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Beneficial Effect of d-Limonene against Experimentally Induced Myocardial Infarction

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A B S T R A C T

Myocardial infarction (MI) is an increasingly common cause of heart failure. d-Limonene is the most abundant monocyclic monoterpene in orange peel oil. Limonene is found in the essential oils of citrus fruits and many other plant species. The present study was undertaken to examine the effect of d-limonene on serum marker enzymes, lipid peroxidation and antioxidant defense system during isoproterenol induced myocardial infarction in rats. Rats were induced with isoproterenol (150 mg/kg body weight) and the changes in lipid peroxidation, serum marker enzymes (lactate dehydrogenase, creatine kinase, aspartate transaminase and alanine trasaminase) and antioxidants (superoxide dismutase, glutathione peroxidase, glutathione-S-transferase, glutathione reductase, catalase and reduced glutathione) were studied. These changes were compared with the d-limonene pre-treatment (10mg/kg body weight). Treatment with d-limonene (10 mg/kg body weight) markedly decreased lipid peroxidation, serum marker enzymes and increased the levels of cellular antioxidants. In conclusion, these findings indicate the protective effect of d-limonene against isoproterenol induced experimental myocardial infarction.

Keywords: Isoproterenol, d-limonene, lipid peroxidation, antioxidants, serum markers.

A R T I C L E I N F O

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1. Introduction

The model of isoproterenol (ISO)-induced myocardial infarction is considered as one of the most widely used experimental model to study the beneficial effects of many drugs and cardiac function [1]. The pathophysiological changes following ISO administration are comparable to those taking place in human myocardial ischemia/infarction [2]. ISO 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 [3]. Myocardial cell protection and prevention of cell ischemia/necrosis have been therapeutic targets for a long time. d-limonene (d-LIM) (1-methyl-4-(1-methylethenyl) cyclohexane) is a monocyclic monoterpene with a lemon-like odor and is a major constituent in several citrus oils (orange, lemon, mandarin, lime, and grapefruit).d-LIM has been clinically used to dissolve cholesterol-containing gallstones [4]. It has also been used to relieve heartburn, because of its potential for gastric acid neutralization and its support for healthy peristalsis. d-LIM has well-established antioxidant property [5] and also possesses chemo preventive activity against many types of cancers [6, 7].Hence the present study is an attempt to explore the effect of d-LIM on ISO induced myocardial infarction in rats.

2. Experimental

Materials

ISO and D-LIM were purchased from M/s. Sigma chemicals, St. Louis, USA. All other chemicals were of analytical grade procured from M/s. SRL Chemicals Pvt. Ltd., Mumbai.

Animals: Adult male albino rats of Wistar strain weighing around 120 to 150 g obtained from Tamil Nadu Veterinary and Animal Sciences University (Tanuvas), Madhavaram, Chennai, India were used in this study. The animals were housed under conditions of controlled temperature (26 ±2oC) with 12-hr day/night cycle. They were fed standard rat/mice pellet diet (M/s. Hindustan Lever Ltd., Mumbai) under the trade name Amrut rat/mice feed and were given access to water ad libitum.

Experimental design

Experimental animals were divided into four groups of six rats each as follows.

Group I: control animals received olive oil throughout the course of the experiment

Group II: animals were induced with ISO (150 mg/kg body weight) intraperitoneally for two consecutive days

Group III:animals received d-LIM alone (10 mg/kg body weight dissolved

in olive oil) intraperitoneally to assess the cytotoxicity (if any) induced by D-LIM

Group IV: animals received ISO (as in Group II) along with d-LIM (10 mg/kg body weight dissolved in olive oil) intraperitoneally. d-LI Mtreatment was started 4 weeks prior to the administration of ISO. At the end of the experimental period, animals were sacrificed by cervical decapitation under ether anesthesia and liver was excised immediately and washed with ice-cold saline. A 10% homogenate of the washed tissue (liver) was prepared in 0.01 M phosphate buffer (pH 7.4). The homogenate was centrifuged at a speed of 12,000 x g for 30 min in a refrigerated high-speed centrifuge at 4oC. Blood was also collected and the serum was separated for other estimations. The following analysis was carried out in the supernatant.

Biochemical analysis

Protein estimation was carried out [8]. LPO was assayed by the method in which the malondialdehyde (MDA) released served as the index [9]. Superoxide dismutase (SOD) and catalase (CAT) were assayed [10, 11].The activities of glutathione peroxidase (GPx), glutathione reductaseand glutathione –S-transferase (GST) were estimated [12-14].

The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated [15]. Lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) activities were determined [16.17].

Data analysis

All data were expressed as mean ± S.D for six rats. The results were computed statistically (SPSS Software Package) using one-way ANOVA. Post-hoc testing was performed for inter comparisons using the LSD. ($P < 0.05$) was considered significant.

3. Results and Discussion

Effect of d-limonene on LPO and antioxidants in rat liver

Table 1 depicts the effect of ISO and D-LIM on LPO and antioxidant status in liver. Significant ($P < 0.05$) reduction in the activities of enzymic antioxidants SOD, CAT, GPx, GR and GST with marked ($P < 0.05$) increase in the levels of LPO were observed in the ISO administered group (Group II). D-LIM pretreatment resulted in a free radical quenching effect and thereby significantly ($P < 0.05$) decreased LPO and reinstated the antioxidant activities to near normalcy in Group IV animals.

Effect of d-limonene on serum marker enzymes

Table 2represents the activities of marker enzymes (AST, ALT, CPK, and LDH) in serum of control and experimental group of animals. Abnormal increase ($P < 0.05$) in marker enzymes was observed in ISO induced rats (Group II) when compared to control rats (Group 1). The marker enzyme activities were found to be at near normal ($P < 0.05$) when pretreated withD-LIM (Group IV). There seems to be no significant difference between theD-LIMalone treated rats (Group III) and control (Group I).

Discussion

LPO is considered as one of the basic mechanism of cellular damage caused by free radicals[18]. An increase in LPO indicates serious damage to cell membranes, inhibition of several enzymes, cellular function and cell death.In recent years, it has been suggested that reactive oxygen species produced by activated macrophages might be the primary cause of LPO leading to ISO- induced myocardial infarction [19]. Our results were in consistent with the previous findings as we observed increased liver LPO in group II ISO induced rats.

When myocardial cells, containing AST, ALT, CPK, and LDH, are damaged or destroyed due to deficient oxygen

supply or glucose, the cell membrane becomes permeable or may rupture, which results in the leakage of enzymes. This accounts for the decreased activities of these enzymes in heart of rats with myocardial infarction-induced by isoproterenol. This might be due to the damage caused to the sarcolemma by the β-agonist that has rendered it leaky [20]. In our present study, treatment with D-LIM reduced liver LPO induced by ISO in group IV animals suggesting its antioxidant efficacy.

The primary defense against oxidative stress in the tissue rests with antioxidants. SOD, an important antioxidant enzyme protects cells against the toxic effects of superoxide anion [21]. CAT catalyses the direct degradation of H2O2 to water. It protects the cellular constituents against oxidative damage [22]. GPx and the cellular NADPH generating mechanisms together form a system for removing the hydroperoxides from the cell[23]. GST is a group of multifunctional proteins that perform tasks ranging from catalyzing the detoxification of electrophilic compounds to protection against peroxidative damage [24]. GR plays a major role in regenerating GSH from GSSG, thus maintaining the balance between the redox couple [25].

The decreased activity of these enzymatic antioxidants in ISO induced myocardial infarction could be due to the accumulation of H2O2 which inturn causes the inhibition of these enzymes. The reduced activities of these enzymes were normalised upon treatment with D-LIM. Thus our present findings clearly demonstrate the role of D-LIM as a potent antioxidant that prevented oxidative stress and in turn paved way for its protective role against ISO induced myocardial infarction in rats.

Table 1: Levels of LPO and antioxidants in the liver of control and experimental groups of rats

Parameters	Group I (Control)	Group II (ISO)	Group III (d-LIM)	Group IV (ISO + d-LIM)
LPO	16.93 ± 0.16	75.28 ± 0.74[a]	16.96 ± 0.16	21.20 ± 0.10[b]
SOD	8.93 ± 0.06	3.88 ± 0.04[a]	6.96 ± 0.09	5.90 ± 0.04[b]
CAT	78.13 ± 1.30	53.03 ± 1.31[a]	71.59 ± 1.23	67.66 ± 1.27[b]
GPx	109.32 ± 1.85	58.71 ± 1.08[a]	104.06 ± 2.53	88.68± 1.77[b]
GR	5.39 ± 0.43	1.28 ± 0.13[a]	4.37 ± 0.43	4.27 ± 0.08[b]
GST	239.27 ± 6.20	149.36 ± 3.81[a]	235.64 ± 7.25	209.51 ± 7.06[b]

Each value is expressed as mean ± S.D for six rats in each group.

Units: LPO –nmoles of MDA formed per mg protein; SOD – units/mg protein. (One unit is equal to the amount of enzyme required to inhibit autoxidation of pyrogallol by 50%); CAT - µg of H_2O_2 consumed/min/mg protein; GPx - µg of reduced GSH utilized/min/mg protein; GR - nmoles of NADPH oxidized/min/mg protein; GST - nmoles of CDNB - GSH conjugate formed/min/mgprotein; Statistical significance at $P<0.05$, as compared with [a]Group I, [b]Group II.

Table 2: Activities of Serum marker enzymes in the liver of control and experimental group of rats

Parameters	Group I (Control)	Group II (ISO)	Group III (d-LIM)	Group IV (ISO + d-LIM)
AST	102.57 ± 9.50	369.84 ± 26.19[a]	103.29 ± 9.32	141.73 ± 8.54[b]
ALT	48.61 ± 3.80	170.34 ± 15.73[a]	50.02 ± 4.01	63.51 ± 4.45[b]
CPK	45.90 ± 4.81	139.00 ± 11.85[a]	48.12 ± 5.1	58.17 ± 6.18[b]
LDH	102.20 ± 7.83	191.00 ± 13.94[a]	101.00 ± 8.71	115.15 ± 11.20[b]

Each value is expressed as mean ± S.D for six rats in each group.
Units-IU/Litre, Statistical significance at $P<0.05$, as compared with [a]Group I, [b]Group II.

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