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Protective Effect of Nobivolol on Lipid Profile and Lipid Metabolizing Enzymes in Isoproterenol Induced Myocardial Infarction in Diabetes Rats

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ABSTRACT

Present study was designed to evaluate the effect of nobivolol on lipid profile and lipid metabolizing enzymes in isoproterenol induced myocardial infarction in normal and streptozotocin-nicotinamide induced diabetic rats. Nobivolol (2 mg/kg, p.o) was administered for 28 days in rats injected with single dose of streptozotocin (65 mg/kg, i.p, STZ) and nicotinamide (110 mg/kg, i.p, NIC) and after isoproterenol (200 mg/kg, s.c.) induced myocardial infarction in rats on 29th and 30th day. At the end of experimental period (i.e. on the day 31) serum and heart tissues sample were collected, and glucose, HbA1c and total cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL) and cholesterol ester synthetase (CES), lecithin cholesterol acyl transferase (LCAT), lipoprotein lipase (LPL) were estimated. Administration of STZ-NIC in rats showed a significant ($p < 0.001$) increase in the levels of serum glucose, glycosylated hemoglobin (HbA1c), total Cholesterol (TC), triglycerides (TG) and low density lipoprotein (LDL) whereas the levels of high density lipoprotein (HDL) were found to be non significant but significant ($p < 0.001$) increase in the level of heart tissues CES and significant ($p < 0.001$, $p < 0.01$) decrease in LCAT and LPL as compared to respective control groups. Treatment with nobivolol significantly ($P < 0.01$) decrease CES level and significantly ($P < 0.001$, $P < 0.01$, $P < 0.01$) decrease LDL, TC and TG and significant ($P < 0.01$) increase in LCAT and LPL level but no significant change in glucose, HbA1c and HDL as compared to diabetic control group.

KEYWORDS: Nobivolol, Lipid profile, Lipid metabolic enzyme.

INTRODUCTION

Three major metabolic abnormalities contribute to the development of hyperglycemia in Type 2 diabetes mellitus such as impaired insulin secretion in response to glucose, increased hepatic glucose production and decreased insulin-stimulated glucose uptake in peripheral tissues. The latter 2 abnormalities are primarily due to insulin resistance (Kahn, 1990; Leibowitz, 1990). Non insulin dependent diabetes mellitus has also been associated with an increased risk for premature arteriosclerosis due to increase in triglycerides and low density lipoprotein levels. About 70-80 % of deaths in diabetic patients are due to vascular diseases. An ideal treatment for diabetes would be a drug that not only controls the glycemic level but also prevent the

development of arteriosclerosis and other complication of diabetes.

Cardiovascular disease is one of the leading causes of death in the western world and diabetes mellitus has been identified as a primary risk factor (Uemura, 2001), due to which there is alteration in vascular responsiveness to several vasoconstrictors and vasodilators (Senses, 2001).

Reactive oxygen species may contribute to the events of atherogenesis and leading to the progression of atherogenic lesions by promoting oxidation of low density lipoproteins (Benelli, 2002). Isoproterenol has been found to cause a severe stress in the myocardium resulting in infarction like necrosis of heart muscles. It also increases the level of serum and myocardial lipids (Prince, 2005) and also increases the level of cholesterol in the tissues which in turn leads to coronary heart disease (Sathish, 2003).

Long before the use of insulin became common, indigenous remedies were used for the treatment of diabetes

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mellitus and antihyperlipidemic. There has been an increasing demand from patients for the use of synthetic drugs with antidiabetic and antihyperlipidemic and impairment other body functions.

Nebivolol (NEB) is a β 1-adrenoceptor blocking drug that possesses certain unusual pharmacological properties by which it differs from conventional β 1-blockers. The haemodynamic properties of Nebivolol appear to be linked not only to the β 1-adrenoceptor blocking action, but also to several mechanisms such as the vasodilatation endothelium-dependent effect. Nebivolol, a highly β 1-selective, non-intrinsic sympathomimetic activity, third generation β -blocker, provided some vasodilating properties, which have been shown to be L-arginine/nitric oxide mediated, (Van de, 1988 and De Cree, 1988) in the treatment of hypertensive patients with associated NIDDM.

Some Literature survey showed that, there was no report regarding the effect of NOB on lipid profile and Lipid Metabolizing Enzymes in experimentally induced myocardial infarction in diabetic rats. Hence, the purpose of the present study was to instigate the effect Nobivolol on serum glucose, HbA1c, lipid profile and lipid metabolizing enzymes in isoproterenol induced myocardial infarction in normal and diabetic rats.

MATERIALS AND METHOD

Drugs and Chemicals

Nobivolol was obtained as a gift sample from Torrent Pharmaceuticals Pvt. Ltd., Ahmadabad, India. STZ and NIC were obtained from SIGMA, St. Louis, MO, USA. All other chemicals and reagents used in the study were of analytical grade.

Experimental Animals

All experiments and protocols described in present study were approved by the Institutional Animal Ethics Committee (IAEC) of Dharmaj Degree Pharmacy College, Anand. Sprague Dawley rats (205 ± 15 g) were housed in-group of 3 animals per cage and maintained under standardized laboratory conditions (12- h light/dark cycle, 24°C) and provided free access to palleted CHAKKAN diet (Nav Maharashtra Oil Mills Pvt., Pune) and purified drinking water *ad libitum*.

Experimental Induction of Type 2 Diabetes in Rats

Type 2 Diabetes was induced in rats by a single intraperitoneal (i.p) injection of Streptozotocin (65 mg/kg, STZ) in overnight fasting rats or mice followed by the i.p administration of Nicotinamide (110 mg/kg, NIC) after 15 minutes. STZ was dissolved in citrate buffer (pH 4.5) and NIC was dissolved in normal saline. After 7 days following STZ and NIC administration, blood was collected from retro-orbital puncture and serum samples were analyzed for blood glucose (Masiello, 1998). Animals showing fasting blood glucose higher than 250 mg/dL were considered as diabetic and used for the further study.

Experimental Protocol

Animals were divided into following groups, each group

containing 6 animals and the treatment period for whole study was 4 weeks.

Group 1: Non-diabetic control [0.5 % Sodium CMC (1 ml/kg/day, p.o) as vehicle for 4 weeks and (ND-CON)] and normal saline subcutaneously on 29th and 30th day.

Group 2: Non-diabetic control treated with NOB (2 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (ND-NOB)] and normal saline subcutaneously on 29th and 30th day.

Group 3: STZ-NIC diabetic control [0.5 % Sodium CMC (1 ml/kg/day, p.o) as vehicle for 4 weeks (D-CON)] and received ISO (200 mg/kg, s.c.) on 29th and 30th day in normal saline.

Group 4: STZ-NIC diabetic rats treated with NOB (2 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (D-NOB)] and received ISO (200 mg/kg, s.c.) on 29th and 30th day in normal saline.

BIOCHEMICAL ESTIMATIONS

Characterization of Type 2 Diabetes Model

Type 2 diabetes was confirmed by measuring fasting serum glucose using standard diagnostic kit (SPAN diagnostics Pvt., India) and the degree of uncontrolled diabetic state was confirmed by measuring HbA1c (Ion Exchange Resin method). After 4 weeks, diabetes was confirmed by measuring glucose and HbA1c as mentioned above.

Estimation of Serum Markers

On 4th week blood samples were collected from retro-orbital plexus under light ether anesthesia and centrifuged at 2500 rpm for 20 minutes to separate serum. Glucose (Kaplan, 1983), HbA1c (Nathan, 1984), TC (Kaplan, 1983), TG (Fossati, 1982), HDL and LDL were estimated using diagnostic kits (SPAN Diagnostics Pvt. India). The activities of lipid metabolizing enzymes such as cholesterol ester synthetase (CES), lecithin: Cholesterol acyl transferase (LCAT) and lipoprotein lipase (LPL) were determined from the heart tissues sample as suggested by Kothari et al (Kothari, 1973), Hitz et al (Hitz, 1983) and Slater et al (Slater, 1996).

Statistical Analysis

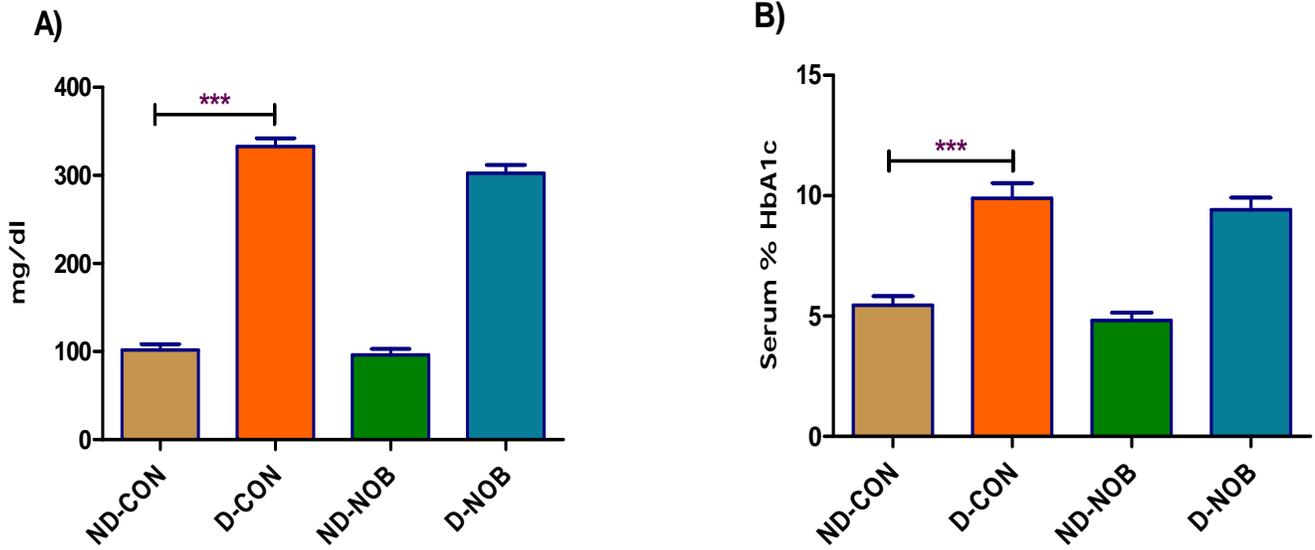
All of the data are expressed as mean \pm SEM. Statistical significance between more than two groups was tested using one-way ANOVA followed by the Bonferroni multiple comparisons test or unpaired two-tailed student's t-test as appropriate using a computer-based fitting program (Prism, Graphpad 5). Differences were considered to be statistically significant when $p < 0.05$.

RESULTS

Characterization of Type 2 Diabetes

Single intraperitoneal (i.p) injection of Streptozotocin (65mg/kg) followed by i.p administration of Nicotinamide (110 mg/kg) to rats produced severe hyperglycemia and increased HbA1c in 70 to 80 % the animals.

Figure.1 Effect of Nobivolol (2mg/kg/day, p.o) on changes in serum glucose and HbA1c level in normal and STZ-NIC induced diabetic rats.



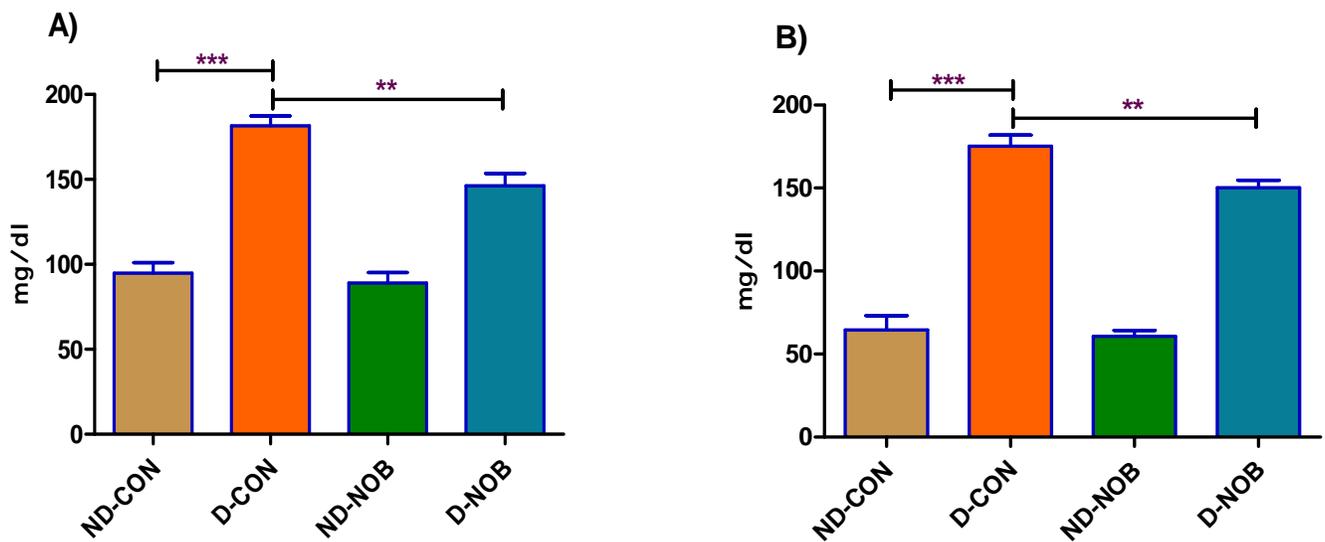
Values are expressed as mean \pm SEM for six animals in the group. * P<0.05, **P<0.001, ***P<0.001 considered statistically significant as compared to Control group.

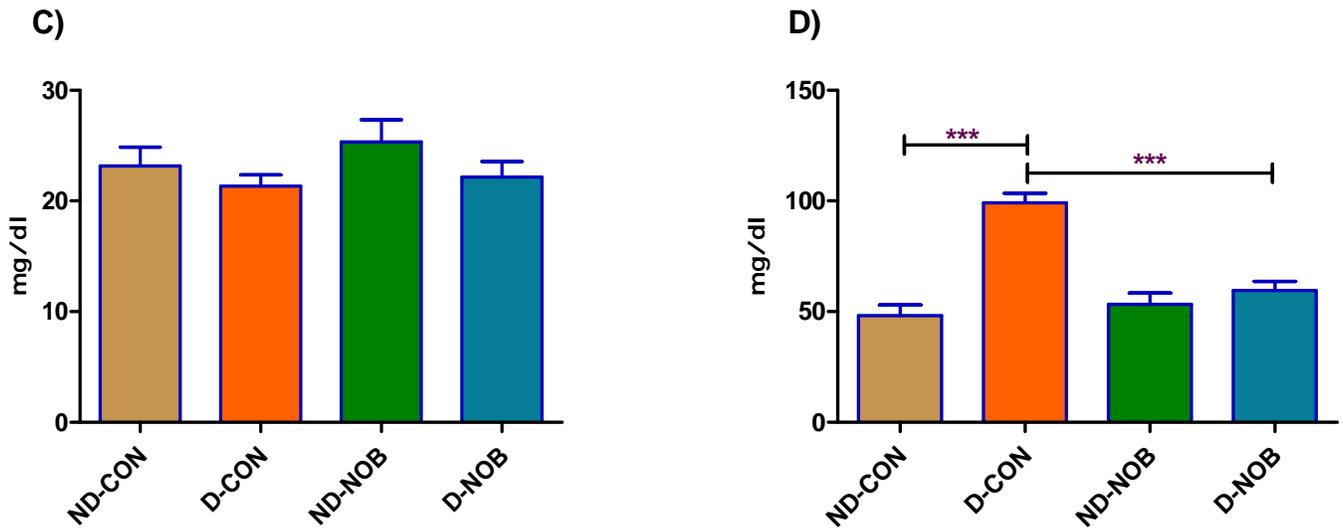
Effect of NOB on serum enzymes

There was a significant (p<0.001) increase in blood glucose and HbA1c level of STZ-NIC injected animals compared to ND-CON group (Fig. 1). Serum total cholesterol, LDL and triglyceride were significant (p<0.001) increase in STZ-NIC diabetic rats as compared to non-

diabetic rats. Administration of NOB in STZ-NIC diabetic rats (D-NOB) no significant on blood glucose and HbA1c, while it significantly (p<0.01) reduced serum total cholesterol, triglyceride and LDL (p< 0.001) in D-NOB group as compared to D-CON group but there was no significant changes in the levels of HDL (Fig. 2).

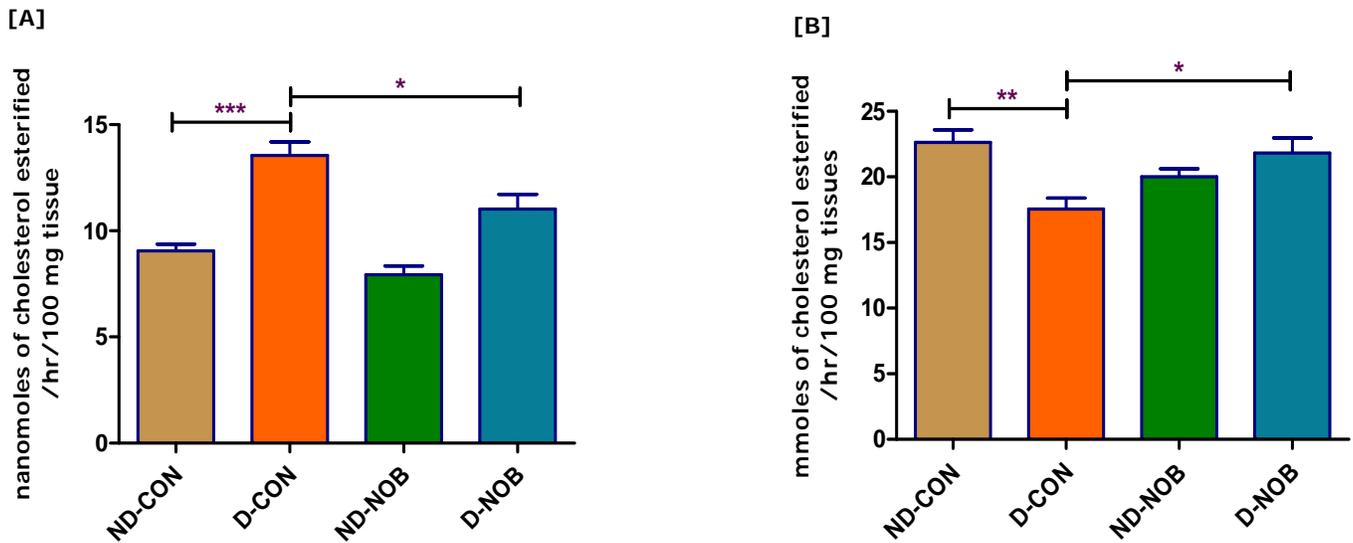
Figure.2 Effect of Nobivolol (2 mg/kg/day, p.o) on changes in serum Total Cholesterol (A), Triglycerides (B), High density lipoprotein (C) and Low density lipoprotein (D) level after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.

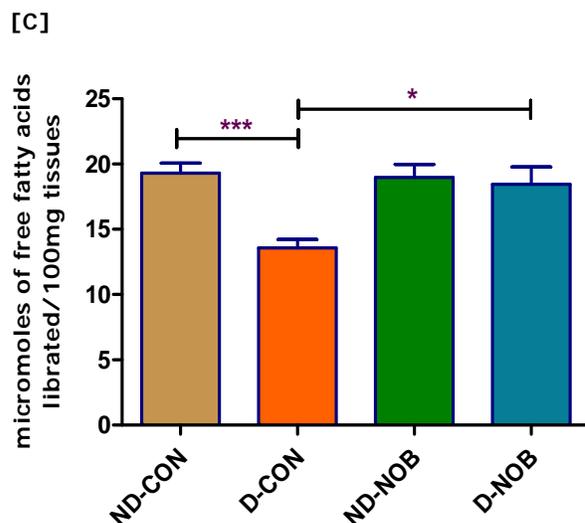




Values are expressed as mean \pm SEM for six animals in the group. * $P < 0.05$, ** $P < 0.001$, *** $P < 0.001$ considered statistically significant as compared to Control group.

Figure.3 Effect of Nobivolol (2 mg/kg/day, p.o) on changes in cholesterol ester synthetase (A), lecithin Cholesterol acyl transferase (B), lipoprotein lipase (C) level after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.





Values are expressed as mean \pm SEM for six animals in the group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ considered statistically significant as compared to respective Control group.

Effect of NOB on Lipid Metabolizing Enzymes enzyme

There was a significant ($p < 0.001$) increase in CES after myocardial infarction in D-CON group as compared to ND-CON group (Fig. 3A). Treatment of NOB is significant ($p < 0.05$) with decreased levels of CES as compared to diabetic rats. There was a significant ($p < 0.05$) increase in LCAT and LPL after myocardial infarction in D-CON group as compared to respective ND-CON group (Fig. 3B and 3C). Treatment of NOB in diabetic rats is significant ($p < 0.01$) with decreased levels of LCAT and LPL.

DISCUSSION

The present study was under taken with the objective of exploring the Nobivolol on lipid profile and Lipid Metabolizing Enzymes in experimentally induced myocardial infarction in diabetic rats.

Diabetes mellitus is a chronic disorder caused by partial or complete insulin deficiency, which produces inadequate glucose control and leads to chronic complication. Premature and extensive arteriosclerosis involving renal, peripheral, and cardiovascular vessels remain the major complication of diabetes mellitus. Alteration in the serum lipid profile is known to occur in diabetes and this is likely to increase the risk for coronary heart disease. A reduction in serum lipids, particularly of the LDL and VLDL fraction and TG, should be considered as being beneficial for the long term prognosis of these patients. Lower of blood glucose and plasma lipid levels thought dietary modification and drug therapy seem to be associated with a decrease in the risk of vascular disease.

Recent studies have suggested that prevalence of type 2 diabetes is rapidly increasing. Patients with diabetes

show an increased mortality concerning cardiovascular events. They more often suffer from myocardial infarction as non-diabetics mostly with a more serious course. Moreover, the post-infarction course is affected with a worse prognosis as in non-diabetics (Abel, 2005).

In the present study, an increase in the levels of serum glucose and HbA1c in STZ-NIC treated rats confirmed the induction of diabetes mellitus. No Significant change was observed in the glucose and HbA1c levels in diabetic rats after treatment with NOB (2 mg/kg) when compared with D-CON rats at the end of experimental period. STZ causes diabetes by the rapid depletion of β -cells and thereby brings about a reduction in insulin release. HbA1c level has been reported to be increased in patients with diabetes mellitus (Paulsen, 1973). It was reported that during diabetes mellitus, the excess of glucose present in the blood reacts with hemoglobin to form HbA1c (Koenig, 1976). The level of HbA1c is always monitored as a reliable index of glycemic control in diabetes (Gabbay, 1976). Elevated levels of HbA1c observed in our study reveal that diabetes animals had prior high blood glucose level.

Lipids play an important role in cardiovascular disease, by modifying the composition, structure and stability of cell membranes. Altered lipid metabolism is considered to accelerate the development of atherosclerosis, a major risk factor in myocardial infarction. High levels of circulating cholesterol and its accumulation in heart tissue is well associated with cardiovascular damage. In the present study there was a significant decrease in cardiac LCAT and LPL activity whereas a significant increase in the activity of CES was found in ISO intoxicated rats. HDL is the main

substrate for LCAT for cholesterol esterification and incorporation (Nestel, 1963).

A significant increase in the level of LCAT in diabetic rats treated with NOB. In the present study hypertriglyceridemia observed in ISO intoxicated rats is due to decrease activity of LPL in the myocardium tissues. Accumulation of ester cholesterol occurs when the rate of esterification by cholesterol ester synthetase exceeds the rate of hydrolysis, which in turn results in myocardial membrane

damage (Upaganlawar, 2009). NOB alters the activities of LCAT, LPL and CES improve compared to normal, indicating the potential lipid lowering effects of NOB.

The chronic diabetic state was also associated with dyslipidemia. This study concluded that NOB at 2 mg/kg may decrease lipid profile and lipid metabolizing enzymes without change in glucose and HbA1c in experimentally induced myocardial infarction in diabetic rats.

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