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Preventive effect of esculetin on lipid peroxides and antioxidants in isoproterenol-induced myocardial infarction in Wistar rats

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ABSTRACT

The present study is undertaken to evaluate the effect of esculetin on the levels of lipid peroxidation and antioxidants in isoproterenol (ISO)-induced myocardial infarction (MI) in Wistar rats. The rats were subcutaneously injected with isoproterenol (ISO) (85 mg/kg) at an interval of 24 hours for two days. A significant increased in the levels of thiobarbituric acid reactive substances (TBARS) and hydroperoxide (HP), whereas the activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione-S-transferase (GST), glutathione peroxidase (GPx) and non-enzymatic antioxidants like reduced glutathione (GSH), vitamin E & C were significantly decreased. Oral pretreatment with esculetin (10 and 20 mg/kg) to ISO-induced rats daily for a period of 21 days significantly decreased the levels of TBARS and HP also increased the levels of both enzymatic and non-enzymatic antioxidant. This could be due to free radical scavenging and antioxidant property of esculetin. Thus, our finding demonstrates that esculetin prevents the alterations in lipid peroxidation and antioxidants status during ISO-induced MI in rats.

Key words: Esculetin; isoproterenol; myocardial infarction; lipid peroxidation; antioxidants.

INTRODUCTION

Cardiovascular disease (CVD) is the class of diseases that involves the heart or blood vessels. Myocardial infarction (MI) is the condition of necrosis of the heart that occurs due to imbalance between coronary blood supply and myocardial demand [2]. The responds with an increase in muscle mass and this process of hypertrophy represents a fundamental compensatory mechanism that permits the ventricle to sustain normal perfusion pressure [3]. Early reperfusion is the best way to reduce the progression of myocardial damage induced by ischemia reperfusion injury [4]. There is extensive evidence to implicate free radicals in the development of degenerative diseases [5].

Oxidative stress resulting from increased production of free radicals associated with decreased antioxidants in the myocardium, play a major role in CVD such as ischemic heart disease, atherosclerosis, congestive heart failure, cardiomyopathy and arrhythmias [6], which are together with other derivatives of oxygen they are inevitable by products of biological redox reactions [7]. Free radicals are continuously produced by the body's normal use of oxygen such as respiration and some cell mediated immune functions. Reactive oxygen species (ROS) such as superoxide anions (O·), hydroxyl radicals (·OH) and nitric oxide (NO). The increased production of toxic oxygen derivatives is considered to be a universal feature of stress conditions [8]. Continuous raise in the cellular reduction potential or decrease in the redox capacity of the redox couples leads to the production of ROS, causing an imbalance in the cell ability to detoxify their toxicity and repair the damage resulting in the oxidative stress [9].

Isoproterenol (ISO), a ß-adrenoceptor agonist that is used to induce MI experimentally in rats. The infarction induced by ISO, which arises from a physiological imbalance between free radical production and the cardiac antioxidative defense system of experimental animals, is similar to that observed in human MI [10]. It is also well known to generate free radicals and to stimulate lipid peroxidation, which may be a causative factor for irreversible damage to the myocardial membrane [11]. Loss of function and integrity of myocardial membranes are the outcomes of ISO-induced myocardial injury involving change in membrane permeability alterations [12]. Antioxidants are beneficial components that neutralize free radicals before they can attack cells and hence prevent damage to cellular proteins, lipids and and carbohydrates. A wide range of antioxidants both natural and synthetic has been proposed for the treatment of human disease. Antioxidant action includes free radical scavenging capacity, inhibition of lipid peroxidation, metal ion chelating ability and reducing capacity [13]. Coumarins are widely distributed in plants and are especially abundant in the bark, leaves and roots of umbeilliferae and rutaceae plants, so far more than 1300 types of coumarins have been identified as natural or synthesized compounds [14]. Coumarins comprise a group of natural compounds that have recently attracted much attention because of their bread biological activites [15].

Esculetin (6, 7 dihydroxy coumarin) is known to be a 5 and 12-lipogenase inhibitor and to inhibit the production of leukotrienes and 5-hydroxyeicosateraenoic acid through the lipogenase pathway [16]. Esculetin also scavenges hydroxyl radicals and inhibits lipid peroxidation in rat liver [17]. In view of the above facts, the present study was designed to evaluate the preventive effect of esculetin on the lipid peroxidases and antioxidants in ISO-induced MI in rats.

MATERIALS AND METHODS

Experimental animals

All the experiments were done with male albino Wistar rats weighing 140-160 g, obtained from the Venkateswara Enterprises, Bangalore were used in this study. They were housed in polypropylene cages (47x34x20 cm) lined with husk, renewed every 24 h under a 12:12 h light/dark cycle at around 22°C and had free access to tap water and food. The rats were fed on a standard pellet diet (Pranav Agro Industries Ltd., Maharashtra, India). The pellet diet consisted of 22.02% crude protein, 4.25% crude oil, 3.02% crude fiber, 7.5% ash, 1.38% sand silica, 0.8% calcium, 0.6% phosphorus, 2.46% glucose, 1.8% vitamins, and 56.17% carbohydrates. It provided a metabolizable energy of 3600 kcal. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

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Drug and Chemicals

(±) Isoproterenol hydrochloride, esculetin, reduced nicotinamide adenine dinucleotide (NADH), and phenazine metthosulphate (PMS) were purchased from Sigma Chemical Company, St. Louis, MO, USA. Thiobarbituric acid (TBA), 1, 1' ,3 , 3' tetramethoxy propane, butylated hydroxy toluene (BHT), xylenol orange, dithionitro bis benzoic acid (DTNB), ascorbic acid and sodium azide were obtained from Himedia laboratory, Mumbai, India. All other chemicals used in this study were of analytical grade.

Induction of Experimental Myocardial Infarction

Isoproterenol (85 mg/kg) was dissolved in normal saline and injected subcutaneously to rats at an interval of 24 h for two days [18].

Experimental Design

A total of 30 rats were used in the present investigation. The animals were randomly divided into 5 groups of 6 rats in each group.

Group 1: Normal control rats
Group 2: Normal rats + Esculetin (20 mg/kg)
Group 3: ISO control rats (85 mg/kg)
Group 4: Esculetin (10 mg/kg) + ISO
Group 5: Esculetin (20 mg/kg) + ISO

Esculetin was dissolved in 0.2% dimethyl sulfoxide and administrated to rats orally using an intragastric tube daily for a period of 21 days [19].

Sample collection

At the end of the experimental period, after 12 h of second ISO-injection, all the rats were anesthetized with sodium pentobarbital (35 mg/kg, i.p.) and sacrificed by cervical decapitation. Blood was collected and plasma separated and used for various biochemical estimations. The heart tissue was excised immediately from the animals, washed off blood with ice-chilled physiological saline and used for further biochemical estimations. A known weight of the heart tissue was homogenized in appropriate buffer solution. The homogenate was centrifuged and the supernatant was used for the estimation of various biochemical parameters.

Biochemical Assays

Biochemical parameters such as plasma thiobarbituric acid reactive substances were estimated by the method of Yagi (1987) [20]. The concentration of TBARS in the heart tissue was estimated by the method of Fraga et al (1988) [21]. The levels of HP were estimated by the method of Jiang et al. (1992) [22]. The activity of SOD was assayed according to the procedure of Kakkar et al. (1984) [23].The activity of catalase was assayed by the method of Sinha (1972) [24]. The activity of GPx was assayed by the method of Rotruck et al. (1973) [25]. The Glutathione-S-transferase (GST) activity was assayed by the method of Habig and Jackoby (1981) [26]. The level of GSH was estimated by the method of Ellman (1959) [27]. The levels of vitamin C were estimated by the method of Omaye et al. (1979) [28]. The levels of vitamin E were estimated by the method of Baker et al. (1980) [29]. And the level of ceruloplasmin was estimated by the method of Ravin (1961) [30] respectively. The levels of protein were determined by the method of Lowery et al. (1951) [31].

Statistical Analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using SPSS software package 9.05. Results were expressed as mean ± S.D. from six rats in each group. *P* values <0.05 were considered as significant.

RESULTS

Effect of esculetin on TBARS and HP

Table1 represent the levels of thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP) in plasma and the heart of normal and ISO-induced rats. Rats induced with ISO, showed a significant increase in the levels of TBARS and HP in plasma and the heart. Oral pretreatment with esculetin (10 and 20 mg/kg) to ISO-induced rats for a period of 21 days, significantly decreased the levels of TBARS and HP in plasma and the heart when compared with ISO-alone induced rats.

Table 1. Effect of esculetin on the levels of thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (HP) in plasma and the heart of normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.

Groups	Plasma TBARS (nM/ml)	Plasma HP (values x 10^{-5} mM/dL)	Heart TBARS (mM/100g wet tissue)	Heart HP (mM/100g wet tissue)
Normal control	2.10 ± 0.12[a]	10.3 ± 0.91[a]	0.65 ± 0.05[a]	16.7 ± 0.31[a]
Normal + Esculetin (20 mg/kg)	2.09 ± 0.14[a]	10.4 ± 0.77[a]	0.63 ± 0.03[a]	16.6 ± 0.37[a]
ISO (85 mg/kg) control	4.36 ± 0.26[b]	18.9 ± 1.07[b]	1.02 ± 0.06[b]	23.3 ± 1.51[b]
Esculetin (10 mg/kg) + ISO	3.74 ± 0.21[c]	15.4 ± 0.80[c]	0.87 ± 0.07[c]	20.4 ± 1.03[c]
Esculetin (20 mg/kg) + ISO	2.45 ± 0.13[d]	12.3 ± 0.31[d]	0.79 ± 0.06[d]	18.2 ± 1.12[d]

Each value is mean ± S.D. for 6 rats in each group.
Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Effect of esculetin on the activities of enzymatic antioxidants

The activities of superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione-S-transferase (GST) in the heart of normal and ISO-induced rats shown in Table 2. Rats induced with ISO, exhibited a significant decrease in the activities of these antioxidant enzymes in the heart. Administration of esculetin to ISO-induced rats significantly increased the activities of these enzymes.

Table 2. Effect of esculetin on the activities of superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione-S-transfarase (GST) in the heart of normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.

Groups	SOD(U/mg protein)	Catalase(µmoles of H_2O_2comsumed /min/mg protein	GPx(µg of GSHConsumed/min/ mg protein)	GST(nmoles of CDNB conjugated min/mg protein)
Normal control	12.08 ± 0.92[a]	5.28 ± 0.37[a]	3.13 ± 0.13[a]	810.3 ±23.5[a]
Normal + Esculetin (20 mg/kg)	12.17 ± 0.83[a]	5.39 ± 0.36[a]	3.17 ± 0.25[a]	823.7 ± 24.2[a]
ISO (85 mg/kg) control	6.57 ± 0.40[b]	3.05 ± 0.25[b]	1.21 ± 0.08[b]	516.0 ± 14.6[b]
Esculetin (10 mg/kg) + ISO	8.75 ± 0.53[c]	4.23 ± 0.27[c]	2.25 ± 0.10[c]	670.7 ± 20.3[c]
Esculetin (20 mg/kg) + ISO	10.31 ± 0.36[d]	4.97 ± 0.34[d]	2.84 ± 0.21[d]	737.2 ± 21.7[d]

SOD units: One unit is defined as the enzymes concentration required to inhibit the optical density at 560 nm of chromogen production by 50% in 1 min.
Each value is mean ± S.D. for 6 rats in each group.
Values not sharing a common superscript (a-d) differ significantly with each other (P<0.05, DMRT).

Effect of esculetin on non-enzymatic antioxidants

The levels of ceruloplasmin in plasma and GSH, vitamin C and E in plasma and heart of normal and ISO induced rats are shown in Tables 3 & 4. Rats induced with ISO, showed a significant decrease in the levels of non-enzymatic antioxidants in plasma and heart. Oral pretreatment with esculetin (10 and 20 mg/kg) to ISO-induced rats significantly increased the levels of plasma ceruloplasmin and plasma and heart GSH, vitamin C and E when compared with ISO-alone induced rats.

Table 3. Effect of esculetin on the levels of plasma ceruloplasmin, reduced glutathione (GSH) in plasma and heart of normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.

Groups	Plasma ceruloplasmin (mg/dL)	Plasma GSH (mg/dL)	Heart GSH (mM/g wet tissue)
Normal control	28.8 ± 1.07[a]	18.3 ± 1.00[a]	8.49 ± 0.48[a]
Normal + Esculetin (20 mg/kg)	28.7 ± 1.19[a]	18.4 ± 0.91[a]	8.53 ± 0.37[a]
ISO (85 mg/kg) control	19.9 ± 0.83[b]	12.1 ± 0.67[b]	4.06 ± 0.27[b]
Esculetin (10 mg/kg) + ISO	23.9 ± 1.26[c]	15.0 ± 0.63[c]	6.20 ± 0.31[c]
Esculetin (20 mg/kg) + ISO	26.7 ± 1.32[d]	17.1 ± 0.54[d]	7.38 ± 0.63[d]

Each value is mean ± S.D. for 6 rats in each group.
Values not sharing a common superscript (a-d) differ significantly with each other (P < 0.05, DMRT).

For all the parameters studied, pretreatment with esculetin at a dose of 20 mg/kg to ISO-induced rats showed better effect than esculetin 10 mg/kg. Esculetin 20 mg/kg to normal rats didn't have any significant effect.

Table 4. Effect of esculetin on the levels of vitamin C and E in plasma and heart of normal and isoproterenol (ISO)-induced myocardial infarction (MI) in rats.

Groups	Plasma Vitamin C (mg/dL)	Plasma Vitamin E (mg/dL)	Heart Vitamin C (µmoles/mg protein)	Heart Vitamin E (µmoles/mg protein)
Normal control	1.93 ± 0.16 [a]	1.53 ± 0.12 [a]	1.12 ± 0.10[a]	0.81 ± 0.03[a]
Normal + Esculetin (20mg/kg)	1.97 ± 0.13[a]	1.56 ± 0.09 [a]	1.10 ± 0.09[a]	0.84 ± 0.05[a]
ISO (85 mg/kg) control	0.89 ± 0.07[b]	0.83 ± 0.03[b]	0.43 ± 0.03[b]	0.40 ± 0.02[b]
Esculetin (10 mg/kg) + ISO	1.32 ± 0.10[c]	1.15 ± 0.10[c]	0.74 ± 0.05[c]	0.61 ± 0.04[c]
Esculetin (20 mg/kg) + ISO	1.82 ± 0.16[d]	1.36 ± 0.11[d]	0.97 ± 0.06[d]	0.73 ± 0.05[d]

Each value is mean ± S.D. for 6 rats in each group.
Values not sharing a common superscript (a-d) differ significantly with each other (P < 0.05, DMRT).

DISCUSSION

Isoproterenol (ISO) causes the cardiotoxicity by several mechanisms including relative hypoxia, coronary microcirculatory disturbances; however intracellular calcium over load is the most accepted cause of catecholamine-induced cardiotoxicity [32]. Lipid peroxidation has been implicated in the pathogenesis of a numerous diseases include atherosclerosis, cancer etc. It is now accepted that lipid peroxidation and its product play an important role in liver, kidney, heart and brain toxicity [33]. Lipid peroxidation *in vivo* has been identified as important and basic deteriorative reactions in cellular mechanisms of myocardial ischemia [34].

Reactive oxygen species (ROS) may attack various biomolecules like proteins, nucleic acids and carbohydrates, but their main target is polyunsaturated fatty acids (PUFA), which is the initiator of lipid peroxide formation. ISO-administration in rats leads to elevated lipid peroxidation process and extensive necrosis of cell membranes. It is well known that administration of ISO produces free radicals, which is involved in membrane damage, leading to elevated levels of thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP) [35-36]. TBARS and HP levels were reflecting the major events of the disease and its complications [37]. Excessive formation of free radicals may leads to severe damage to the myocardium.

Pretreatment with esculetin to ISO-induced rat significant decreased the levels of TBARS and HP in plasma and heart. This could be due to free radical scavenging properties of esculetin. Phytochemicals like flavonoids and coumarins possess free radical scavenging properties. Esculetin, being a coumarin, scavenges the excess amounts of free radicals produced by ISO. Reactive oxygen species are generated from the leakage of electrons into oxygen from various systems in our body and the endogenous antioxidant enzymatic defense is a very important source to neutralize the oxygen free radical mediated injury [38]. Organs cannot continue to function without adequate blood flow, and if a vital organ such as heart or brain is severely compromised, death is inevitable. Following ischemia, ROS are produced during reperfusion phase [39]. The extent of ROS-induced oxidative damage can be exacerbated by a decreased efficiency of antioxidant defense mechanisms [40-41]. Several evidences shows that antioxidants can protect against free radical production, which is responsible for reperfusion-induced damage and lipid peroxidation, and may thereby inhibit thrombosis, myocardial damage and arrhythmias during MI [42].

In this study, we have observed decreased activities of superoxide dismutase (SOD) and catalase in ISO-induced rats. Free radical scavenging enzymes such as SOD and catalase are the first line of cellular defense against oxidative injury, which is involved in the decomposing superoxide radicals and H_2O_2 before interacting to form the more reactive hydroxyl radical. Superoxide radicals ($O_2^{\cdot -}$) are eliminated by SOD in cytosol (Cu/Zn-SOD) and in mitochondria (Mn-SOD) and the resultant H_2O_2 is removed by catalase [43]. Superoxide radical produced at the region of cardiac injury alters SOD and catalase leads to inactivation of these enzymes, which inturn leads to accumulation of superoxide anion and damages the heart.

Reduced glutathione (GSH) is one of the most important endogenous antioxidants, which play an important role against free radical damage. Depletion of GSH levels results in increased lipid peroxidation further the excessive lipid peroxidation can cause increased GSH consumption. Glutathione peroxidase (GPx) catalyzes the process of peroxide reduction utilizing GSH as the substrate and converting it to GSSG [44]. GSH act as a substrate in the scavenging reaction catalyzed by GPx and as a scavenger of vitamins C and E radicals [45]. The decrease levels of GSH decreases the activities of glutathione dependent enzymes such as GPx and GST in ISO-induced rats. Decreased availability of GSH also reduces the activities of GPx and GST in ISO-induced MI. In this study, the activities of GPx and GST were significantly decreased in ISO-induced rats, along with reduction in the levels of GSH in plasma and heart. Our observations are in correlation with previous report [46].

Esculetin pretreated ISO-induced rats showed significant elevations in the activities of antiperoxidative enzymes like SOD, catalse, GPx and GST in heart and also the GSH in plasma and heart. This could be due to free radical scavenging and antioxidant properties of esculetin. It's well known that, esculetin posses free radical scavenging and antioxidant properties [47]. Coumarins are the phytoconstituents, which posses free radical scavenging and antioxidant properties in oxidative stress condition in rats.

Antioxidant Vitamins like vitamin C and E and cereloplasmin are important natural antioxidants, which inhibit lipid peroxidation, and high intake of these vitamins, particularly vitamin E, is related to reduced incidence of ischemic heart disease. The observed decrease in the levels of these antioxidant vitamins and ceruloplasmin in this study could be due to superoxide radicals and hydroxyl radical produced by ISO.

The cardioprotective effects of vitamin E may be due to its beneficial effect in reducing excess tissue aldehydes. Vitamin C reduces the risk of CVD by reducing cholesterol, BP and the formation of oxidized LDL. Ceruloplasmin donate copper ion to SOD and inhibit the process of lipid peroxidation. Esculetin pretreatment to ISO-induced rats significantly increased the levels of ceruloplasmin in plasma and vitamin C and E in plasma and heart of ISO-induced rats. This could be due to the antioxidant properties of esculetin.

CONCLUSION

In conclusion, oral pretreatment with esculetin had antilipoperoxidative and adntixoidant effect in ISO-induced MI. Esculetin pretreatment with ISO induced rats decrease in lipidperoxidation and increases antioxidants might be due to free radical scavenging and antioxidant properties of the drug. Thus esculetin possess protective role against ISO-induced MI in rats.

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