

## Comparative evaluation of spironolactone, atenolol, metoprolol, ramipril and perindopril on diabetes-induced cardiovascular complications in type 1 diabetes in rats

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### Abstract

The present study was carried out to study the effect of spironolactone, atenolol, metoprolol, ramipril and perindopril on cardiovascular complications associated with type 1 diabetes mellitus in rats. Single tail vein injection of 45 mg/kg streptozotocin (STZ) produced type 1 diabetes in Wistar rats of either sex. Spironolactone (SL; 20mg/kg/day), atenolol (10mg/kg/day), metoprolol (10mg/kg/day), ramipril (1mg/kg/day) and perindopril (1mg/kg/day) were administered for 6 weeks after which various biochemical and cardiac parameters were measured. STZ produced hyperglycemia, hypoinsulinemia, dyslipidemia, hypertension, bradycardia and cardiac hypertrophy. Chronic treatment with only metoprolol produced a reduction in glucose and spironolactone and metoprolol significantly decreased insulin levels. Spironolactone, ramipril and perindopril significantly reduced cholesterol levels. Spironolactone, metoprolol and perindopril significantly reduced triglyceride levels. Spironolactone and perindopril also increased the serum HDL levels. Spironolactone and metoprolol significantly reduced the serum creatinine levels. Blood pressure was controlled by all the drug treatment. However, heart rate and cardiac hypertrophy were controlled only by spironolactone, metoprolol and perindopril treatment. In conclusion, spironolactone, metoprolol and perindopril prevent not only the STZ-induced metabolic abnormalities but also cardiovascular complications and they appear to be beneficial agents as compared to atenolol and ramipril.

**Key words:** *Type 1 diabetes mellitus, cardiovascular complications, anti-hypertensives*

### Introduction

Over the last three decades, a number of epidemiological, clinical and autopsy studies have proposed the presence of diabetic heart disease, as a distinct clinical entity.<sup>1</sup> Hypertension is an extremely common comorbid condition in diabetes affecting approximately 20-60% of patients with diabetes, depending on obesity, ethnicity and age.<sup>2</sup> Hypertension is approximately twice as frequent in patients with diabetes as compared with patients without the disease.<sup>3</sup> Diabetic patients develop congestive cardiac failure more readily and have significantly worse prognosis than their non-diabetic counterparts once they develop coronary disease.<sup>4,5</sup> Thus controlling blood pressure in diabetics is positively more beneficial as far as progression of diabetic complications is concerned.

The use of anti-hypertensive therapy such as  $\alpha$ - and  $\beta$ -adrenoceptor blockers, angiotensin converting enzyme (ACE) inhibitors, calcium channel blockers, vasodilators and diuretics may provide some benefit in reducing blood pressure and coronary artery disease.

Spironolactone is a mineralocorticoid receptor antagonist

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and is reported to reverse cardiac fibrosis in both left and right ventricles.<sup>6</sup> It could be helpful in preventing cardiac remodelling in an inflammatory cardiomyopathy, as in the case of Chagas' cardiomyopathy, reducing mortality in the chronic phase and reducing inflammatory infiltration.<sup>7</sup> Randomized Aldactone Evaluation Study (RALES), involving 1663 patients with moderately severe or severe congestive heart failure and receiving a low dose of spironolactone (25mg/day) in addition to standard therapy, showed that spironolactone substantially reduces the risk of both morbidity and death among patients with severe heart failure.<sup>8</sup> Beta blockers have been convincingly shown to reduce total and cardiovascular morbidity and mortality in hypertensive diabetic patients. After myocardial infarction these agents confer a twice as high protective effect when compared to non-diabetic patients.<sup>9</sup>  $\beta$ -blocker use improves outcomes even more for the patient with diabetes mellitus than for the patient without diabetes with a history of acute myocardial infarction or coronary artery disease.<sup>10</sup> However, most paradoxically, beta blocking agents are used less frequently in diabetes. ACE inhibitors seem to be metabolically neutral in hypertensive diabetics.<sup>11</sup> It has been shown that enalapril improve lipid profile in hypertensive subjects<sup>12</sup> and in streptozotocin diabetic rats.<sup>13</sup> Perindopril is reported to produce an improvement in insulin sensitivity, prevent dyslipidaemia and cardiac dysfunctions associated with STZ-diabetes in Wistar and SH rats.<sup>14</sup>

Despite these, the choice of anti-hypertensive depends and varies from clinician to clinician. The choice of anti-hypertensives, especially in diabetic population needs to be

considered in context of providing cardioprotection. Hence, in present investigation we have carried out comparative evaluation of spironolactone, atenolol, metoprolol, ramipril and perindopril on cardiovascular complications associated with STZ-induced type 1 diabetic rats.

### Material and methods

The protocol of the experiment was approved by our institutional animal ethical committee as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (Protocol No. IPS/PCOL/PhdD08/003 dated on 8<sup>th</sup> March 2008).

Wistar rats of either sex weighing 180-220 g were made diabetic by single tail vein injection of STZ (45mgkg<sup>-1</sup>) dissolved in 0.1mol/lit citrate buffer. Control rats were injected with 0.1mol/lit citrate buffer alone. The induction of diabetes was checked 48h after the STZ injection by measuring the extent of glycosuria with Diastix (Bayers Diagnostics Ltd, New Delhi, India). Rats displaying glycosuria >2% were considered as diabetic. The rats were then randomly divided into eight groups as follows:

- Control (CON),
- Control treated with spironolactone (COS),
- Control treated with atenolol (COA),
- Control treated with metoprolol (COM),
- Control treated with ramipril (COR)
- Control treated with perindopril (COP)
- Diabetic control (DIC),
- Diabetics treated with spironolactone (DIS),
- Diabetics treated with atenolol (DIA),
- Diabetics treated with metoprolol (DIM),
- Diabetics treated with ramipril (DIR),
- Diabetics treated with perindopril (DIP),

The drugs were dissolved in distilled water and were administered orally (*per os*) at the doses mentioned in Table 1.

Animals had free access to food and water *ad libitum*. At the end of 6 weeks of the treatment, animals were fasted for 12h and blood samples were collected from the retroorbital plexuses. The serum was separated and analyzed for glucose, cholesterol, triglycerides and creatinine, spectrophotometrically using available biochemical diagnostic kits. Serum insulin was estimated by radioimmunoassay technique using kits obtained from Board of Radiation and Isotope Technology, Mumbai, India and gamma counter (Packard, USA). Blood pressure and heart rate were recorded by carotid artery cannulation using iworx 118 data acquisition system. After recording blood pressure and heart rate and withdrawal of blood samples from retro-orbital plexus, animals were sacrificed, hearts

**Table 1:** Drug dosage

Drug	Dose
Spironolactone	20 mg/kg/day
Atenolol	10 mg/kg/day
Metoprolol	10 mg/kg/day
Ramipril	1 mg/kg/day
Perindopril	1 mg/kg/day

were excised, extraneous tissues were separated and wet weight of the entire heart was noted down to calculate the index of cardiac hypertrophy as wet heart weight to body weight ratio. Results are presented as Mean  $\pm$  SEM. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey's test. Data were considered statistically significant at *p* value <0.05.

### Results

#### General features of experimental rats

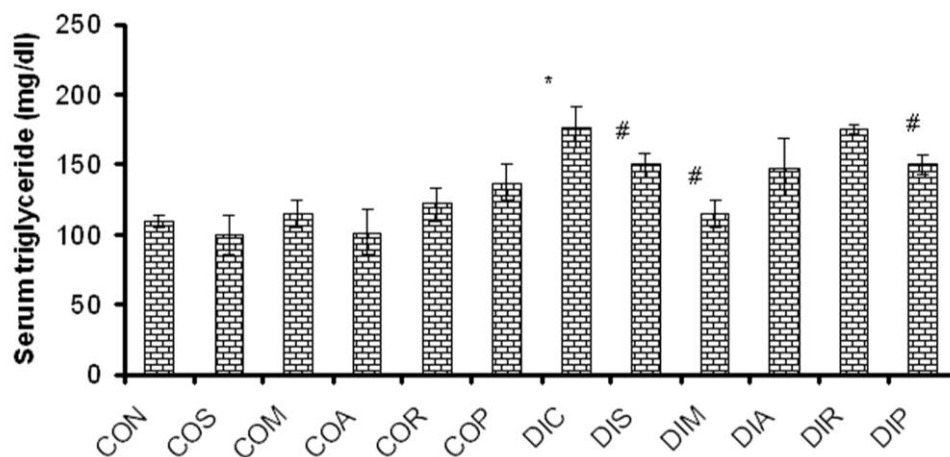
Injection of streptozotocin (45 mg/kg) into rats produced glucosuria (>2%) in all the animals. No glucose was detectable in the urine of control animals. Diabetic rats showed a loss of body weight, polyphagia, and polydipsia. Chronic treatment with spironolactone, metoprolol, atenolol and perindopril failed to prevent loss of body weight in STZ-diabetic rats. Treatment with ramipril however, prevented the loss of body weight. Metoprolol and atenolol did not reduce the elevated food and water intake in STZ-diabetic rats while spironolactone, ramipril and perindopril reduced the elevated food and water intake in STZ-diabetic rats (Table 2).

#### Biochemical parameters of the experimental rats

STZ-diabetic rats were found to exhibit significant hyperglycemia as compared to control rats. Treatment with metoprolol produced significant decrease in elevated serum glucose levels whereas treatment with spironolactone, atenolol, ramipril or perindopril did not produce any significant effect on serum glucose levels (Table 3). Hyperglycemia was associated with significant hypoinsulinemia in STZ-diabetic rats as compared to control rats. Chronic treatment with spironolactone and metoprolol produced a significant increase in decreased serum insulin levels. The treatment with atenolol, ramipril or perindopril did not produce a significant increase in decreased serum insulin levels (Figure 1).

STZ-diabetic rats exhibited significantly higher cholesterol and triglycerides levels as compared to those of control rats. Chronic treatment with spironolactone, ramipril and perindopril could significantly reduce the elevated cholesterol levels in diabetic rats. Chronic treatment with spironolactone, ramipril and perindopril significantly reduced the elevated cholesterol levels in diabetic rats. A significant reduction in HDL-cholesterol levels was observed in STZ-diabetic rats as compared to control rats and chronic treatment with spironolactone significantly elevated the reduced HDL-cholesterol levels in diabetic animals. Moreover, STZ-diabetic rats show elevated triglyceride levels which by chronic treatment with spironolactone, metoprolol and perindopril were reduced significantly (Figure 2). Further, treatment of control rats with all of these agents did not produce any significant effects on the lipid profile of the animals (Table 3).

STZ-diabetic rats exhibited significantly higher serum creatinine levels as compared to those of control rats. Chronic treatment with spironolactone and metoprolol significantly reduced the elevated creatinine levels of diabetic rats (Table 3).



**Figure 1:** Effect of spironolactone, atenolol, metoprolol, ramipril and perindopril on serum insulin levels. Each bar represents mean  $\pm$  SEM of six experiments. \*- significantly different from control ( $p < 0.05$ ) #- significantly different from diabetic control ( $p < 0.05$ ) Values are expressed as Mean  $\pm$  S.E.M,  $n=6$  in each group. Control (CON), Control treated with spironolactone (COS), Control treated with atenolol (COA), Control treated with metoprolol (COM), Control treated with ramipril (COR), Control treated with perindopril (COP), Diabetic control (DIC), Diabetics treated with spironolactone (DIS), Diabetics treated with atenolol (DIA), Diabetics treated with metoprolol (DIM), Diabetics treated with ramipril (DIR), Diabetics treated with perindopril (DIP)

**Table 2:** Effect of spironolactone, metoprolol, atenolol, ramipril and perindopril on general features of the animals

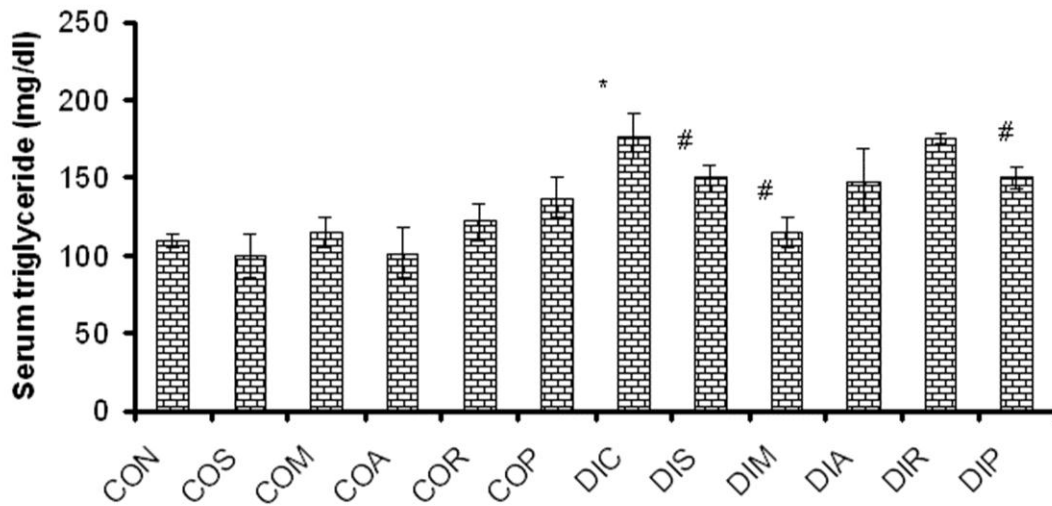
Parameter	CON (n=6)	COS (n=6)	COM (n=6)	COA (n=6)	COR (n=6)	COP (n=6)	DIC (n=6)	DIS (n=6)	DIM (n=6)	DIA (n=6)	DIR (n=6)	DIP (n=6)
Body weight After treatment (g)	302.5 $\pm$ 24.5	295.9 $\pm$ 4.96	291.3 $\pm$ 19.7	274.4 $\pm$ 5.6	293.4 $\pm$ 10.6	289.3 $\pm$ 6.7	183.8 $\pm$ 13.6 *	192.88 $\pm$ 6.95	195 $\pm$ 22.8	146.9 $\pm$ 9.8	245.67 $\pm$ 10.5#	201.36 $\pm$ 12.6
Food intake (gm/animal/day)	17.4 $\pm$	16.42 $\pm$ 0.81	18.4 $\pm$ 0.5	17.9 $\pm$ 0.4	18.2 $\pm$ 1.6	17.52 $\pm$ 2.1	26.5 $\pm$ 0.6 *	21.37 $\pm$ 1.20#	27.6 $\pm$ 1.0	27.4 $\pm$ 1.3	19.33 $\pm$ 1.2#	18.91 $\pm$ 4.16#
Water intake (ml/animal/day)	39.2 $\pm$	52.71 $\pm$ 1.52	36.2 $\pm$ 0.9	35.6 $\pm$ 5.3	34.3 $\pm$ 6.1	40.6 $\pm$ 1.94	89.1 $\pm$ 3.2 *	65.89 $\pm$ 1.55#	98.8 $\pm$ 3.1	89.4 $\pm$ 2.4	65.24 $\pm$ 3.2#	55.35 $\pm$ 5.2#

significantly different from control ( $p < 0.05$ ); #- significantly different from diabetic control ( $p < 0.05$ ); Values are expressed as Mean  $\pm$  S.E.M,  $n=6$  in each group; Control (CON); Control treated with spironolactone (COS); Control treated with atenolol (COA); Control treated with metoprolol (COM); Control treated with ramipril (COR); Control treated with perindopril (COP); Diabetic control (DIC); Diabetics treated with spironolactone (DIS); Diabetics treated with atenolol (DIA); Diabetics treated with metoprolol (DIM); Diabetics treated with ramipril (DIR); Diabetics treated with perindopril (DIP)

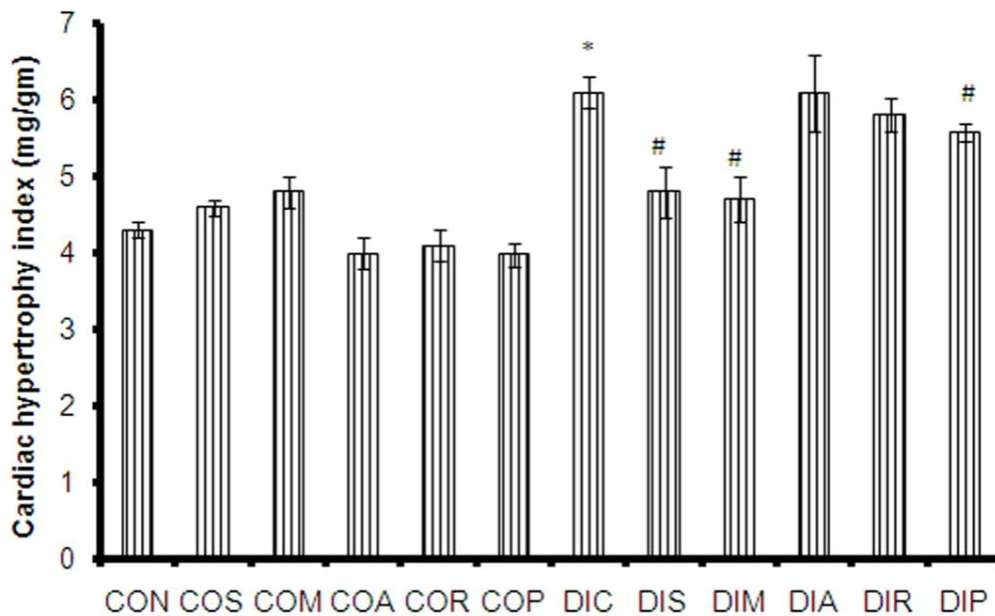
**Table 3:** Effect of spironolactone, metoprolol, atenolol, ramipril and perindopril on biochemical parameters of the animals

Parameter	CON (n=6)	COS (n=6)	COM (n=6)	COA (n=6)	COR (n=6)	COP (n=6)	DIC (n=6)	DIS (n=6)	DIM (n=6)	DIA (n=6)	DIR (n=6)	DIP (n=6)
Serum glucose (mg/dl)	97.3 $\pm$ 8.4	76.62 $\pm$ 5.89	86.6 $\pm$ 11.9	110.2 $\pm$ 2.7	93.26 $\pm$ 4.6	85.64 $\pm$ 2.3	439.0 $\pm$ 13.2	354.95 $\pm$ 34.26	365.0 $\pm$ 29.9#	445.6 $\pm$ 0.6	428.3 $\pm$ 15.3	350.2 $\pm$ 11.0
Serum cholesterol (mg/dl)	47.8 $\pm$ 2.7	42.70 $\pm$ 4.99	46.2 $\pm$ 2.4	49.4 $\pm$ 4.1	50.1 $\pm$ 5.12	54.2 $\pm$ 1.11	64.1 $\pm$ 3.3*	43.25 $\pm$ 3.08#	61.5 $\pm$ 3.0	66.2 $\pm$ 9.2	45.3 $\pm$ 10.3#	46.73 $\pm$ 6.55#
HDL-Cholesterol (mg/dl)	48.8 $\pm$ 4.3	35.71 $\pm$ 2.18	47.2 $\pm$ 5.6	48.2 $\pm$ 5.5	41.3 $\pm$ 3.84	50.32 $\pm$ 5.6	39.6 $\pm$ 2.2*	46.40 $\pm$ 1.87#	37.6 $\pm$ 2.7	38.7 $\pm$ 3.0	38.92 $\pm$ 2.98	47.32 $\pm$ 2.5
Serum Creatinine (mg/dl)	1.6 $\pm$ 0.1	1.28 $\pm$ 0.27	1.6 $\pm$ 0.1	1.6 $\pm$ 0.18	1.76 $\pm$ 0.2	1.87 $\pm$ 0.16	2.6 $\pm$ 0.3*	1.67 $\pm$ 0.13#	1.6 $\pm$ 0.2#	2.2 $\pm$ 0.24	2.4 $\pm$ 0.1	2.4 $\pm$ 0.45

\*- significantly different from control ( $p < 0.05$ ); #- significantly different from diabetic control ( $p < 0.05$ ); Values are expressed as Mean  $\pm$  S.E.M,  $n=6$  in each group; Control (CON); Control treated with spironolactone (COS); Control treated with atenolol (COA); Control treated with metoprolol (COM); Control treated with ramipril (COR); Control treated with perindopril (COP); Diabetic control (DIC); Diabetics treated with spironolactone (DIS); Diabetics treated with atenolol (DIA); Diabetics treated with metoprolol (DIM); Diabetics treated with ramipril (DIR); Diabetics treated with perindopril (DIP)



**Figure 2:** Effect of spironolactone, atenolol, metoprolol, ramipril and perindopril on serum triglyceride levels. Each bar represents mean  $\pm$  SEM of six experiments. \*- significantly different from control ( $p < 0.05$ ) #- significantly different from diabetic control ( $p < 0.05$ ) Values are expressed as Mean  $\pm$  S.E.M, n=6 in each group. Control (CON), Control treated with spironolactone (COS), Control treated with atenolol (COA), Control treated with metoprolol (COM), Control treated with ramipril (COR), Control treated with perindopril (COP), Diabetic control (DIC), Diabetics treated with spironolactone (DIS), Diabetics treated with atenolol (DIA), Diabetics treated with metoprolol (DIM), Diabetics treated with ramipril (DIR), Diabetics treated with perindopril (DIP).



**Figure 3:** Effect of spironolactone, atenolol, metoprolol, ramipril and perindopril on cardiac hypertrophy index. Each bar represents mean  $\pm$  SEM of six experiments. \*- significantly different from control ( $p < 0.05$ ) #- significantly different from diabetic control ( $p < 0.05$ ) Values are expressed as Mean  $\pm$  S.E.M, n=6 in each group. Control (CON), Control treated with spironolactone (COS), Control treated with atenolol (COA), Control treated with metoprolol (COM), Control treated with ramipril (COR), Control treated with perindopril (COP), Diabetic control (DIC), Diabetics treated with spironolactone (DIS), Diabetics treated with atenolol (DIA), Diabetics treated with metoprolol (DIM), Diabetics treated with ramipril (DIR), Diabetics treated with perindopril (DIP)

**Table 4:** Effect of spironolactone, metoprolol, atenolol, ramipril and perindopril on cardiovascular parameters of the animals

Parameter	CON (n=6)	COS (n=6)	COM (n=6)	COA (n=6)	COR (n=6)	COP (n=6)	DIC (n=6)	DIS (n=6)	DIM (n=6)	DIA (n=6)	DIR (n=6)	DIP (n=6)
Heart rate (beats/min)	348 ± 4.2	295.9 ± 4.96	331 ± 16.4	342 ± 6.7	351 ± 9.8	367 ± 10	206 ± 26.4	287 ± 6.2#	282 ± 12.2#	178 ± 11.4	203 ± 5.8	297 ± 10#
Blood pressure (mmHg)	102 ± 2.8	111 ± 4.2	99 ± 4.7	97 ± 3.3	96 ± 3.6	92 ± 3.4	221 ± 15.8	112 ± 5.1#	110 ± 5.9#	143 ± 4.8#	117.4 ± 5.2#	180 ± 5.72#

\*- significantly different from control ( $p < 0.05$ ); #- significantly different from diabetic control ( $p < 0.05$ ); Values are expressed as Mean  $\pm$  S.E.M,  $n=6$  in each group; Control (CON), Control treated with spironolactone (COS), Control treated with atenolol (COA), Control treated with metoprolol (COM); Control treated with ramipril (COR); Control treated with perindopril (COP); Diabetic control (DIC); Diabetics treated with spironolactone (DIS); Diabetics treated with atenolol (DIA); Diabetics treated with metoprolol (DIM); Diabetics treated with ramipril (DIR); Diabetics treated with perindopril (DIP)

#### **Hemodynamic parameters and hypertrophy index**

Heart rate was found to be significantly lower in diabetic rats as compared to controls. Chronic treatment with spironolactone, metoprolol and perindopril in diabetic rats exhibited significant increase in heart rate as compared to diabetic control animals. The mean blood pressure was significantly increased after six weeks study in diabetic rats as compared to control rats. Treatment with spironolactone, metoprolol, atenolol, ramipril and perindopril prevented the STZ-induced increase in blood pressure in diabetic animals (Table 4).

The ratio of heart weight to body weight which is a measure of cardiac hypertrophy was significantly higher diabetics as compared to those of control rats. Chronic treatment with spironolactone, metoprolol and perindopril significantly reduced the elevated cardiac hypertrophy index while atenolol and ramipril did not reduce the cardiac hypertrophy index (Figure 3).

#### **Discussion**

In the present investigation STZ produced cardinal signs and characteristics of diabetes viz polyphagia, polyuria, polydipsia, hyperglycemia, hypoinsulinemia, dyslipidemia and cardiovascular alterations like bradycardia, hypertension and hypertrophy of heart which are consistent with those reported earlier.<sup>14</sup> Chronic treatment with spironolactone, metoprolol and atenolol did not prevent the loss of body weight, polyuria, polyphagia or polydipsia in STZ-diabetic rat. Chronic treatment with ramipril and perindopril did not prevent the loss of body weight; however, they reduced the elevated food intake and water intake of the animals.

Intravenous injection of STZ produces fragmentation of DNA of  $\beta$ -cells of pancreas which stimulates poly (ADP-ribose) and depletes NAD ultimately leading to destruction of  $\beta$ -cells and it is evidenced by clinical symptoms of hyperglycemia and hypoinsulinaemia.<sup>15,16</sup> There exists a direct relationship between aldosterone and insulin resistance and in patients with hypertension, this relationship might contribute to maintenance of high blood pressure and increased cardiovascular risk.<sup>17</sup> In present study, STZ produced a significant increase in glucose levels associated with decrease in insulin levels in type I diabetic rats. Treatment with spironolactone significantly reduced the serum glucose levels. It also produced significant

elevation in the serum insulin levels of STZ-diabetic rats. Without affecting insulin sensitivity, spironolactone is reported to increase basal and insulin-stimulated glucose uptake in cultured in vitro-differentiated adipocytes.<sup>18</sup> Thus, reduction in the serum glucose levels may be attributed to increase in glucose uptake. However, further studies are required to be carried out to find out precise mechanism of action. Treatment with metoprolol could reduce the serum glucose levels to some extent while treatment with atenolol produced a slight increase but it was not significant. Metoprolol treatment could elevate the serum insulin levels of diabetic rats while atenolol did not produce any significant effect on the serum insulin levels. These results show that metoprolol possesses a beneficial effect over atenolol on the glycemic status of diabetic hypertensive rats. However, it could further prove hazardous by producing hypoglycemia if given along with other antidiabetic drugs or exogenous insulin in case of type 1 diabetes. Further investigations are required to elaborate the mechanism underlying this effect of metoprolol. Ramipril neither reduced the serum glucose levels nor elevated the serum insulin levels while perindopril significantly reduced the serum glucose levels without increase in insulin levels. Numerous published trials show that perindopril has either a neutral or, in many cases, a positive effect on insulin sensitivity, glycaemic control and/or glucose metabolism in patients with hypertension and varying degrees of insulin resistance.<sup>19-22</sup> The improvement in insulin sensitivity might be the possible mechanism for reduction in glucose levels in spite of low levels of insulin.

It has been reported that in STZ-diabetic rats, insulin deficiency is associated with hypercholesterolemia and hypertriglyceridemia.<sup>16</sup> A low level of plasma high-density lipoprotein cholesterol (HDL-C) is one component of a cluster of coronary disease risk factors that also includes abdominal obesity, hypertension, hyperinsulinemia, and insulin resistance.<sup>23</sup> Aldosterone levels are relatively high in subjects with the coronary disease risk factor cluster<sup>24</sup> and unesterified fatty acids can inhibit aldosterone secretion in vitro.<sup>25</sup> In the present investigation serum cholesterol and triglyceride levels of diabetic rats were found to be significantly decreased by the treatment with spironolactone. Extensive trials suggest that non selective beta blockers without intrinsic sympathomimetic activity increase serum triglyceride levels and decrease high density

lipoprotein levels.<sup>26</sup> There are however, controversial reports regarding the effects of selective beta adrenoceptor blockers metoprolol and atenolol.<sup>27</sup> In the present investigation it was interesting to note that metoprolol produced a decrease in triglycerides in STZ-diabetic rats while treatment with atenolol in diabetic rats produced a only a slight reduction in the triglyceride levels. Thus, metoprolol could have beneficial effect on heart inspite of no reduction in cholesterol levels. It is possible that with the blockade of  $\beta$ -adrenoceptors, the unopposed alpha activity might result in the increase in LDL (low density lipoproteins). An increase in LDL-cholesterol levels has also been demonstrated with the treatment with beta blockers.<sup>28</sup> Ramipril treatment significantly reduced cholesterol levels but did not improve the HDL and triglyceride levels in diabetic animals. However, perindopril improved the cholesterol levels as well as the HDL and triglyceride levels in diabetic animals. Thus perindopril appears to be more beneficial than ramipril as far as the improvement in lipid profile is concerned. Data from the Framingham study suggested that high plasma triglycerides is one of the risk factors for coronary heart disease in women<sup>29</sup> and in case of men, it is the risk factor when hyperlipidaemia is associated with low levels of HDL-cholesterol concentration.<sup>30</sup> Thus lowering of cholesterol and triglyceride levels with increase in the HDL-cholesterol by spironolactone and perindopril may produce beneficial effects in coronary heart diseases.

Rats treated with STZ also develop changes in renal function including altered renal hemodynamics and structural changes which can be attributed to the development of diabetes. In the present investigation, we found significant elevation in the serum creatinine levels in diabetic rats and treatment with spironolactone reduced the elevated serum creatinine levels. Spironolactone is reported to prevent diabetic nephropathy by down-regulation of the genes that are involved in the inflammatory process, such as monocyte chemotactic peptide-1, migration inhibitory factor, the Tumor Necrosis Factor and interleukins and its receptor, TGF- $\beta$ , matrix metalloproteinase and vascular endothelial growth factor.<sup>31</sup> Metoprolol treatment in diabetic group prevented this rise in serum creatinine levels while atenolol did not produce any effect. Perindopril improved the creatinine levels while ramipril did not produce any effect on the creatinine levels. Hence, spironolactone, metoprolol and perindopril could be beneficial in providing some protection against diabetic nephropathy. However, further studies are required to prove their efficacy in diabetic nephropathy.

Increase in blood pressure after treatment with STZ has been reported by several workers.<sup>32,33</sup> In our study also, blood pressure of STZ-diabetic animals was found to be significantly higher as compared to non-diabetic animals. Spironolactone, metoprolol, atenolol, ramipril and perindopril prevented the rise in blood pressure in diabetic animals.

Bradycardia is frequently observed in STZ diabetic rats.<sup>34</sup> In our study, also heart rate of STZ-diabetic animals was found

to be significantly lower in diabetic rats as compared to non-diabetic. Spironolactone treatment in STZ-diabetic rats exhibited a significant increase in heart rate which was near to normal. Chronic aldosterone administration depresses baroreceptor and baroreflex functions without inducing hypertension,<sup>35</sup> blunts human baroreflex response<sup>36,37</sup> and potentiates the effect of catecholamines.<sup>38</sup> Our reports are in consistence with others who have reported that spironolactone can prevent this aldosterone-induced baroreceptor and baroreflex depression and improves heart rate and heart rate variability.<sup>39</sup> Metoprolol treatment exhibited an increase in heart rate but was found to be still quite lower than normal rats while atenolol treatment produced a further reduction in heart rate. Beta blockers, especially lipophilic ones, are reported to up regulate the cardiac  $\beta_1$  receptors and also inhibit the stimulatory auto  $\beta_1$  receptor auto antibodies. This may be a possible mechanism responsible for the beneficial effect of metoprolol in diabetes induced cardiac dysfunction. Ramipril produced an increase in heart rate but it was not significant where as perindopril produced a significant increase in the heart rate. Thus, perindopril appears to be better than ramipril in improving the bradycardia.

Data from Framingham study indicate that, left ventricular hypertrophy (LVH) is not a benign compensatory process but an independent risk factor for congestive heart failure, coronary artery disease, and sudden death.<sup>40,41</sup> In the presence of diabetes, in hypertensive subjects, damage to the myocardium due to hypertension appears to be accelerated. Diabetic hypertensive patients have greater interventricular septum and posterior wall thickness than non-diabetic hypertensive patients. Consequently, left ventricular mass index may be greater in patients with hypertension and diabetes mellitus than in those without diabetes mellitus.<sup>42</sup> In the present study, the wet heart weight to body weight ratio, an index of cardiac hypertrophy, was found to be increased in diabetic hearts. Spironolactone treatment significantly reduced the cardiac hypertrophy and left ventricular hypertrophy in treated animals. A peripheral infusion of aldosterone is reported to cause cardiac hypertrophy and fibrosis without increasing the blood pressure in rats.<sup>43</sup> Further, spironolactone prevents myocardial fibrosis<sup>44</sup> and late cardiac remodeling after ventricular restoration surgery post myocardial infarction in rats.<sup>45</sup> Metoprolol treatment significantly reduced the cardiac hypertrophy index whereas, treatment with atenolol did not produce any effect in diabetic animals. Hence, while atenolol seems to worsen diabetes induced cardiomyopathy, metoprolol appears to delay the progression the congestive heart failure in diabetic condition possibly by preventing development of cardiomyopathy and hypertrophy. Perindopril treatment also significantly reduced the cardiac hypertrophy index in diabetic animals while ramipril did not produce any improvement in cardiac hypertrophy. It has been reported that ramipril therapy reduced cardiac fibrosis and left ventricular hypertrophy (LVH) and improves post-infarction survival and LV function in spontaneously hypertensive rats (SHR).<sup>46</sup> Additionally ramipril induced regression of LVH in patients with type 2 diabetes mellitus

but without hypertension or albuminuria in a double-blind, placebo controlled trial.<sup>47</sup> Hence it might be possible that ramipril improves the cardiac hypertrophy in hypertension but not in presence of diabetes. In rats with streptozotocin-induced diabetes, perindopril attenuated mesenteric vascular hypertrophy and it was suggested that this antitrophic effect resulted from inhibition of transforming growth factor- $\beta$  expression.<sup>48</sup> All these results further support the contention that spironolactone, metoprolol and perindopril are beneficial in STZ-induced cardiovascular dysfunction which are in consistence with our previous reports.<sup>49,50,51</sup>

In conclusion, our data suggests that spironolactone, metoprolol and perindopril prevent not only the STZ induced metabolic abnormalities but also cardiovascular complications as evident from the reduction in cholesterol, triglyceride and decrease in cardiac hypertrophy which are the initial symptoms of congestive heart failure. Metoprolol and perindopril appears to be beneficial agents as compared to atenolol and ramipril.

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