



RESEARCH ARTICLE

**Evaluation of Ivabradine against Insulin Resistance Syndrome in Experimental
Animals**

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ABSTRACT

Insulin resistance syndrome comprises of high glucose level, hypertension and dyslipidemia. The present study was aimed to evaluate effect of Ivabradine in insulin resistance syndrome in experimental animals. Male Sprague-Dawley rats of 6-8 weeks (150-180g) were randomly allocated based on serum glucose levels in 8 groups (n=6). All groups except normal control were fed high fructose diet (HFD) along with drugs for 28 days. Body weight, food intake, mean blood pressure and serum lipid levels were measured weekly. Blood glucose was measured on every 3rd day. On 28th day, OGTT, serum ions, kidney function markers, antioxidant parameters and histopathology were performed. Statistical analysis was done by ANOVA followed by post hoc Dunnett's test. Feeding HFD to normal rats for 28 days induced insulin resistance (shown by OGTT) and oxidative stress (increased malondialdehyde, decreased catalase, superoxide dismutase and reduced glutathione) leading to hyperglycemia, dyslipidemia (increased triglyceride, total cholesterol, VLDL, LDL), deteriorated kidney function (increased creatinine, albumin and urea) and hypertension (Systolic BP > 130 mm/Hg and Diastolic BP > 80 mm/Hg). Ivabradine therapy prevented HFD induced insulin resistance and oxidative stress thus leading to improved glycemic control, correction of dyslipidemia, better control of blood pressure and reasonably improved kidney function test. The results were supported by histopathology.

KEYWORDS

Fructose, Insulin Resistance, Hypertension, Dyslipidemia, Ivabradine

INTRODUCTION

Diabetes and hypertension are common diseases that coexist at a greater frequency than chance alone would predict. Hypertension affects approximately 70% of patients with diabetes and is approximately twice as common in persons with diabetes as in those without.¹ 60% to 80% of people with diabetes die of

cardiovascular complications and up to 75% of specific cardiovascular complications have been attributed to high blood pressure.²

The mechanisms by which insulin resistance leads to hypertension³⁻⁶ are- increased sympathetic nervous system activity, enhanced renin-angiotensin aldosterone system (RAAS) activity and Angiotensin II (Ang II) levels, increased sodium reabsorption, impaired endothelium-dependent relaxation, altered cellular electrolyte transport and composition and generation of free radicals.

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The current regimen for hypertension in diabetes includes inhibitors of the production of angiotensin II (ACE inhibitors), inhibitors of angiotensin II action (ARBs), and the aldosterone receptor antagonists along with antidiabetic drugs.⁷ Though these drugs tackled diabetes induced hypertension very effectively, there are many side effects associated with regimen.⁷⁻¹⁰

Ivabradine, I_f current inhibitor can be useful to treat the complications associated with diabetes without such side effects.¹¹ The drug blocks whole pathway of aldosterone synthesis starting from gene expression to receptor level.^{11,12} Ivabradine was demonstrated to improve endothelial function.¹³ Pre-clinical data reveal no special hazard for humans, based on conventional studies of safety pharmacology, repeated dose toxicity, genotoxicity, carcinogenic potential.¹¹ Ivabradine is not associated with erectile dysfunction nor is dosage adjustment required in the patient with renal dysfunction and hepatic impairment which is most common problem with present regimen.^{11,14} Thus, the present study was done to investigate the effects of Ivabradine on diabetes induced hypertension.

MATERIALS AND METHOD

Drugs and Preparation of Solutions

Ivabradine, Metformin and Lisinopril pure powders were obtained from Biocon, Sun Pharmaceutical and Berrock Pharmaceuticals respectively.

Drug solutions were prepared freshly everyday by suspending the drug in distilled water. Ivabradine, Metformin and Lisinopril were prepared in stock solution of 10 and 20 mg/kg, 350 mg/kg and 10 mg/kg respectively.

Chemicals and Kits

All the chemicals used in this project were of analytical grade and were obtained from Astron chemicals, Ahmedabad and SD fine chemicals, Mumbai. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained as a pure chemical from Sigma Aldrich. All the biochemical tests were performed using the standard reagent kits

purchased from Coral clinical systems, Goa. Insulin kit was obtained Genxbio Health Sciences Pvt. Ltd, Delhi.

Animals

Healthy male Sprague Dawley rats^{15,16} of 6-8 weeks weighing 150 ± 30 g were used for the study. The animals were housed in a group of 3 rats per cage under well-controlled conditions of temperature ($22 \pm 2^\circ\text{C}$), humidity ($55 \pm 5\%$) and 12hrs/12hrs light-dark cycle. Animals had free access to conventional laboratory diet and distilled water *ad libitum*.

The protocol (No. 1205, dated 24th Nov. 2012) of the experiment was approved by 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.

Experimental Procedure

Groups

Animals were allocated based on serum glucose levels in 8 groups, with n=6 animals in each group, as follows:

- Group I: Normal Control (NC); received rat chow diet and distilled water,
- Group II: Model Control (MC); received HFD for 28 days,
- Group III: Standard I-(Met); received HFD and metformin (350mg/kg; p.o.) for 28 days.
- Group IV: Standard II (Met + Lis); received HFD and a combination of Metformin (350mg/kg; p.o.) and Lisinopril (10mg/kg; p.o.) for 28 days.
- Group V: Test I (Iva 10); received HFD and Ivabradine (10mg/kg; p.o.) for 28 days.
- Group VI: Test II (Iva 20); received HFD and Ivabradine (20mg/kg; p.o.) for 28 days.
- Group VII: Test III (Met + Iva10); received HFD and a combination of Metformin (350mg/kg; p.o.) and Ivabradine (10mg/kg; p.o.) for 28 days.

- Group VIII: Test IV (Met + Iva20); received HFD and a combination of Metformin (350mg/kg; p.o.) and Ivabradine (20mg/kg; p.o.) for 28 days.

Induction of Diabetic Hypertension by HFD

Diabetic Hypertension was induced by feeding a high fructose diet (HFD) for 28 days.¹⁶ The fructose diet¹⁷ consisted of 329g of fructose, 329g corn starch, 188g casein, 1.9 g methionine, 14.1g gelatin, 41.4 g Safflower oil, 37.6g Wheat bran, 9.4g Vitamin Mixture and 49.4g Mineral mixture.

Vitamin mixture consists of 3g thiamine mononitrate, 3g riboflavin, 3.5g pyridoxine, 15 g nicotinamide, 8 g d-calcium pantothenate, 1 g folic acid, 0.1 g d-biotin, 5mg cyanocobalamin, 12.5 mg cholecalciferol, 25mg acetomenaphthone, 0.6g vitamin A acetate, 25g RRR-a-tocopherol acetate, 10 g choline chloride.

Mineral Mixture consists of 30.5g MgSO₄.7H₂O, 65.2g NaCl, 105.2g KCl, 200.2g KH₂PO₄, 38.8g MgCO₃.Mg(OH)₂.3H₂O, 40.0g FeC₆H₅O₇.5H₂O, 512.4g CaCO₃, 0.8g NaF, 0.9g CuSO₄.5H₂O, 0.4g MnSO₄, 0.05g CoNH₃.

The rats were fed fructose diet for 28 days. Food intake was measured daily. Body weight was measured weekly during treatment period.

Biochemical Analysis

Serum glucose, total cholesterol (TC), triglyceride (TG) and HDL-cholesterol (HDL), sodium, potassium, creatinine, urea were estimated by kits. Malondialdehyde (MDA), Superoxide dismutase (SOD), reduced glutathione (GSH) and Catalase¹⁸ levels were estimated in liver homogenate.

Measurement of Blood Pressure¹⁹

Blood Pressure was measured in all conscious rats using the indirect tail-cuff method on a 37°C preheated plate every week for 28 days. The mean of three consecutive readings was recorded. The rats were preconditioned to the experimental procedure before actual measurement was conducted. (Biopac Instrument; MP36)

Oral Glucose Tolerance Test²⁰

Oral glucose tolerance test (OGTT) was performed on 16 h fasted albino rats using 1.5 g glucose/kg body weight fed orally (dissolved in water for injection) through a cannula fitted needle attached to a syringe. Blood samples were collected from the animals by retro-orbital plexus at 0, 30, 60 and 90 min after glucose load. Blood glucose and blood insulin levels were measured using kits. Insulin resistance was further confirmed by HOMA-IR (Homeostatic Model Assessment)^{21,22} and QUICKI (Quantitative Insulin Sensitivity Check Index).²²

DPPH Activity²³

Quantitative measurement of radical scavenging assay was done using different concentrations of Ivabradine ranging from 5mg/ml to 25mg/ml. The commercial known antioxidant, ascorbic acid was used for comparison (positive control). The DPPH solution in the absence of drug was used as control and the 80% methanol was used as blank. Discoloration was measured at 517 nm by using spectrophotometer (UV-1601 Shimadzu, Japan) after incubation for 30 min in the darkroom. The percentage of the DPPH free radical was calculated by following formula:

$$DPPH \text{ scavenging effect (\%)} = ((A_0 - A_1) / A_0) \times 100$$

Where A₀=absorbance of the control

A₁=absorbance in the presence of the drug solution.

The actual decrease in absorption induced by the test was compared with the positive controls.

Histopathological Analysis¹⁸

Kidney and pancreas were isolated immediately and samples were prepared for histopathological assessment.

Statistical Analysis

The statistical difference between the means of the various groups were analysed using one way analysis of variance (ANOVA) followed by Dunnett's post hoc test with P value <0.05. In all figures and tables, results are presented as mean ± SEM (N=6); Significant values were

compared with #P<0.05 normal control vs. model control *P<0.05 model control vs. all other groups.

RESULTS

Food Intake and Body Weight

Throughout the experiment, all groups had similar food intake and after 4 weeks, an increase in body weight was similar in all groups.

Effect of Ivabradine on Serum Glucose Levels in Fructose Fed Hypertensive Rats

Fructose rich diet resulted in significant hyperglycemia (P<0.001) in MC as compared to NC rats. The delta change in glucose level is depicted in Fig 1. Ivabradine alone and its combination with Metformin significantly (P<0.001) suppressed increase in blood glucose levels which was comparable to Metformin.

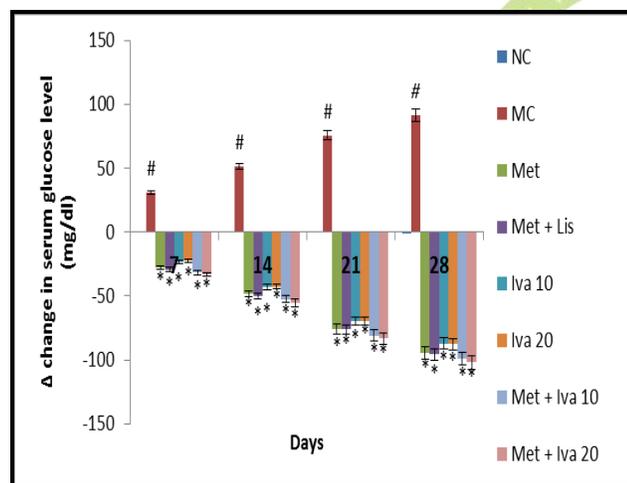


Figure 1: Effect of Ivabradine on serum glucose levels in fructose fed hypertensive rats

Effect of Ivabradine on Oral Glucose Tolerance Test in fructose fed hypertensive rats

Analysis of oral glucose tolerance pattern during 90 min. test period in NC and MC showed significant difference (P<0.05) in glucose and insulin levels (Figure 3 (a) and (b)). The peak level of glucose in MC was found at 30 min. which is much higher than NC. The glucose level was found to be decreased in MC at 60 and 90 min. However, it was significantly greater than NC. Ivabradine alone and its combination

with metformin supplemented rats showed a lower glucose elevation and faster disposal rates, thereby displaying significant improvement (P<0.001) in glucose tolerance pattern as compared to MC.

The MC secreted more insulin than NC, as indicated by the greater peak insulin response (P<0.05). Ivabradine and its combination with metformin significantly decreased insulin secretion in the rats as compared to MC.

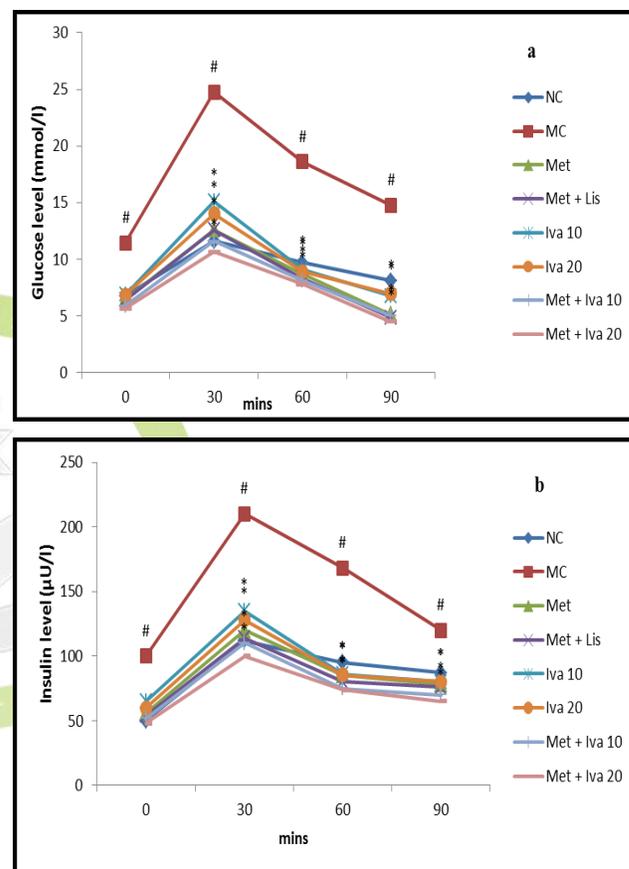


Figure 2: Effect of Ivabradine on (a) serum glucose levels (b) serum insulin levels after glucose overload in fructose fed hypertensive rats

Furthermore, HOMA-IR levels were significantly (P<0.05) increased in MC. Ivabradine and its combination with metformin significantly prevented this increase in HOMA-IR levels.

MC showed significant decrease in QUICKI levels as compared to NC. Treatment with Ivabradine and its combination with metformin significantly prevented decrease in this level.

Effect of Ivabradine on Blood Pressure in Fructose Fed Hypertensive Rats

The delta change in blood pressure in MC is significantly ($P < 0.001$) higher as compared to NC for 28 days (Fig 2). Iva10 and Iva20 prevented the rise in mean blood pressure significantly ($P < 0.001$) as compared to MC. Ivabradine alone and its combination with metformin also reduced the elevation in mean blood pressure significantly ($P < 0.001$) as compared to MC.

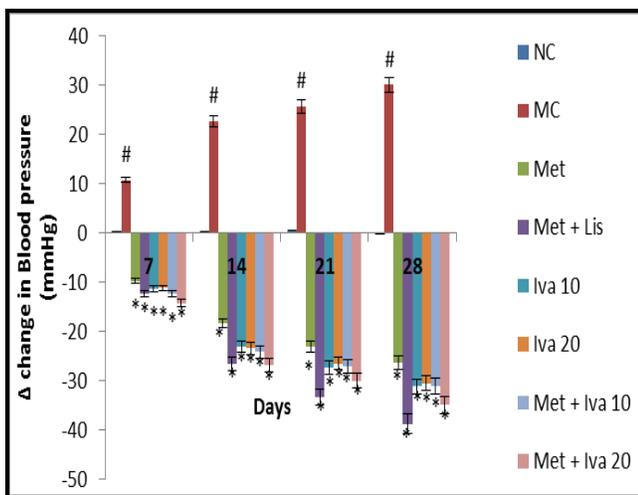


Figure 3: Effect of Ivabradine on mean blood pressure in fructose fed hypertensive rats

Effect of Ivabradine on Serum Lipid Levels in Fructose Fed Hypertensive Rats

The serum levels of TG, TC, LDL and VLDL increased significantly ($P < 0.05$) in MC while there was no significant difference in HDL levels in HFD rats. (Table 1) Ivabradine alone and its combination with Metformin, significantly ($P < 0.05$) decreased rise in lipid levels.

Effect of Ivabradine on Kidney Markers in Fructose Fed Hypertensive Rats

Fructose consumption in rats significantly ($P < 0.05$) increased serum creatinine (Fig 4), serum urea (Fig 5) and urine albumin levels (Fig 6). However, the consumption of Ivabradine and its combination with metformin resulted in the reversal of urine and serum parameters of renal function that were comparable to control levels.

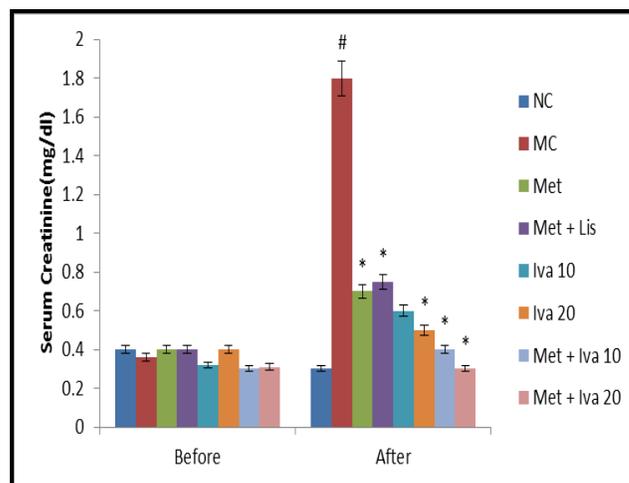


Figure 4: Effect of Ivabradine on serum creatinine levels in fructose fed hypertensive rats

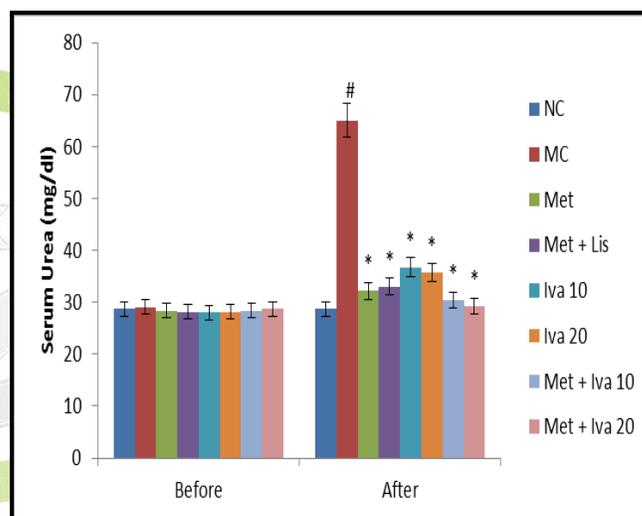


Figure 5: Effect of Ivabradine on serum urea levels in fructose fed hypertensive rats

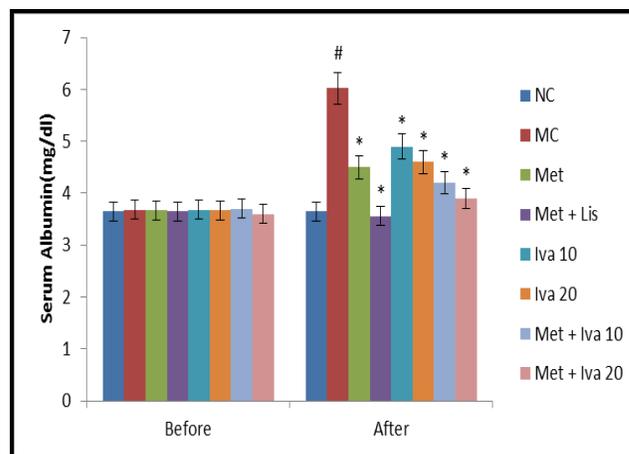


Figure 6: Effect of Ivabradine on urine albumin levels in fructose fed hypertensive rats

Table 1: Effect of Ivabradine on lipid levels in fructose induced hypertensive rats

Parameters (mg/dl)	Days	NC	MC	Met	Met + Lis	Iva 10	Iva 20	Met + Iva 10	Met + Iva 20
TG	1	57.89 ±1.63	57.92 ±1.17	57.89± 1.62	57.67 ±1.89	57.78 ±1.89	57.34 ±0.24	57.03± 1.87	57.13 ±1.25*
	7	56.06 ±1.65	66.67 ±0.24 [#]	60.54± 1.62*	60.32 ±1.89*	64.45 ±1.95*	62.45 ±1.62*	59.04 ±1.62*	58.13 ±1.62*
	14	57.30 ±1.23	96.12 ±1.43 [#]	78.45± 1.39*	76.78 ±1.92*	77.18 ±0.24*	75.45 ±1.25*	68.79 ±1.62*	60.9 ±1.56*
	21	56.92 ±1.45	103.17 ±1.23 [#]	73.45± 0.24*	72.47 ±1.62*	78.45 ±1.89*	75.67 ±1.62*	65.45 ±1.56*	57.98 ±0.24*
	28	56.83 ±1.63	131.25 ±1.75 [#]	68.12± 1.75*	67.02 ±1.85*	75.14 ±1.20*	72.01 ±1.24*	60.13 ±1.56*	57.66 ±1.51*
TC	1	70.00 ±1.21	71.00 ±1.12	71.01± 0.12	72.00 ±1.56	72.00 ±1.12	73.00 ±1.2	72.00 ±1.23	73.00 ±1.32
	7	71.23 ±1.25	85.43 ±1.56 [#]	74.00± 1.56*	73.45 ±1.56*	76.15 ±1.63*	75.56 ±1.25*	74.13 ±1.47*	74.00 ±1.12*
	14	71.02 ±1.92	98.23 ±1.56 [#]	78.15± 1.63*	78 ±1.12*	80.19 ±1.25*	78.15 ±1.39*	76.14 ±1.56*	75.84 ±1.51*
	21	71.43 ±1.63	110.01 ±1.92 [#]	79.17± 1.51*	80.12 ±1.56*	83.14 ±1.12*	76.14 ±1.25*	76.51 ±1.63*	75.14 ±1.92*
	28	72.29 ±1.63	119.93 ±1.75 [#]	67.15± 2.75*	67.13 ±1.85*	85.01 ±1.11*	84.14 ±1.45*	75.13 ±1.20*	75.12 ±1.24*
VLDL	1	11.58 ±1.23	11.58 ±1.57	11.58 ±1.13	11.53 ±1.57	11.56 ±1.40	11.47 ±1.90	11.41 ±1.97	11.43 ±1.43
	7	11.21 ±1.22	13.33 ±1.44 [#]	12.11 ±1.57*	12.06 ±1.12*	12.89 ±1.56*	12.49 ±1.36*	11.81 ±1.38*	11.63 ±1.68*
	14	11.46 ±1.90	19.22 ±1.30 [#]	15.69 ±1.65*	15.36 ±1.34*	15.44 ±1.54*	15.09 ±1.98*	13.76 ±1.29*	12.18 ±1.57*
	21	11.38 ±1.65	20.63 ±1.92 [#]	14.69 ±1.50*	14.49 ±1.57*	15.6 ±1.67*	15.13 ±1.58*	13.09 ±1.16*	11.60±1 .90*
	28	11.37 ±1.59	26.25 ±1.78 [#]	13.62 ±1.70*	13.40 ±1.75*	15.03 ±1.76*	14.40 ±1.69*	12.03 ±1.78*	11.53 ±1.67*
LDL	1	2.28 ±1.24	1.25 ±1.50	2.26 ±1.55	2.30 ±1.37	3.26 ±1.88	4.38 ±1.87	2.68 ±1.70	3.43 ±1.84
	7	2.90 ±1.25	14.96 ±1.45 [#]	4.73 ±1.55*	4.20 ±1.70*	6.09 ±1.67*	5.94 ±1.42*	5.18 ±1.49*	5.18 ±1.86*
	14	2.42 ±1.88	21.87 ±1.29 [#]	5.26 ±1.20*	5.46 ±1.87*	7.64 ±1.77*	5.91 ±1.76*	5.25 ±1.79*	6.45 ±1.32*
	21	2.91 ±1.68	32.24 ±1.90 [#]	7.35 ±1.86*	8.47 ±1.23*	10.32 ±1.9*	3.86 ±1.23*	6.29 ±1.23*	6.40 ±1.29*
	28	3.74 ±1.60	36.54 ±1.76 [#]	11.29 ±1.7*	12.57 ±1.89*	12.84 ±1.48*	12.59 ±1.45*	5.99 ±1.49*	6.36 ±1.50*

Effect of Ivabradine on Serum Electrolytes Level in Fructose Fed Hypertensive Rats

Sodium levels were found to be significantly increased in MC while potassium levels were decreased compared to NC (Table 2).

Treatment with Ivabradine alone and its combination with metformin markedly reduced ($P < 0.05$) sodium and elevated potassium levels as compared to MC. Met + Lis showed hyperkalemia.

Table 2: Effect of Ivabradine on sodium and potassium levels in fructose induced hypertensive rats

Groups Parameters	Sodium (mg/dl)		Potassium (mg/dl)	
	Before	After	Before	After
NC	143.00±1.63	145.90±1.63	4.8±1.93	4.75±0.23
MC	144.65±1.63	185.32±1.15#	4.33±1.63	2.34±0.25#
Met	144.05±1.85	180.65±1.75*	4.71±1.75	2.43±0.82*
Met + Lis	145.84±1.95	150.55±1.44*	4.90±1.23	6.5±0.55*
Iva10	145.54±1.20	165.12±1.20*	4.52±1.14	3.7±1.24*
Iva20	146.43±1.16	162.12±1.14*	5.09±0.34	3.2±0.36*
Iva10+Met	145.68±1.12	164.30±2.15*	4.72±1.44	3.1±1.96*
Iva20+Met	145.9±1.16	162.08±1.63*	4.8±1.71	2.8±1.88*

Table 3: Effect of Ivabradine on antioxidant enzyme levels in fructose induced hypertensive rats

Groups Parameters	MDA (µg/ml)	GSH (µg/ml)	SOD (µg/ml)	Catalase (mg of H ₂ O ₂ /min/gm of tissue)
NC	0.56±1.63	6.34±0.05	12.34±0.25	59.45±0.36
MC	3.67±1.15#	2.83±0.013#	5.33±0.13#	29.35±0.24#
Met	0.56±1.34*	5.43±0.01*	10.12±0.12*	49.65±0.43*
Met + Lis	0.65±2.55*	5.13±0.02*	9.45±0.23*	49.13±0.14*
Iva10	0.762±1.20*	5.13±0.34*	9.45±0.86*	45.53±0.45*
Iva20	0.752±1.14*	5.10±0.36*	9.75±0.36*	45.90±0.13*
Iva10+Met	0.613±2.15*	5.28±0.76*	11.43±0.86*	50.13±0.26*
Iva20+Met	0.615±2.05*	5.26±0.73*	11.42±0.73*	50.56±0.16*

Effect of Ivabradine on antioxidant levels in fructose fed hypertensive rats

The activity of antioxidant enzymes was significantly decreased in MC as compared to NC. Treatment with Ivabradine alone and its combination with metformin markedly showed elevated levels of antioxidant enzymes ($P < 0.05$). MDA levels were significantly increased ($P < 0.05$) in MC which was significantly decreased by Ivabradine alone and its combination with metformin.

DPPH Scavenging Activity

As shown in Fig 7, different concentrations of Ivabradine showed significant scavenging property when compared with different concentrations of standard. This showed comparable antioxidant activity of Ivabradine with Standard (Ascorbic acid).

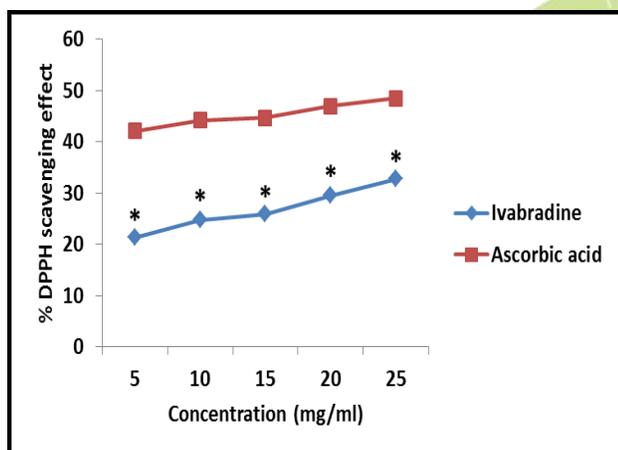


Figure 7: Antioxidant property of Ivabradine by DPPH assay

Values are expressed as mean \pm SEM (N=3). Values are statistically evaluated using ANOVA analysis followed by Dunnett's Post hoc test.

Effect of Ivabradine on Morphological Changes in Kidney and Pancreas in Fructose fed Hypertensive Rats

The glomerulus of the kidney in the MC shows thickening or damage of the glomerular basement membrane. (Fig 8 (a)) Treatment with Ivabradine showed no damage to glomeruli but thickening of the endothelial membrane was observed with Iva10. The combination of

Ivabradine and metformin exhibited similar morphological structure as normal kidney.

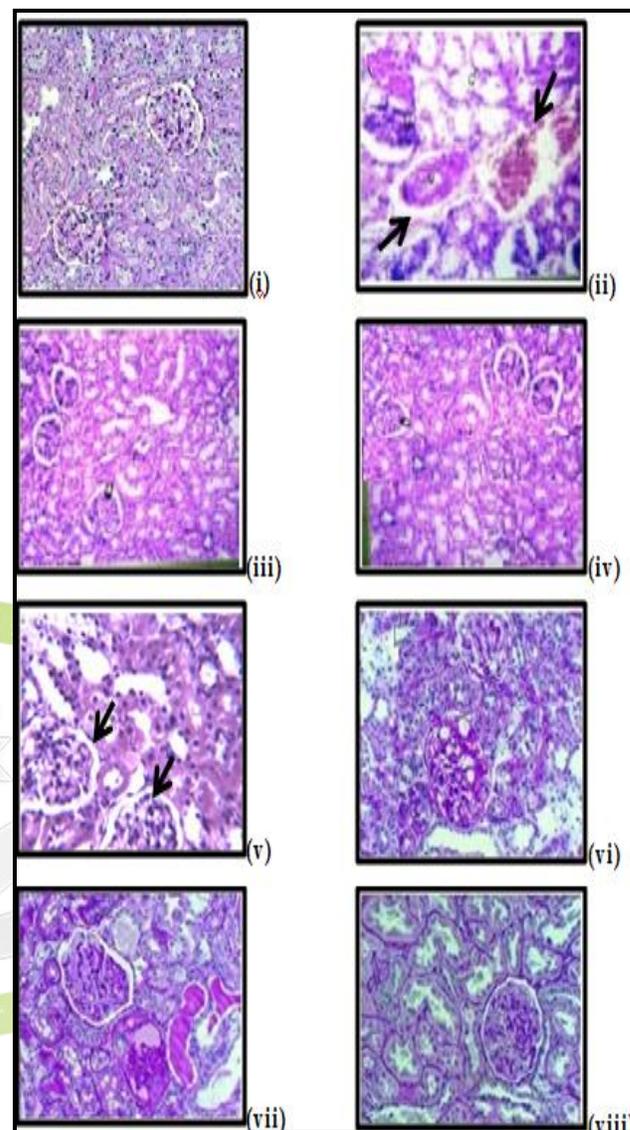


Figure 8 (a) Effect of Ivabradine on morphological changes in kidney in fructose fed hypertensive rats: (i) NC: The kidney architecture is normal with normal glomeruli and endothelial membrane; (ii) MC: Kidney shows damage and thickening of glomerular membrane; (iii) Met: No kidney damage; architecture is same as NC; (iv) Met + Lis: No kidney damage; architecture is same as NC; (v) Iva10: No damage to glomeruli but thickening of endothelial membrane is observed; (vi) Iva20: No kidney damage; architecture is same as NC; (vii) Iva10 + Met: No kidney damage; architecture is same as NC; (viii) Iva20 + Met: No kidney damage; architecture is same as NC

In the histological section of pancreas in MC, hypertrophy of islet of Langerhans with fibrosis of the lobules is observed. (Fig 8 (b)) Ivabradine treatment showed restoration of pancreatic acini. However, fibrosis was still observed in Iva10. The combination of Ivabradine with metformin completely reversed these pathological changes.

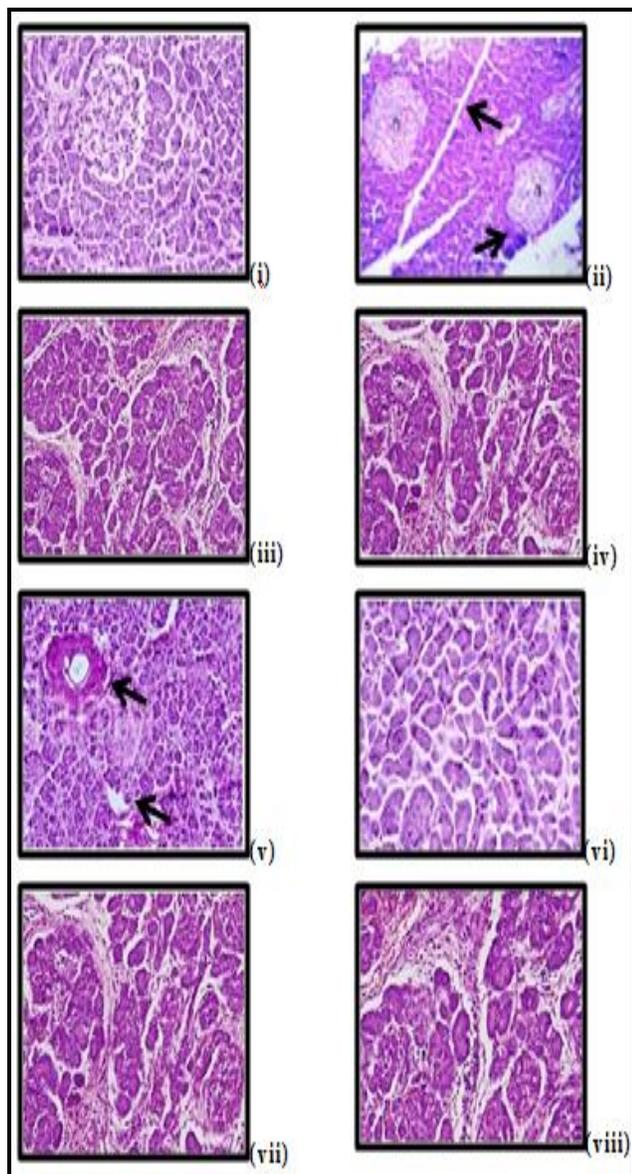


Figure 8 (b): Effect of Ivabradine on morphological changes in pancreas in fructose fed hypertensive rats: (i) NC: The pancreas architecture is normal with normal islet of Langerhans and lobules.; (ii) MC: Pancreas shows hypertrophy of Islet of Langerhans with fibrosis of lobules; (iii) Met: No pancreas damage; architecture is same as NC; (iv) Met + Lis: No pancreas damage; architecture is same as

NC; (v) Iva10: No damage to Islet of Langerhans but fibrosis is still observed; (vi) Iva20: No pancreas damage; architecture is same as NC; (vii) Iva10 + Met: No pancreas damage; architecture is same as NC; (viii) Iva20 + Met: No pancreas damage; architecture is same as NC

DISCUSSION

Metabolic syndrome is fast emerging as an epidemic of the new millennium fraught with serious consequences to human health worldwide. Metabolic syndrome (formerly known as syndrome X or insulin resistant syndrome), represents a cluster of cardiovascular risk factors like, insulin resistance, dyslipidemia and hypertension.²⁴

Cardiovascular disease (CVD) is the ultimate cause of mortality in people with metabolic syndrome and one of the associated manifestations leading to CVD could be hypertension.^{1,25} Importantly, hypertension in patients with diabetes causes a significant increase in the risk of vascular complications in this population, and together both conditions predispose to chronic kidney disease.²⁶ The overlap between hypertension and diabetes substantially increases the macrovascular and microvascular complications. Macrovascular complications include coronary artery disease, myocardial infarction, stroke, congestive cardiac failure and peripheral vascular disease. Microvascular complications include nephropathy, retinopathy and neuropathy.²⁶

Recent evidences tend to identify consumption of carbohydrates, mostly refined sugars with high fructose content, as an important culprit in the development of metabolic syndrome.^{24,27}

Fructose-induced hypertensive rats is a diet-induced model, considered equivalent to human metabolic syndrome as marked by the expression of all the manifestations such as hyperglycemia, insulin resistance, hyperinsulinemia, dyslipidemia, and hypertension; and hence a suitable model for evaluating the efficacy of preventive/ameliorating agents.^{19,27} Moreover,

high dietary fructose intake does not result in a significant weight gain; so can be used to investigate the relationship between metabolic alterations and the development of hypertension without the confounding influences of obesity or genetic predispositions.^{27,28}

Hyperglycemia seen in association with hyperinsulinemia is suggestive of impaired insulin action in fructose fed rats.^{24, 28} The observed hyperglycemia in the present study could be a consequence of fructose diet-induced metabolic alterations leading to gluconeogenesis and poor glucose oxidation. Ivabradine (10mg/kg and 20mg/kg) and its combination with metformin normalize blood glucose level; thereby preventing HFD induced increment in blood glucose level. The effect of metformin was found to be highest. This effect of Ivabradine can be contributed to its anti-hyperinsulinemic effect and thus improving glucose uptake and glucose oxidation in liver.

Insulin resistance may occur for different reasons^{29,30} including defects in insulin binding caused by decreased receptor number or affinity, defects in signal transduction involving receptor auto phosphorylation and tyrosine kinase activity, or post receptor defects at the level of substrates of phosphorylation or effector molecules such as glucose transporters (i.e., GLUT-4) and enzymes involved in glucose metabolism. Hepatic insulin resistance has also been reported following fructose consumption in humans. The present study portrays prevalence of insulin resistance in fructose fed rats, which is clearly indicated by the poor glucose tolerance curve as recorded in an oral glucose tolerance test. Treatment with Ivabradine exhibited improved glucose tolerance pattern which was further confirmed by formulae given by Matthews et al.²¹

The hyperinsulinemia associated with insulin resistance has been found in fructose-fed rat and has been linked to hypertension development in this model.^{4,19,28} Insulin resistance and compensatory hyperinsulinemia may act as predisposing factors for the development of hypertension possibly through activation of the

sympathetic nervous system (SNS). The present study demonstrated that fructose feeding for 28 days significantly increases blood pressure in rats and that fructose-induced hypertension could be reversed to normal blood pressure level by Ivabradine treatment.

Another possibility for development in hypertension is renal sodium reabsorption²⁷ induced by the increased sympathetic activity arising from the insulin resistance. Ivabradine reversed this increment. It also doesn't increase potassium levels. There is substantial evidence in both humans and animals showing that an increase in plasma triglyceride concentration is the expected consequence of insulin resistance and hyperinsulinemia due to overproduction of hepatic very low density lipoprotein (VLDL) triglyceride, impairment in the rate of removal of VLDL-triglyceride and resistance to the action of insulin on lipoprotein lipase; thereby causing dyslipidemia.^{17,20} Ivabradine prohibited dyslipidemia by decreasing overproduction of VLDL level and improving glucose profile.

Fructose-feeding in rats has been found to increase oxidative stress as reflected by the generation of reactive oxygen species that play a key role in the cardiovascular abnormalities associated with insulin resistance.¹⁸ Ivabradine shows beneficial effects evidenced by reduction of lipid peroxidation and restoration of antioxidant enzyme levels. The antioxidant activity of drugs was also proven in-vitro by DPPH assay.

Fructose-treated rats also showed renal dysfunctions such as greater urinary excretion of albumin and higher serum urea and creatinine.²⁷ Although the exact mechanisms for renal damage caused by fructose treatment have not been established; oxidative stress, the lipogenic effect, release of inflammatory cytokines, and endothelial dysfunction may be underlying mechanisms. All these effects raise were forbidden by Ivabradine alone and its combination with metformin. The histological reports also revealed the protective effect of Ivabradine.

CONCLUSION

Ivabradine was found to be effective in insulin resistance syndrome in experimental animals.

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