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**Effects of Chronic Treatment with Nitrendipine in Streptozotocin-Induced Diabetic Rats**

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S.S. JOSHI, T.S. SHAH AND R.K. GOYAL\*

Dept. of Pharmacology, L.M. College of Pharmacy, P.O. Box No. 4011, Navrangpura, Ahmedabad - 380 009.

The present investigation involves the study of the effects of chronic treatment with oral nitrendipine (15 mg/kg/day) in streptozotocin (STZ) induced diabetic rats. Single tail vein injection of STZ (45 mg/kg) produced a diabetic state exhibiting all the cardinal symptoms such as loss of body weight, polyuria, polydipsia, glucosuria, hypoinsulinemia, and hyperglycemia. The diabetic state was also found to be associated with hypothyroidism, hypercholesterolemia, bradycardia, hypertension, cardiac depression and cardiomyopathy. Nitrendipine treatment prevented the hypercholesterolemia, hypertension and bradycardia observed in untreated STZ-diabetic rats. It could not prevent however, loss of body weight, hyperglycemia, hypoinsulinemia and hypothyroidism. The cardiac depression and cardiomyopathy were found to be partially prevented by nitrendipine. These data suggests that nitrendipine has certain beneficial effects in diabetic rats.

**HYPERTENSION** which co-exists with diabetes-mellitus is not only an indicator of increased risk of mortality but also a contributory factor to the development of diabetic complications, such as cardiomyopathy<sup>1</sup>, retinopathy<sup>2</sup> and nephropathy<sup>3</sup>. The processes leading to coronary heart disease, stroke, peripheral vascular disease and congestive heart failure are all accelerated in hypertensives as compared to normotensive diabetic subjects. The pathogenesis of diabetic cardiomyopathy is a complex phenomenon and may involve a decreased  $Ca^{+2}$ ,  $Mg^{+2}$  ATPase activity of the sarcoplasmic reticulum<sup>4</sup>, shifting of the myosin isoenzyme distribution to the isoenzyme associated with lower ATPase activity<sup>5</sup>, increased metabolism of fatty acids by the myocardium<sup>6</sup>, hypothyroidism<sup>7</sup>, microangiopathy, macroangiopathy and associated cardiovascular conditions.<sup>8</sup> From these reports it becomes apparent that controlling blood pressure in diabetes may play a crucial role in arresting the progression of cardiomyopathy and other secondary diabetic complications concerned. A new therapeutic approach to the

treatment of such patients should be such that it does not worsen the condition but leads to improvement.

Cardiac performance was found to be improved in diabetic animals treated with hydralazine<sup>9</sup> and enalapril.<sup>10</sup> Atenolol was found to worsen further hyperlipidaemia and depression of cardiac function.<sup>11</sup> There are controversial reports with respect to calcium channel blockers, regarding their effects on blood glucose levels and insulin secretion.<sup>12, 13</sup> It was earlier reported that nifedipine prevented hyperlipidaemia, hyperglycemia, cardiac dysfunction and cardiomyopathy in STZ induced diabetic rats.<sup>14</sup> However, discrepancies remain about insulin and blood sugar levels.

Nitrendipine, a second generation calcium channel blocker is reported to be devoid of the possible myocardial depression associated with nifedipine.<sup>15</sup> Further short-term studies with nitrendipine indicated that it is metabolically neutral and reverses left ventricular hypertrophy. The present investigation was undertaken up to study the effect of chronic treatment

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\* For Correspondence

with nitrendipine in streptozotocin induced diabetic rats.

## MATERIAL AND METHODS

### Induction of diabetes

Healthy female albino rats of Wistar strain, weighing (180-200 g) were made diabetic by a single tail vein injection of STZ (45 mg/kg). Control rats were injected with citrate buffer. Diabetic rats were checked for the intensity of glucosuria using enzymatic test strips (Miles India Ltd.). Animals showing glucosuria (> 2%), 48 hours after the injection of STZ were selected as diabetic rats for the experiments. All these animals were randomly divided into four sub-groups:- Control, Nitrendipine - treated control, Diabetic control and Diabetic treated with nitrendipine. Nitrendipine maleate (dissolved in distilled water) was given in the dose of 15 mg/kg daily for six weeks by oral route. Food and water was provided *adlibitum* throughout the study period. The animals were observed throughout the six week study period for their water intake, food intake, changes in body weight and mortality.

### Blood Sample Collection and Analysis

At the end of six weeks, blood samples were collected from the retro-orbital plexus of rats and serum was separated by centrifugation at 3000 R.P.M. for 15 min. Serum glucose levels were determined by glucose oxidase peroxidase method using a commercially available GOD/POD kit (Span Diagnostics, India). Serum immunoreactive insulin was assayed by radioimmunoassay method using the kit obtained from BARC, Bombay. Serum T<sub>3</sub> was estimated by Solid Phase Competitive Binding Enzyme linked immunoassay method using the kit from Miles India Ltd. Serum cholesterol, total lipids, and triglycerides were measured enzymatically by spectrophotometric end-points using their respective kits.

### Measurement of Blood pressure, Heart rate and Cardiac function

After completion of the six weeks treatment blood pressure and heart rate were recorded by tail cuff method after which animals were sacrificed by a sharp blow to the head followed by decapitation. Hearts were excised quickly and perfused according to modified Neely's Working Heart mode as described earlier.<sup>9</sup> Hearts were perfused with Chenoweth Koelle solution (pH 7.4) which was constantly bubbled with carbogen and maintained at 37°C. Hearts were allowed to stabilize for 10 min at the perfusion pressure of 10 cm H<sub>2</sub>O. The left ventricular developed pressure (LVDP) was then recorded at different atrial filling pressure (5 cm H<sub>2</sub>O to 25 cm H<sub>2</sub>O). The left atrial filling pressure was changed in a stepwise manner using 2.5 cm H<sub>2</sub>O steps each time which was achieved by changing the height of the constant level buffer reservoirs.

### Histological study of myocardium

To carry out the histological study of the heart, it was isolated and fixed in Bouin's fixative and then dehydrated using different grades of alcohol. Tissues were then embedded in molten paraffin and blocks were prepared. Sectioning was done using a microtome. The sections were stained with Haemotoxyllin and Eosin stains. Perfectly stained slides were mounted with DPX (Di Phenyl Xylene) and observed under a light microscope.

### Statistical Analysis

The results were analysed statistically using one way analysis of variance followed by Tucky's test. The value of probability less than 5% ( $P < 0.05$ ) was considered as significant.

## RESULTS

### 1. General Features of the Diabetic Rats

Injection of STZ to rats produced glucosuria (> 2%) in all the animals. Polyuria, polydipsia and polyphagia, which are the classical symptoms of a dia-

betic state were also observed in the STZ-treated rats. Weight gain during the study period was significantly less in both diabetic groups than in controls (Table 1). There was no significant difference in weight gain between the controls and nitrendipine treated controls. Diabetic rats exhibited reduced heart weight. However, the ratio of heart weight to body weight was not significantly different in either of the groups. The mean blood pressure was increased significantly in STZ-treated animals. The diabetic animals treated with nitrendipine showed blood pressure comparable to non-diabetic controls (Table 1). Heart rate was found to be decreased in diabetic animals and this bradycardia was prevented by nitrendipine.

### Effects of nitrendipine on cardiac Function

In response to increased left atrial filling pressure there was an increase in LVDP. Hearts from untreated diabetic rats showed lower LVDP at higher filling pressures. Treatment of the diabetic rats with nitrendipine did not alter the status of LVDP at any of the filling pressures. There was no significant difference in the LVDP between the control and nitrendipine treated control group of animals (Fig. 1).

### Effect of nitrendipine on Various Biochemical Parameters

Serum glucose levels measured at the time of sacrifice were found to be significantly elevated in the diabetic animals. This was associated with a decrease in insulin levels. Treatment of animals with nitrendipine failed to prevent STZ-induced hyperglycaemia. There was an increase in serum glucose levels in control treated animals but it was not significant. Serum  $T_3$  level was also significantly decreased in diabetic rats. Serum cholesterol level was significantly higher in diabetics than in controls. Nitrendipine treatment, in diabetic animals could not prevent hyperglycemia, hypoinsulinemia and hypothyroidism. However, serum cholesterol level was significantly decreased to normal level by nitrendipine treatment.

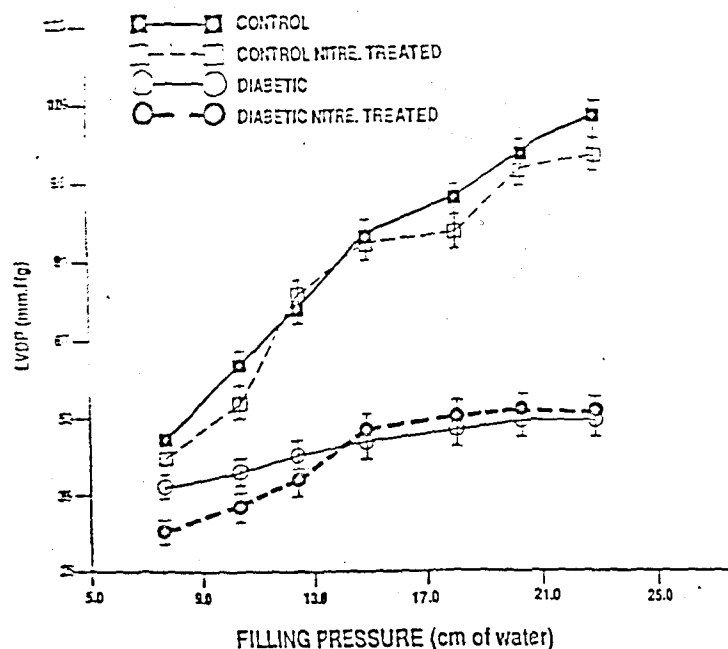


Fig. 1 :- Effects of streptozotocin-diabetes and nitrendipine treatment of Left Ventricular Developed Pressure of rat heart. Each point and the bar represents mean  $\pm$  SEM of 7 experiments.

### Histological alterations in the myocardium

STZ-diabetes caused distortion of myocardial fibres. The nuclei were clustered, intercalations disrupted and vacuoles were formed. Six weeks treatment with nitrendipine prevented these distortions in the myocardium.

### DISCUSSION

Rats treated with streptozotocin (STZ) have been reported to displayed many of the features seen in human subjects with uncontrolled diabetes mellitus, including hyperglycemia, polydipsia, polyuria and weight loss.<sup>16</sup> In the present investigation also STZ-induced diabetic rats showed characteristic symptoms of diabetes such as polydipsia, polyuria, glucosuria and weight loss. Hofteizer and Carpenter<sup>17</sup> suggested that the loss of body weight could be due to dehydration and the catabolism of fats and proteins seen during diabetes mellitus. Treatment with nitrendipine could not prevent the weight loss.

**Table - 1: Effect of Nitrendipine on General Characteristics of Control and Diabetic Rats**

	Controls		Diabetics	
	Untreated (n = 7)	Nitrendipine treated (n = 7)	Untreated (n = 7)	Nitrendipine treated (n = 7)
Body weight (g)	210.5 ± 8.5	208.0 ± 12.1	170.0 ± 8.7*	153.3 ± 2.1*
Water intake (ml/day)	35 ± 2	30 ± 1	58 ± 9*	60 ± 5**
Wet heart weight/ body weight (mg/g)	4.08 ± 0.15	4.24 ± 0.11	6.12 ± 0.7*	5.55 ± 0.19*
Mean blood pressure (mm Hg)	128 ± 7.5	102 ± 9.0	143 ± 4.0*	98.3 ± 4.5**
Heart rate (Beats/min)	320 ± 15	340 ± 10	253 ± 8*	310 ± 27**

\* Significantly different from controls (P < 0.05)

\*\* Significantly different from diabetic controls (P < 0.5)

**Table 2: Effects of Nitrendipine on Various Biochemical Parameters in Control and Diabetic Rats**

Parameter	Control (n = 6)	Nitrendipine Control (n = 6)	Diabetic Control (n = 6)	Diabetic Treated (n = 6)
Serum Glucose (mg/dl)	86 + 9.9	149 + 25.5	339.3 + 8.9*	329 + 68*
Serum Insulin (uU/ml)	41.2 + 6.9	40.7 + 8.1	15.38 + 1.8*	21.1 + 2.4
Serum Cholesterol (mg/dl)	83.6 + 5.0	77.35 + 10.3	129.8 + 17.7*	51.7 + 9.0**
Serum T <sub>3</sub> (ng/dl)	1.66 + 0.2	1.18 + 0.1	0.64 + 0.07	0.68* + 0.08*
Serum Creatinine (mg/dl)	2.90 + 0.19	2.68 + 0.09	3.07 + 0.02*	2.86 + 0.02**
Serum SGPT (U/ml)	31.32 + 5.9	37.89 + 5.5	53.5 + 6.6*	44.1 + 7.1*

\* Significantly different from control (P < 0.05)

\*\* Significantly different from diabetic control (P < 0.5)

STZ-induced diabetic rats exhibited significant glucosuria (> 2%) from the second day onward. There was a significant reduction in glucose levels after nitrendipine treatment. This was not associated with increase in insulin levels. In control non-diabetic rats also similar results were obtained. These results are identical to those reported with nifedipine.<sup>14</sup> The effect of calcium channel blockers on glucose tolerance and insulin release has been a subject of controversy. Calcium ions play an important role in both the phases of glucose-induced release of insulin from the beta cells of pancreas. There is a possibility that calcium channel blockers may decrease the release of insulin and thereby the glucose tolerance in normal and diabetic individuals.<sup>12-13</sup> However, some of the workers did not observe any significant change in glucose tolerance or insulin level in diabetic and non diabetic individuals after treatment with nifedipine.<sup>18</sup> As far as nitrendipine is concerned, five years of antihypertensive therapy has not been found to alter carbohydrate homeostasis in diabetic patients.<sup>19-20</sup> The results of the present investigation indicate that nitrendipine does not cause any change or sensitivity to insulin as reported for nifedipine.<sup>14</sup>

There are several reports on the genesis of hypertension in STZ-induced diabetes mellitus rat model. Kawashima *et al*<sup>21</sup> were the first to report that rats treated with STZ developed hypertension. Since then many other workers have observed raised BP in STZ treated rats.<sup>22,23</sup> In the present study we observed similar changes in BP. Nitrendipine treatment could prevent the rise in BP in the diabetic treated animals (**Table 1**).

The heart rate of diabetic rats was found to be significantly less as compared to control rats. Nitrendipine treatment prevented the bradycardia seen in STZ-diabetic rats. Although the underlying mechanism remains obscure, in the short term, it is possible that bradycardia results from an increase in vagal activity and/or enhanced sensitivity to the chronotropic effects of acetylcholine.<sup>24</sup> The decrease in heart rate has also been attributed to the decrease

in the number of beta receptors that have been found in the hearts of STZ diabetic animals. Another possible mechanism for the bradycardia is the hypothyroid state of the animals.<sup>25</sup> Hypothyroidism has been reported to cause a decrease in responses to beta-adrenoceptors<sup>26</sup> and an increase in alpha-adrenoceptor.<sup>27</sup> In the present study, the improvement in heart rate in diabetic treated rats despite the unaltered hypothyroid status shows that besides hypothyroidism, metabolic changes which are the sequel of the diabetic state such as hyperglycemia, ketosis, weight loss or other factors may also play an important role in the genesis of bradycardia.

Hypothyroidism and diabetes also share in common, a number of alterations such as reduction in left ventricular contractility and relaxation rate,<sup>28</sup> a reduction in calcium binding and uptake by the sarcoplasmic reticulum and a decrease in myosin and actomyosin calcium ATPase activity.<sup>5</sup> Indeed, in the present study we found that hypothyroid diabetic rats showed cardiac depression at higher filling pressures. Histological studies also revealed that STZ-induced diabetes caused a degeneration of fibres, loss of fibre intactness and vacuolization. Nitrendipine treatment of diabetic rat could prevent this derangement only partially, but along with hypothyroid status, cardiac depression at higher filling pressures remain unimproved. It has been suggested that the occurrence of an intracellular calcium overload may result in the development of a cardiomyopathy. It is associated with depletion of high energy phosphate stores and derangement of cardiac dysfunction. The partial reversal of cardiomyopathy by nitrendipine treatment could be explained by the virtue of its ability to reduce intracellular calcium overload because of its calcium channel blockade.

In addition to hypothyroidism, alterations in lipid metabolism is another possible mechanism involved in cardiac depression by modifying the structure of cardiac plasma membrane and subcellular membrane. In the present investigation nitrendipine treatment significantly reduced serum cholesterol level

in diabetic rats as compared to diabetic controls. The proposed hypothesis is that they act by preventing the generation of plasma borne cytotoxic compounds, which are probably free radicals. This might serve to protect native low density lipoprotein within the arterial intima from undergoing the oxidative modification that in turn seems to be important in the development of atherosclerotic lesions.<sup>29</sup> Calcium antagonists may prevent endothelial damage and thus prevent the progression of atherosclerosis. This might be accountable for the partial improvement in diabetic cardiomyopathy seen with nitrendipine treatment.

In conclusion, chronic treatment with nitrendipine could prevent STZ-induced hypercholesterolemia and bradycardia. It could not prevent however, loss of body weight and depressed heart functions although cardiomyopathy was partially prevented. Our data suggests beneficial role of nitrendipine in STZ-induced metabolic alterations.

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