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Original Article

Combination therapy of losarton and lycopene in isoproterenol-induced cardiomyopathy in Wistar rats

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ABSTRACT

Lycopene, a potent antioxidant provides protection against free radicals and has been used in the treatment of cancer, coronary heart disease and hypercholestrimia. Losarton has been used for the treatment of essential hypertension, cardiac hypertrophy and various cardiovascular disorders. The present study was designed to evaluate the cardioprotective effect of combination of Losartan with Lycopene in isoproterenol induced cardiomyopathy in rats. Myocardial infarction was induced by isoproterenol (ISO, 85 mg/kg, sc) twice at an interval of 24 hrs in Wistar rats. ISO produced significant alteration in the hemodynamic parameters (Blood pressure and heart rate), creatine kinase, lactate dehydrogenase, TBARS, GSH, SOD and catalase. Oral treatment with losartan (10 mg/kg) and lycopene (4 mg/kg) for 21 days in ISO treated rats attenuated above mentioned parameters. The treatment with combination of losartan and lycopene reduced the cardiotoxicity induced by ISO which was confirmed by reduction in hemodynamic parameters, TBARS, LDH, CK and increase in GSH, SOD and Catalase enzymes. The effect could be due to the myocardial membrane protection provided by antioxidant and free radical scavenging property. Thus, combination therapy with losartan and lycopene provides new intervention in the treatment of cardiomyopathy.

Key Words: Cardiomyopathy, Lycopene, Losartan, Lactate dehydrogenase

INTRODUCTION

Cardiovascular disease (CVD) affects millions of people lives and is one of the largest causes of death and disability worldwide. This disease includes coronary heart disease, stroke, hypertension, hypercholestrimia and myocardial infarction (MI) [1]. About 17.3 million people died from CVD worldwide, representing 30% of all global deaths. Out of these about 7.3 million people were die due to coronary heart disease and 6.2 million were due to stoke. It is estimated that more than 23 million people will die annually from CVD by 2030 [2].

Myocardial infarction (MI) is one of the most common manifestations of cardiovascular disease. The morbidity and mortality due to MI is now reaching epidemic proportion throughout the world. MI is the myocardium necrosis that occurs as a result of imbalance between coronary blood supply and myocardial demand. Study reported that reactive oxygen species (ROS) in pathogenesis of myocardial injury [3].

There is substantial evidence that ischemic tissue generates oxygen-derived free radicals (oxygen radicals). Free radicals and reactive oxygen species have been implicated in cardiac diseases and metabolic disorders, which result due to exposure to chemicals and environmental agents [3].

Isoproterenol (ISO) is synthetic catecholamine compound and a potent β-adrenergic receptor agonist with peripheral vasodilator and cardiac stimulating properties, has been found to cause a severe stress in the myocardium resulting in infarct like necrosis of the heart muscle and is also well known to generate free radicals and stimulate lipid peroxidation [4,5]. This may be a causative factor for irreversible damage to the myocardial membrane in experimental myocardial infarction [3].

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In recent years, evidence indicates that incidence and progression of cardiovascular disease may, to some extent, be modified by dietary means. In particular, attention has focused on the apparent beneficial effects of antioxidants supplementation in reducing the incidence of cardiovascular disease [6].

Losarton, a selective non-peptide angiotensin II receptor antagonist and has been used in variety of CVDs such as essential hypertension, cardiac hypertrophy and MI [7]. Several clinical and preclinical studies reported that losartan affects rennin-angiotensin aldosterone system and has antioxidant properties [8]. Furthermore, the blockade of AT1 receptors on the sympathetic nerve terminal by Losarton reduces catecholamine release, which subsequently attenuates the generation of free radical and thereby reduce myocardial damage and heart failure [9].

Lycopene is an unsaturated straight chain hydrocarbon and very potent antioxidant because it contains more conjugate bonds. A number of studies reported that lycopene is twice as potent as β-carotene and ten times more potent than α-tocopherol because of its singlet oxygen quenching ability [10].

Although, Losarton has cardioprotective effect and lycopene antioxidant properties. Cardioprotective effect of Losartan and Lycopene studied alone but their combination therapy has not been explored yet. Hence, the present study was designed to evaluate the combination therapy of Losarton and Lycopene in isoproterenol-induced myocardial infarction.

MATERIALS AND METHODS

Animals: Male albino Wistar rats (8- 12 week old), weighing 180-250g, were procured from the animal house facility, KIET School of Pharmacy, Ghaziabad. They were housed in clean polypropylene cages under standard conditions of humidity (50±5%), temperature (25±2˚C) and light (12 h light/12 h dark cycle) and fed with standard rat chow diet (Amrut Laboratory Animal Feed, Nava Maharashtra Chakan Oil Mills, Pune, India) and water *ad libitum*. The protocol was approved by Institutional Animal Ethics committee (IAEC) of KIET School of Pharmacy (Registration number 1099/07/CPCSEA, dated 27/07/2007), Ghaziabad (UP).

Drugs and Chemicals: Isoproterenol and losartan were procured from Fresinius Kabi Oncology Ltd., India; Unichem Pharmaceuticals Ltd., Ghaziabad, India respectively. Lycopene was obtained from Krishgir Pharmaceuticals, Kangra, India. All other chemicals used were of AR grade.

Experimental Protocol: Animals were randomly divided into five groups comprising of six animals in each group and treated in the following way: Group I: Normal control (normal saline, 2 ml/kg, p.o.) given on 13th and 14th day. Group II: ISO control group- received ISO (85 mg/kg, s.c.) on 13th and 14th day. Group III i.e. (ISO+LOS) - Losarton (10 mg/kg, p.o.) was given for 14 days and isoproterenol (85 mg/kg, s.c.) on 13th and 14th day. Group IV i.e. (ISO+LOS 5+LYC 2) - Lycopene (2 mg/kg, p.o.) and losartan (5 mg/kg, p.o.) were administered for 14 days and isoproterenol (85 mg/kg, s.c.) on 13th and 14th day. Group V (ISO+LOS 10+LYC 4) - Lycopene (4 mg/kg, p.o.) and losartan (10 mg/kg, p.o.) were administered for 14 days and isoproterenol (85 mg/kg, s.c.) was given on 13th and 14th day.

Hemodynamic measurements: Hemodynamic measurements were carried out using tail cuff method on Non-Invasive Blood Pressure instrument (AD Instruments, Australia). All the rats were initially trained in the restrainer for a period of 15 min every day at least 10 days prior to the day of measurement of the hemodynamic parameter (systolic, diastolic, mean blood pressure and heart rate).

Biochemical estimation in serum: Blood was collected from retro-orbital plexus under light ether anesthesia and allowed to clot for 30 min at room temperature. The serum was separated by centrifugation at 3000 rpm at 30 ˚C for 15 min and used for the estimation of marker enzymes viz., LDH and CK.

Estimation of lactate dehydrogenase (LDH) and CK (Creatinine kinase) Serum: The concentrations of LDH (Coral Clinical System, Goa, India) and CK (Erba Mannheim, Germany) in serum were measured with commercial kits. The assay is carried out as per the procedure given by the manufacturer. The results were expressed as IU/L for LDH and CK.

Heart weight to body weight ratio measurement: Heart weight, composed of atria, ventricles, and septum, was recorded. Total heart weight–to–body weight ratio is used to express the degree of myocardial hypertrophy [11].

Tissue preparation: The hearts were removed, rinsed in isotonic saline and weighed. A 10% (w/v) tissue homogenate was prepared with 0.1M phosphate buffer (pH 7.4). The post nuclear fraction was obtained by centrifugation of the

homogenate at 12000×g for 20min at 4 ˚C. An aliquot was used for the estimation of biochemical estimations.

Biochemical estimation in cardiac tissues homogenates: Measurement of lipid peroxidation was carried out by determination of myocardial malondialdehyde (MDA) content by the method of Ohkawa *et al.* [11]. Antioxidant enzymes viz. GSH, SOD and CAT were estimated in cardiac tissue as per standard protocol. GSH was estimated by the Sedlack and Lindsay method [12]. The activity of SOD was measured according to the method of Marklund and Marklund [13]. CAT activity was measured according to Clairbone [14].

Statistical Analysis: Statistical analysis was carried out by using Graph Prism 3.0 (graph pad software, San Diego, CA). All results were expressed as mean ± SEM. Data was compared with the analysis of variance (ANOVA) followed by dunett's t- test. Values were considered statistically significant when $P<0.05$.

RESULTS

Effect on haemodynamic parameters: The systolic, diastolic, mean BP and heart rate were significantly increased in ISO treated rats (i.e. Group II) as compared to the normal control rats (i.e. Group I). Treatment with LYC (4 mg/kg) along with LOS (10 mg/kg) showed significant ($p<0.01$) reduction in the systolic, diastolic, mean BP and heart rate as compared to the ISO treated group (Table 1).

Effect on lactate dehydrogenase (LDH) activities: Treatment of rats with ISO (85 mg/kg) caused a significant ($p< 0.05$) increase in the LDH enzyme activity as compared to the normal control group. LOS (10 mg/kg) treatment significantly ($P<0.05$) decrease the serum LDH levels as compared to ISO control group. Administration of LOS along with LYC (2 and4 mg/kg) resulted in significant ($p<0.01$) decrease in LDH activity as compared with ISO control group (Figure 1).

Effect on creatinine kinase (CK) activities: Treatment of rats with ISO (85 mg/kg) caused a significant ($p< 0.05$) increase in the CK enzyme activity as compared to the normal control group. LOS (10 mg/kg) treatment significantly ($P<0.05$) decrease the serum CK levels as compared to ISO control group. Administration of LOS along with LYC (2 and4 mg/kg) resulted in significant ($p<0.01$) decrease in CK activity as compared with ISO control group (Figure 2).

Effect on heart weight/body weight ratio: There was a significant ($p<0.05$) decrease in the mean heart weight/body weight ratio in the ISO treated group as compared to normal control group (Figure 3). The mean heart weight/body ratio in the ISO+ LOS (10 mg/kg), ISO+ ALK+LYC (2 mg/kg) treated groups were significantly restored ($p<0.05$) as compared with ISO treated group. While the mean heart weight/body weight ratio was most significantly ($p < 0.01$) increased in ISO+ ALK+LYC (4 mg/kg) treated group.

Effect on oxidative stress and antioxidant enzyme levels: Oxidative stress was determined by determining myocardial MDA content (Table 2). MDA level was significantly ($p<0.05$) increased in the ISO treated group as compared to the normal control group. MDA level was significantly ($p<0.05$ and $p<0.01$) decreased in ISO+LOS (10 mg/kg), ISO+LOS +LYC (2 and 4 mg/kg) treated groups compared with the ISO treated group respectively. In ISO treated group antioxidant enzymes viz. CAT, GSH and SOD were significantly decreased as compared to normal control group. While the antioxidant enzyme levels were significantly restored in ISO+LOS (10 mg/kg) and ISO+LOS +LYC (2 and 4 mg/kg) treated groups (Table 2).

DISCUSSION

The present study was aimed to explore the cardioprotective effect of combination therapy of losartan and lycopene on ISO induced myocardial infarction in rats. It was found in the present study that isoproterenol increased the heart rate and blood pressure while combination therapy with losartan and lycopene reversed the hemodynamic changes.

Heart weight–to–body weight ratio is used to express the degree of myocardial hypertrophy [11]. Alteration in HW/BW ratio could arise due to consecutive loss of myocardial connective tissue in damaged myocardium. In the present study, there was a significant rise in HW/BW ratio in ISO treated rats which were markedly reduced on treatment with losartan and lycopene combination, suggesting their myocardial tissue protecting effect. In the present study, ISO treated rats there was an increase in activities of the marker enzymes LDH and CK in the serum. The plasma LDH and CK enzyme activities are important measures for both early and late phases of cardiac injury as myocardium contains an increased level of diagnostic marker enzymes of myocardial infarction viz. CK and LDH and once metabolically damaged, releases its content into extracellular fluid (ECF) [16]. The release of the cardiac specific isoenzymes LDH1 and LDH2-into

the circulation might be due to the necrosis induced by ISO [17]. These finding confirm the onset of myocardial necrosis and leaking out of the marker enzymes from heart to blood [18].

Combined treatment with losartan and lycopen significantly lowered these enzymes level. It demonstrated that combination therapy with losartan and lycopene could maintain membrane integrity, thereby restricting the leakage of this enzyme.

Increased activities of serum marker enzymes accompanied by their concomitant reduction in heart homogenate in ISO treated rats, confirm the onset of myocardial necrosis. The increased levels of MDA indicate excessive formation of free radicals by auto-oxidation of ISO and activation of the lipid peroxidation process, resulting in irreversible damage to heart in animals subjected to ISO stress [18]. LOS+LYC+ISO treatment significantly decreased the MDA levels by preventing formation of lipid peroxides from fatty acids.

In the present study, SOD activity was decreased significantly in the ISO control animals may be due to an excessive formation of superoxide anions. A decrease in SOD activity results in the decreased removal of superoxide anions, which can be harmful to the myocardium [19]. The activity of H_2O_2 scavenging by catalase was decreased significantly in ISO treated animals. The decline in these enzyme levels may be explained by the fact that excessive superoxide anions may inactivate SOD, thus, resulting in an inactivation of the H_2O_2 scavenging enzymes [20]. Administration of LOS+LYC to ISO treated rats prevented the decrease in SOD, catalase and GSH levels, which may be correlated directly to the scavenging of radicals by LOS and LYC resulting in protection of these enzymes.

Combination therapy of losartan and lycopene significantly enhanced the level of GSH SOD and catalase levels thereby showing the reversal of oxidative stress by its free scavenging or neutralizing properties and by enhancing the enzyme activities.

CONCLUSION

In conclusion, the treatment with combination of losartan and lycopene attenuated the cardiotoxicity induced by Isoproterenol which was confirmed by reduction in blood pressure, heart rate, HW/BW ratio, TBARS and LDH, CK levels and increase in GSH, SOD and Catalase enzyme. The effect could be due to myocardial membrane protection provided by antioxidant and free radical scavenging property of the drugs.

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Conflict of interest: The authors declare that they have no conflicts of interest concerning this article.

Table 1. Combined effect of Losarton & Lycopene on isoproterenol induced changes on systolic, diastolic, mean BP and heart rate of Wistar rats

Groups	Heart Rate (BPM)	Systolic BP (mmHg)	Diastolic BP (mm Hg)	Mean BP (mmHg)
Normal Control Group (saline 2ml/kg, i.p)	365.89±6.72	120.56±3.46	101.76±1.78	113.26±2.04
ISO control Group (ISO 85 mg/kg, s.c.)	538.70±10.43[a]	166.47±4.96[a]	129.58±2.65[a]	144.15±2.28[a]
ISO+LOS (10 mg/kg, p.o.)	482.45±8.07[b]	138.92±3.82[b]	116.04±2.17[b]	128.35±2.16[c]
ISO+LOS5+LYC2 (2mg/kg, p.o.)	454.09±7.37[c]	128.02±3.16[c]	106.48±2.01[c]	118.25±1.86[c]
ISO+LOS10+LYC4 (LYC 4mg/kg, p.o.)	418.21±5.14[c]	122.98±2.44[c]	103.92±1.53[c]	114.44±2.05[c]

ISO: Isoproterenol; LOS: Losartan; LYC: lycopene; BPM: beats per minute; BP: blood pressure.
All values were expressed as Mean ±S.E.M. (n=6),[a]P<0.01 as compared to the normal control group, [b]P<0.05 and [c]P<0.01 as compared to the ISO control group (ANOVA followed by Dunnett's test).

Table 2: Combined effect of Losarton and Lycopene on isoproterenol induced changes on myocardial TBARS, GSH, SOD, & Catalase levels

GROUPS	TBARS (nmol MDA/mg protein)	GSH (nmole/mg protein)	SOD (IU/mg protein)	CATALASE (nmole H_2O_2 consumed/ min/ mg protein)
Normal Control Group (saline 2ml/kg, i.p)	1.04 ± 0.28	1.022±0.16	7.64 ± 0.45	4.72± 0.47
ISO control Group (ISO 85 mg/kg, s.c.)	6.26 ± 0.42 [a]	0.355±0.28[a]	4.12 ± 0.23[a]	2.92 ± 0.36[a]
ISO+LOS (10 mg/kg, p.o.)	4.36 ± 0.26[b]	0.628±0.37[b]	5.94 ± 0.36[b]	3.66 ± 0.16[b]
ISO+LOS5+LYC2 (2mg/kg, p.o.)	3.64 ± 0.16[c]	0.899±0.28[c]	6.24 ± 0.54[c]	3.88 ± 0.18[c]
ISO+LOS10+LYC4 (LYC 4mg/kg, p.o.)	2.57 ± 0.18 [c]	0.954±0.42[c]	7.38 ± 0.80[c]	4.47 ± 0.22[c]

All values were expressed as Mean ±S.E.M. (n=6), [a]P<0.01 as compared to the normal control group, [b]P<0.05and [c]P<0.01 as compared to the ISO control group (ANOVA followed by Dunnett's test).

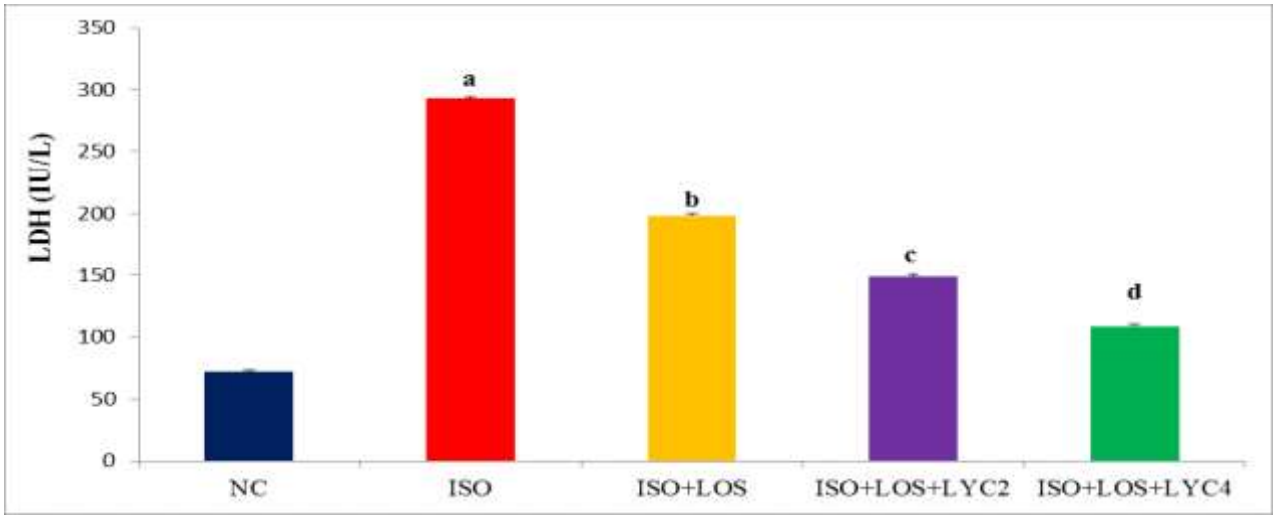

Figure 1: Combined effect of Losarton and Lycopene on isoproterenol induced changes on cardiac LDH levels
All values were expressed as Mean ±S.E.M. (n=6), [a]P<0.01 as compared to the normal control group, [b]P<0.05, [c]P<0.01, [d]P<0.01 as compared to the ISO control group (ANOVA followed by Dunnett's test).

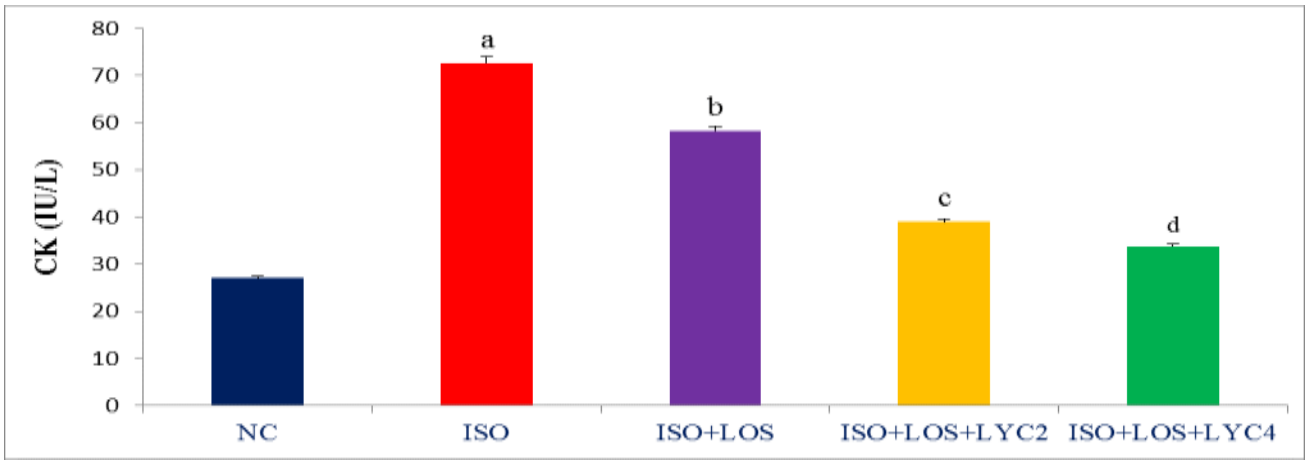

Figure 2: Combined effect of Losarton and Lycopene on isoproterenol induced changes on cardiac CK levels
All values were expressed as Mean ±S.E.M. (n=6), [a]P<0.01 as compared to the normal control group, [b]P<0.05, [c]P<0.01, [d]P<0.01 as compared to the ISO control group (ANOVA followed by Dunnett's test).

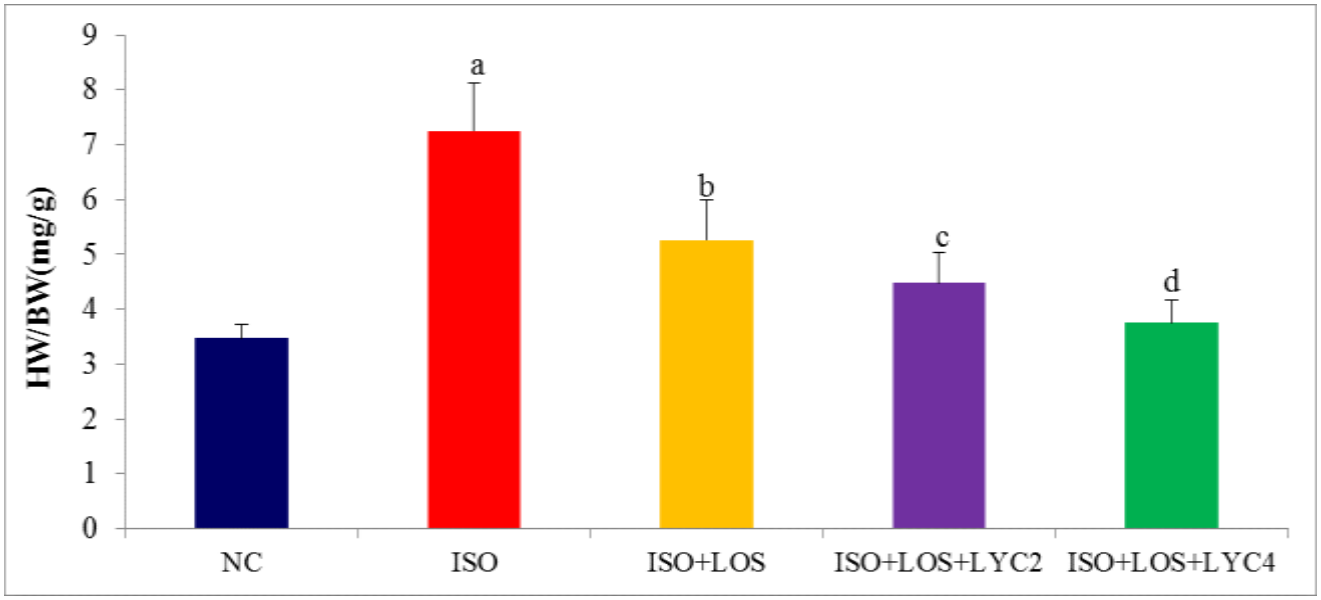

Figure 3: Combined effect of Losarton and Lycopene on isoproterenol induced changes on cardiac HW/BW ratio. All values were expressed as Mean ±S.E.M. (n=6), [a]P<0.01 as compared to the normal control group, [b]P<0.05, [c]P<0.01, [d]P<0.01 as compared to the ISO control group (ANOVA followed by Dunnett's test).

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