defense has been revealed to trigger myocardial damage during MI (Peer et al., 2008). It is the most dreaded sequel among ischemic heart diseases invariably followed by several biochemical alterations such as hyperlipidemia, increased lipid peroxidation, free radical damage, thrombosis etc. leading to qualitative and quantitative alteration of myocardium. Plentiful experimental and clinical

studies have revealed that enormous amounts of ROS such as superoxide, hydrogen peroxide and hydroxyl radicals are generated in failing myocardium (Rajadurai and Stanely Mainzen Prince, 2006). The recognition that free radicals mediated myocardial injury has created opportunities to interrupt the injury cascade and preserve the myocardium at risk (Mari Kannan and Darlin Quine, 2011).

Isoproterenol (ISO), a synthetic sympathomimetic β-adrenoreceptor agonist, has been found to induce myocardial injury in rat as a result of disturbance in physiological balance between production of free radicals and antioxidative defense system (Rathore et al., 1998). It is the acute condition of myocardial necrosis which causes cardiac dysfunctions, increased lipid peroxidation, altered activities of cardiac enzymes and antioxidants (Todd et al., 1998). ISO-induced myocardial necrosis involves membrane permeability alterations that bring about loss of function and integrity of myocardial membranes. ISO induced myocardial infarction is a well standardized model to study the protective effects of many drugs and cardiac function, since it mimics the clinical conditions of myocardial infarction due to ischemia in humans (Ithayarasi and Devi, 1997). Among the various mechanisms proposed to explain isoproterenol induced cardiac damage, generation of highly cytotoxic free radicals through auto-oxidation of catecholamines has been implicated as one of the important causative factors. Several recent studies demonstrate the protective action of natural products from a variety of sources on incidence of MI. Antioxidants not only suppress the formation of ROS but have a modulatory effect on the survival and death signaling of ROS (Ulrich-Merzenich et al., 2009).

Lipid peroxidation, an important pathogenic event associated with altered membrane structure and enzyme inactivation in MI. Lipid peroxidation of membranes is regulated by the availability of free radicals and the increased levels of lipid peroxides in ISO induced damage might be due to free radical-mediated chain reactions that could damage the myocardium. This is an indication of the severity of ISO induced necrotic damage of the heart (Albayrak et al., 2009). As a defense mechanism against the toxic ROS, cells are provided with non-enzymic antioxidants such as vitamin-C.

Diet and nutrition have substantial impact on reducing the incidence of cardiovascular diseases (CVD). The most active principles having antioxidant property found in botanical products are not only vitamins and also chemicals like polyphenols, organosulfur compounds and flavonoids (Basha and Priscillla, 2011). Many dietary antioxidants and some non-nutrient-based antioxidants from plants – such as sulfur-containing compounds in garlic, phytoestrogens in soy and green tea, anthocyanins in red berries, lycopene in tomatoes, and red and white wines from grape seeds – are increasingly being recognized as potential health promoters by reducing the risk of cardiovascular disease (Walker, 1996). In another study, randomized double-blind placebo-controlled multicenter pilot myocardial infarction and vitamins (MIVIT) trial has shown that supplementation with antioxidant vitamins C and E improves the clinical outcome of patients with acute MI (Jaxa-Chamiec et al., 2005).Recently, much attention has been focused on the protective effects of natural products in MI. 7-hydroxycoumarin (7-HC), is a major biotransformed product of coumarin (1,2-benzopyrone) and a widely distributed natural product. Coumarin and its derivatives result in a wide range of bioactivities, such as anticoagulant, estrogenic, dermal photosensitizing, vasodilator, molluscicidal, antihelmintic, sedative, hypnotic, analgesic, hypothermic, antimicrobial, anti-inflammatory, antifungal, and antiulcer activities (Kostova et al., 2001).

7-HC which is found in edible fruits and plants such as golden apple (*Aegle marmelos correa.*) and little orange (*Citrus aurantium*), carrot, coriander, garden angelica (*Angelica archangelica*), mouse ear hawk weed (*Pilosella officinarum*) (Vasconcelos et al., 2009). It is one of the components of asafoetida, the dried latex from the giant fennel (*Ferula communis*). A major transformed product is an intermediate of coumarin in human body is 7-HC and consequent glucuronidation in the intestine and

liver (Toyama Dde et al., 2011). Furthermore, it is rapidly absorbed after oral administration in humans and the maximum plasma concentration is reached after 105 min. A crossover study with various source of coumarin metabolized to 7-HC revealed their bioavailability of 66.1% in urine samples (Abraham et al. 2011). Also there are no scientific reports available on the effects of this polyphenolic compound in MI. In view of the fact that free radical generation has been concerned in the progression of MI, we have decided to carry out this present study to evaluate the protective effects of 7-hydroxycoumarin on plasma lipid peroxidation and non-enzymic antioxidants in ISO induced MI. This study on 7-hydroxycoumarin is the first study on MI.

Materials and Methods

Drug and Chemicals

7-hydroxycoumarin and isoproterenol hydrochloride were purchased from Sigma Chemical Company, St. Louis, MO. Thiobarbituric acid, 2, 4-dinitro phenyl hydrazine and L-ascorbic acid were obtained from S.D Fine Chemicals, Mumbai, India. All the other chemicals used were of analytical grade.

Experimental Animals

All the experiments were performed with healthy male albino Wistar rats (*Rattus norvegicus*) weighing 180-200g, aged 8-10 weeks and were maintained at the Central Animal House, Rajah Muthiah Institute of Health Sciences, Annamalai University, Tamil Nadu, India. The experimental animals were kept in polypropylene cages (47x34x20 cm) lined with husk, renewed every 24 h under a 12:12 h light and dark cycle at around 25°C. The rats had free access to tap water and food. They were fed on a standard pellet diet (Pranav Agro Industries Ltd., Maharashtra, India). The experiment was performed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India and approved by the Animal Ethical Committee of Annamalai University (Proposal No.801, Approval date; 20th April, 2011).

Induction of MI in Wistar Rats

ISO (100 mg/kg body weight) dissolved in saline was subcutaneously injected into rats at an interval of 24 h for 2 days. ISO induced MI was confirmed by elevated activity of serum creatine kinase (CK) in rats (Mari Kannan and Darlin Quine, 2011).

Experimental Design

The experimental design consists of four groups of six rats.

Group I : Normal control rats

Group II : Rats were pre- and co- treated orally with 7-HC (16 mg/kg bodyweight) daily for a period of eight days by an intragastric tube ;

Group III : Rats were subcutaneously injected with ISO alone (100 mg/kg bodyweight) twice at an interval of 24 h (on 7th and 8th day) ;

Group IV : Rats were pre- and co-treated with 7-HC (16 mg/kg bodyweight) orally by an intragastric tube daily for a period of eight days and subcutaneously injected with ISO (100 mg/kg bodyweight) two times at an interval of 24 h (on 7th and 8th days).

Both normal control and ISO control rats were received one ml saline alone daily for a period of 8 days. 7-HC was dissolved in saline and one ml was administered to each rat orally using an intragastric tube daily for a period of eight days.

At the end of treatment, 12 hours after second ISO administration all the rats were anesthetized and the rats were sacrificed by cervical decapitation. The blood was collected from the experimental animals and centrifuged. The plasma and serum was separated and stored for further analysis. Heart tissues were excised from the rats, washed in cold saline. A known weight of heart tissue was homogenated and stored in 5ml of 0.1 M Tris-Hcl

buffer solution (pH 7.4). The homogenate was centrifuged and the supernatant was used for the estimation of various biochemical parameters.

Biochemical Estimations

Assay of Cardiac Marker Enzyme

The activity of serum CK was assayed by using a standard commercial kit (Agappe Diagnostics, Kerala, India).

Estimation of Lipid Peroxidation Products

The levels of plasma thiobarbituric acid reactive substances (TBARS) by the method of Yagi (1987).

Estimation of Lipid Peroxidation Products and Non-enzymic Antioxidant System

The levels of vitamin-C in the heart were estimated by the method of Omaye et al. (1979) by were estimated.

Results

A pilot study was conducted with three different doses of 7-HC (4 mg, 8 mg, and 16 mg/kg body weight) to determine its dose dependent effect in ISO induced myocardial infarcted rats (Figure 1). It was observed that after 8 days of experiment, 7-HC pre- and co-treated rats at the doses of 4 mg, 8 mg and 16 mg/kg body weight significantly ($P<0.05$) lowered the elevated levels of plasma TBARS in ISO induced myocardial infarcted rats (Group-VI, VII and VIII) compared to ISO alone induced myocardial infarcted rats (Group-V). But, 7-HC (16 mg/kg body weight) pre- and co-treatment decreased level of plasma TBARS in ISO induced myocardial infarcted rats (Group-VIII) compared to ISO alone induced myocardial infarcted rats (Group-V). 7-HC (4 mg, 8 mg and 16 mg/kg body weight) alone treated rats (Group-II, III, and IV) revealed no significant effects compared to normal control rats (Group-I). Since 16 mg/kg body weight of 7-HC showed the highest effect, we have chosen this dose for our further study.

ISO induced myocardial infarcted rats showed significant ($P<0.05$) increase in the activity of serum CK (Group-III) compared to normal control rats (Group-I). Pre-and co- treatment with 7-HC (16 mg/kg body weight) decreased the activity of these serum CK in ISO induced myocardial infarcted rats (Group-IV) compared to ISO alone induced myocardial infarcted rats (Group-III) (Figures 2).

ISO induced myocardial infarcted rats showed significant ($P<0.05$) decrease in the levels of vitamin-C in the plasma (Group-III) and pre-and co-treatment with 7-HC increased the levels of vitamin-C in the plasma of ISO induced myocardial infarcted rats (Group-IV) (Figures 3).

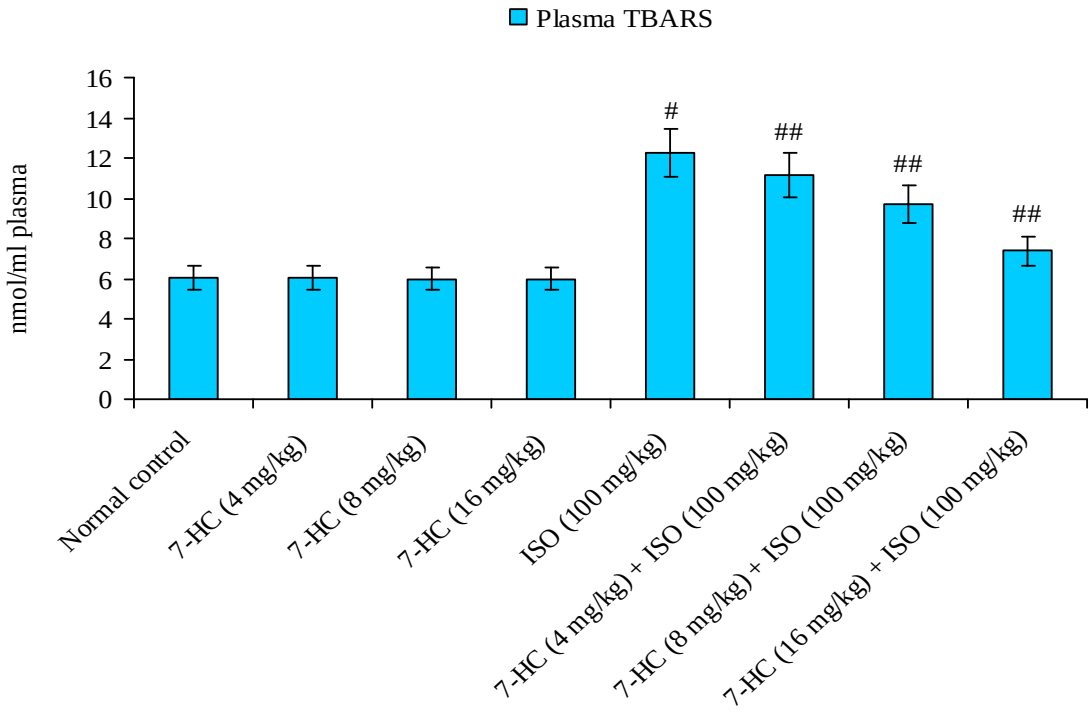
For the all biochemical parameters studied, 7-HC (16 mg/kg body weight) alone treated rats (Group-II) did not show any significant effect compared to normal control rats (Group-I).

Discussion

MI is characterized by cardiac dysfunction, increased lipid peroxidation, altered activities of cardiac injury markers and depletion of endogenous antioxidants (Ojha et al., 2008; Panda and Naik, 2008; Goyal et al., 2010). Administration of supramaximal doses of ISO had been reported to induce severe oxidative stress (Rathore et al., 1998). Overproduction of ROS can cause severe impairment of cellular functions and necrotic lesions in the myocardium of rats. Polyphenols have the ability to reduce the oxidative stress by removing the superoxide anion and hydrogen peroxide formed during the auto-oxidation of ISO. Thus the 7-HC has revealed its cardioprotective nature. Most of the related compounds are anti-coagulant and anti-platelet activity.

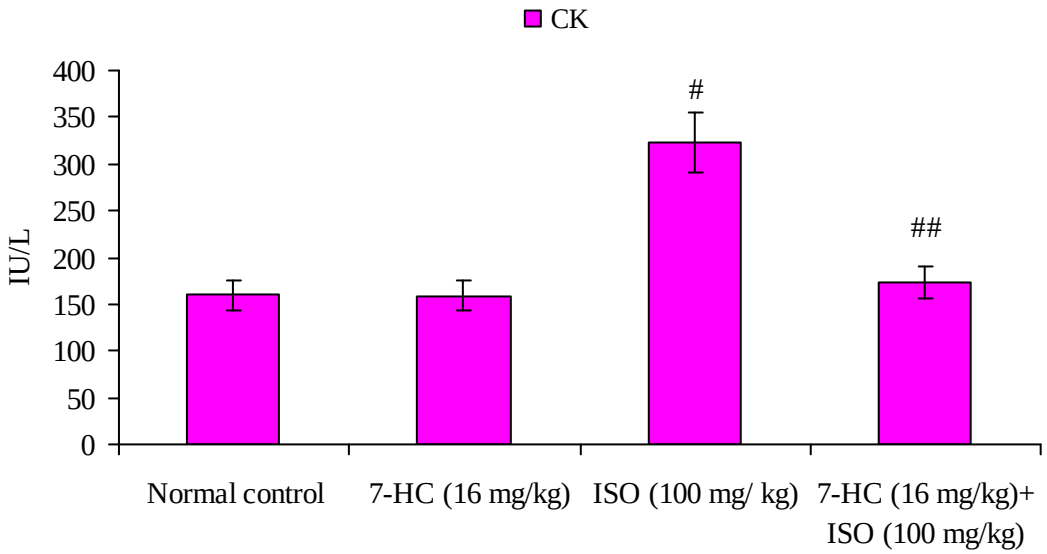
In this present study we have observed an elevated activity of cardiac marker enzyme in the serum of ISO-induced myocardial infarcted rats. Amoung all the macromolecules that are found to escape from damaged tissue cardiac marker enzymes are the best markers of tissue damage due to their tissue specificity and catalytic activity (Iwase et al., 2001). CK is an important marker of myocyte injury or death in the clinical investigation of MI.

Fig 1. Levels of plasma thiobarbituric acid reactive substances (Dose dependent study)



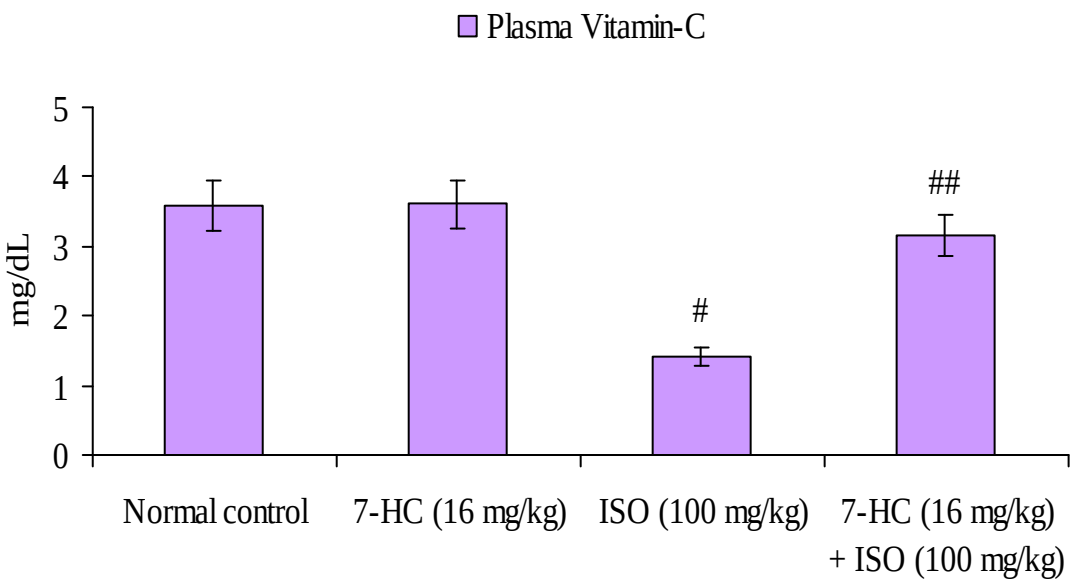
Each column is mean ± standard deviation for six rats in each group; #P<0.05 as compared to normal control (Group-I), ##P<0.05 as compared to isoproterenol control (Group-V) (DMRT).

Fig 2. Activity of serum creatine kinase



Each column is mean ± standard deviation for six rats in each group; #P<0.05 as compared to normal control (Group-I), ##P<0.05 as compared to isoproterenol control (Group-III) (DMRT).

Fig.3 Levels of vitamin-C in the plasma



Each column is mean ± standard deviation for six rats in each group; $^{\#}P<0.05$ as compared to normal control (Group-I), $^{\#\#}P<0.05$ as compared to isoproterenol control (Group-III) (DMRT).

As a response to β-adrenergic stimulation, the cellular enzymes are released that reflects the alterations in the plasma membrane permeability and integrity (Ebenezar et al., 2003). A marked elevation of cardiac marker in serum CK was observed in ISO induced myocardial infarcted rats. This might be due to the damage sarcolemma caused by the β-agonist that has rendered its permeability. ISO induction produces free radicals via the β-adrenoceptor mechanism, which are cytotoxic and affects the cell metabolism, producing myocardial necrosis (Sumitra et al., 2001). Pre- and co- treatment with 7-HC (16 mg/kg body weight) decreased the activity of serum CK in ISO induced myocardial infarcted rats by its antioxidant effect.

The degree of lipid peroxidation was reflected by increased levels of TBARS in the plasma of ISO induced myocardial infarcted rats. Lipid peroxidation, is an indication of the severity of isoproterenol induced necrotic damage of the heart, and has been linked with altered membrane structure and enzyme inactivation. The increased levels of lipid peroxides in ISO-induced myocardial necrosis might be due to free radical-mediated membrane damage (Zhou et al., 2006; Karthikeyan et al., 2007). The results of our present study are concomitant with previous reports stating increase of lipid peroxides in ISO-induced rats could be attributed to the accumulation of lipids in the heart and irreversible damage to the myocardial membranes. Furthermore, increased levels of lipid peroxidation products injure blood vessels, causing increased adherence and aggregation of platelets to the injured sites (Sathish et al., 2003). The decreased levels of plasma lipid peroxidation products observed in 7-HC pre-and co-treated rats are due to its antilipid peroxidation and membrane stabilizing effects. Thus, 7-HC scavenges excessive free radicals produced by ISO and protects the heart.

Hydrophilic antioxidant such as vitamin-C is the first line of antioxidant defense against reperfusion damages during the return of blood flow (Janero, 1991). Decreased levels of vitamin-C could be due to increased utilization of vitamin-C as an antioxidant defense against increased ROS or could be due to decreased levels of GSH, because GSH is required for the recycling of vitamin-C. Scavenger antioxidants are important because they react with the free radicals directly and also as they act synergistically with one another. The increased

vitamin-C levels in 7-HC pre- and co- treated rats can be correlated to decreased lipid peroxidation in ISO induced myocardial infarcted rats.

In conclusion, the above findings obtained from our study indicate that 7-HC (16 mg/kg body weight) offers protection to the myocardium against ISO induced oxidative stress in rats. This could be due to inhibition of lipid peroxidation system by its potent antioxidant effect. Thus, 7-HC may be useful for the prevention of MI.

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