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Research Article

Investigation effect of pioglitazone, glimepiride, nobivolol, valsartan and hesperidine on glucose, HbA1c and blood pressure in experimentally induced myocardial infarction in type 2 diabetic rats

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ABSTRACT

Present study was designed to evaluate effect some synthetic drugs and some herbal compound on Glucose, HbA1c and Systolic and diastolic blood pressure in isoproterenol induced myocardial infarction in normal and Streptozotocin-Nicotinamide induced diabetic in rats. Pioglitazone (10 mg/kg, p.o), Glimepiride (0.5 mg/kg, p.o), Nobivolol (2 mg/kg, p.o), Valsartan (8 mg/kg, p.o) and Hesperidin (100 mg/kg, p.o) were administered for 28 days in rats injected with single dose of Streptozotocin (65 mg/kg, i.p, STZ) and Nicotinamide (12mg/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 sample were collected, and glucose, HbA1c were find out and measurement of systolic and diastolic blood pressure. Administration of STZ–NIC in rats showed a significant ($p<0.001$) increased in the levels of serum glucose and glycosylated hemoglobin (HbA1c) and significant ($p<0.01$) increased in the level systolic and diastolic blood pressure as compared to respective control groups. Treatment with Pioglitazone, Glimepiride and Hesperidin significantly ($P<0.05$) decreased HbA1c, glucose, systolic and diastolic blood pressure but treatment with Nobivolol and Valsartan significantly ($p<0.01$) decreased without change on glucose and HbA1c. This study concluded that Pioglitazone, Glimepiride and Hesperidin may show reduced diabetes marker and systolic and diastolic blood pressure but Nobivolol and Valsartan may show reduced only systolic and diastolic blood pressure on experimentally induced myocardial infarction in type 2 diabetic rats.

Keywords: Blood pressure; Glucose; HbA1c; Streptozotocin; Nicotinamide.

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). 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 2003), due to which there is alteration in vascular responsiveness to several vasoconstrictors and vasodilators (Senses 2001).

Hypertension, a component of metabolic/insulin resistance syndrome, is an important risk factor for cardiovascular disease contributing to the increased morbidity and mortality. Oxidative stress and glucose has emerged as an important pathogenic factor in the development of hypertension (United 1998, Perneger 1994, Perneger 1993). Hyperglycaemia, central (visceral) obesity, hypertension, dyslipidaemia, hyperinsulinaemia, endothelial dysfunction and impaired fibrinolysis are found in both type 2 diabetes and hypertension (Ferranini 1999), as well as in the prediabetic state (Haffner 2000), but insulin resistance is the common metabolic disorder in patients with these two conditions (Guidelines 1999).

Recently, a protective effect of Pioglitazone against oxidative stress in liver and kidney of diabetic rabbits (Gumieniczek 2003) has been reported. Pioglitazone (PIO) hydrochloride is a widely used drug in the treatment of insulin resistance diabetes. Pioglitazone lowers blood pressure and restores blunted endothelium-dependent vasodilatation in fructose-fed rats (Kotchen 1997), insulin-resistant rheus monkey (Kemnitz 1994), SHR (Grinsell 2002) and sucrosefed SHR (Uchida 2007). Glimepiride (GLI) an oral blood glucose lowering drug of the sulfonylurea class is reported to have pancreatic and extra pancreatic effects as well. The blockages of K_{ATP} channels of pancreatic cells by sulphonylurea are

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critical in the regulation of glucose regulated insulin secretion.

Nebivolol (NEB) is a β1-adrenoceptor blocking drug that possesses certain unusual pharmacological properties by which it differs from conventional β1-blockers. Recent evidence suggest that blockade of the rennin-angiotensin system ameliorates diabetes induced cardiac dysfunction. Because activation Valsartan (VAL) - Angiotensin II receptor (AT 1) blocker blocks the vasoconstrictor and aldosterone-secreting effects of angiotensin II by selectively blocking the binding of angiotensin II to the AT1 receptor in many tissues, such as vascular smooth muscles and the adrenal gland.

Hesperidin (HES) is an abundant and inexpensive by-product of Citrus cultivation and isolated from the ordinary orange Citrus aurantium and other species of the genus Citrus (family: Rutaceae). It is reported to have anti-allergic, radio protective, immunomodulator, anti-hypertensive and anti-oxidant properties. When Hesperidin is administered orally, it is hydrolyzed by intestinal micro flora to yield a major active metabolite Hesperidin.

So far the effect of PIO, GLI, NOB, VAL and HES on diabetic marker and blood pressure in experimentally induced myocardial infarction in type 2 diabetic rats has not been studied. Hence, the purpose of the present study was to instigate the effect of PIO, GLI, NOB, VAL and HES on serum diabetic marker and blood pressure in Isoproterenol induced myocardial infarction in type 2 diabetic rats.

MATERIALS AND METHOD

Drugs and Chemicals

Nobivolol was obtained as a gift sample from Torrent Pharmaceuticals Pvt. Ltd., Ahmadabad, India. Hesperidin was obtained from ACROS Lab, US. Pioglitazone hydrochloride and Valsartan was obtained as a gift sample from Alembic Pharmaceuticals Pvt. Ltd., Baroda, 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 (210 ± 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 libitium*.

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. Drugs were administered for 28 days in diabetic rats and after isoproterenol induced myocardial infarction in rats on 29th and 30th day.

At the end of experimental period (i.e. on the day 31) blood samples were collected and carried out glucose and HbA1c diabetic marker estimations.

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: 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 3: Non-diabetic control treated with PIO (10 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (**ND-PIO)**] and normal saline subcutaneously on 29th and 30th day.

Group 4: STZ-NIC diabetic rats treated with PIO (10 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (**D-PIO)**] and received ISO (200 mg/kg, s.c.) on 29th and 30th day in normal saline.

Group 5: Non-diabetic control treated with GLI (0.5 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (**ND-GLI)**] and normal saline subcutaneously on 29th and 30th day.

Group 6: STZ-NIC diabetic rats treated with GLI (0.5 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (**D-GLI)**] and received ISO (200 mg/kg, s.c.) on 29th and 30th day in normal saline.

Group 7: Non-diabetic control treated with VAL (8 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (**ND-VAL)**] and normal saline subcutaneously on 29th and 30th day.

Group 8: STZ-NIC diabetic rats treated with VAL (8 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (**D-VAL)**] and received ISO (200 mg/kg, s.c.) on 29th and 30th day in normal saline.

Group 9: Non-diabetic control treated with NEB (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 10: 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.

Group 11: Non-diabetic control treated with HES (100 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (**ND-HES)**] and normal saline subcutaneously on 29th and 30th day.

Group 12: STZ-NIC diabetic rats treated with HES (100 mg/kg/day, p.o) as a suspension [0.5 % Sodium CMC for 4 weeks (**D-HES)**] 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.

Measurement of systolic and diastolic blood pressure

Systolic and diastolic blood pressure was measured indirectly in a conscious, pre-warmed, and slightly restrained rat by tail cuff method (Harvard rat-tail blood pressure monitor, USA). An average of eight consecutive reading was noted after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.

Statistical Analysis

All of the data are expressed as mean ± 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

Effect of synthetic drugs and herbal on diabetic marker

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. There was a significant ($p<0.001$) increase in Glucose and HbA1c after myocardial infarction in D-CON group as compared to ND-CON group. Treatment of PIO, GLI and HES in STZ-NIC diabetic rats significant ($p<0.001$, $p<0.05$) decreased levels of serum Glucose and HbA1c compared to respective diabetic control (Table 1).

Table 1: Effect of Pioglitazone (10 mg/kg/day, p.o), Glimepiride (0.5 mg/kg/day, p.o), Nobivolol (2 mg/kg/day, p.o), Valsartan (8 mg/kg/day, p.o) and Hesperidin (100 mg/kg/day, p.o) on changes in Glucose and HbA1c in experimentally induced myocardial infarction in normal and STZ-NIC induced diabetic rats

Groups	Glucose	HbA1c
ND-CON	101.8 ± 6.799	5.455 ± 0.3729
D-CON	332.8 ± 9.167***	9.900 ± 0.6323***
ND-PIO	95.67 ± 7.654	4.937 ± 0.4211
D-PIO	189.3 ± 8.3530***	6.618 ± 0.3421***
ND-GLI	97.67 ± 8.429	4.825 ± 0.4115
D-GLI	162.0 ± 11.72***	6.133 ± 0.3325***
ND-NOB	96.17 ± 6.954	4.820 ± 0.3265
D-NOB	302.3 ± 9.622	9.413 ± 0.4993
ND-VAL	93.00 ± 7.967	4.715 ± 0.3950
D-VAL	301.8 ± 11.48	9.363 ± 0.4487
ND-HES	98.17 ± 6.650	4.865 ± 0.3047
D-HES	294.0 ± 12.94*	7.872 ± 0.425*

Values are expressed as mean ± SEM for six animals in the group. * P<0.05, *** P<0.001 compared to respective control group.

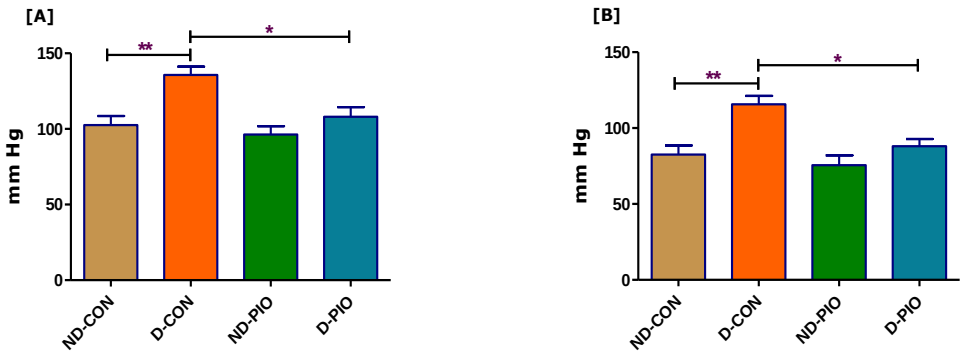

Figure 1: Effect of Pioglitazone (10 mg/kg/day, p.o) on changes in Systolic [A] and diastolic [B] blood pressure level after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.

Values are expressed as mean ± 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.

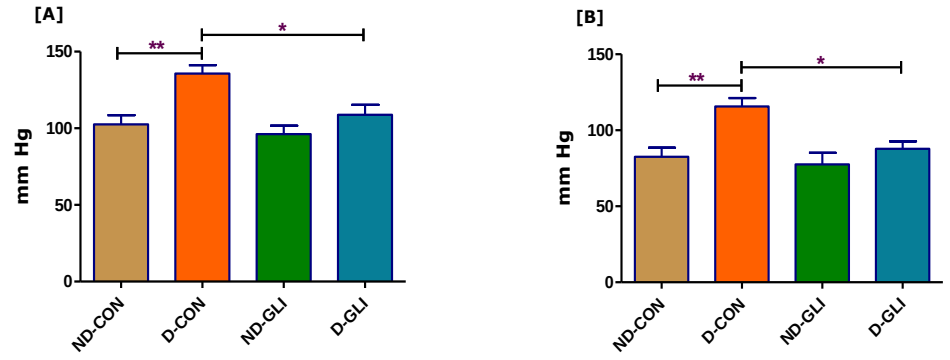

Figure 2: Effect of Glimepiride (0.5 mg/kg/day, p.o) on changes in Systolic [A] and diastolic [B] blood pressure level after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.

Values are expressed as mean ± 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.

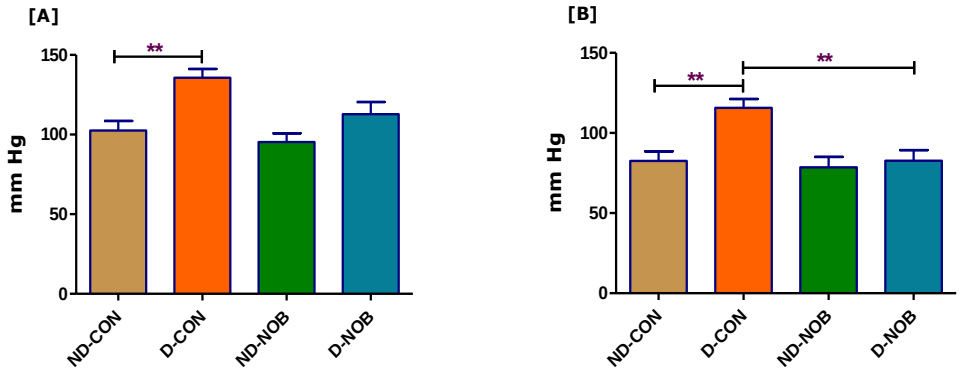

Figure 3: Effect of Nobivolol (2 mg/kg/day, p.o) on changes in Systolic [A] and diastolic [B] blood pressure level after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.

Values are expressed as mean ± 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.

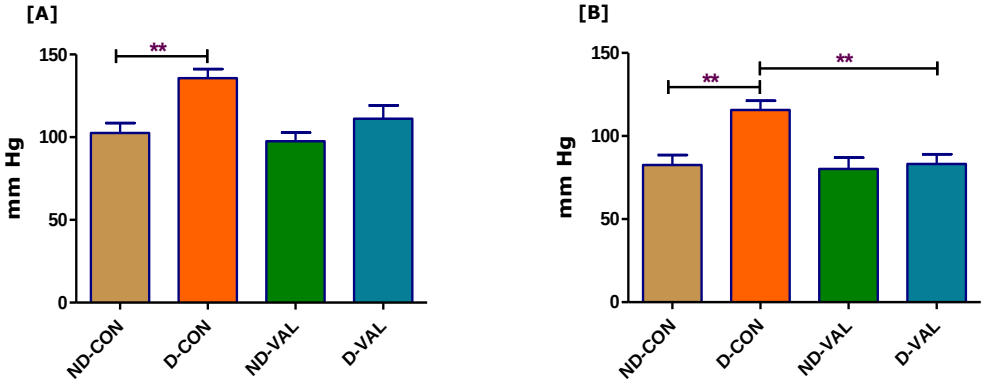

Figure 4: Effect of Valsartan (8 mg/kg/day, p.o) on changes in Systolic [A] and diastolic [B] blood pressure level after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.

Values are expressed as mean ± 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.

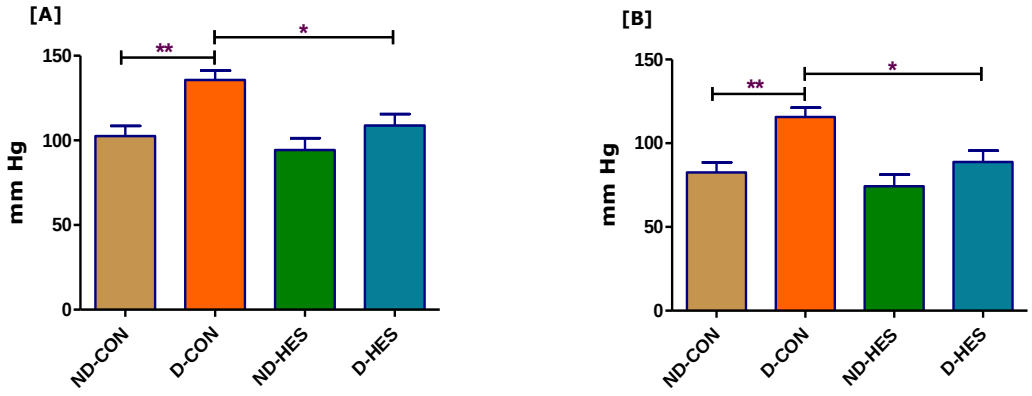

Figure 5: Effect of Hesperidin (100 mg/kg/day, p.o) on changes in Systolic [A] and diastolic [B] blood pressure level after completion of myocardial infarction in normal and STZ-NIC induced diabetic rats.

Values are expressed as mean ± 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 drug and herbal on systolic and diastolic blood pressure

There was a significant (p<0.01) increase in systolic and diastolic blood pressure after myocardial infarction in D-CON group as compared to ND-CON group (Fig. 1-5). Treatment of PIO, GLI and HES in STZ-NIC diabetic rats (D-PIO, D-GLI, D-HES) significant (p<0.05) decrease levels of systolic and diastolic blood pressure as compared to diabetic control (Fig. 1-2, 5). Treatment of VAL and NOB in diabetic rats significant (p<0.01) decrease levels of systolic and diastolic blood as compared to respective ND-CON group (Fig. 4, 5).

DISCUSSION

The present study was under taken with the objective of exploring the Pioglitazone, Glimepiride, Nobivolol, Valsartan and Hesperidin on diabetic marker, systolic and diastolic blood pressure in experimentally induced myocardial infarction in diabetic rats.

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. 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 1993). It was reported that during diabetes mellitus, the excess of glucose present in the blood reacts with hemoglobin to form HbA1c (Koening 1976). The level of HbA1c is always monitered 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. Treatment of PIO, HES and GLI decreased glucose and HbA1c in diabetic rats as compared to diabetic control. Treatment of VAL and NOB no significantly change as compared to diabetic rats.

Hypertension is a risk factor for the development and worsening of many diabetes complications, and likewise having diabetes increases your risk of developing high blood pressure. The risk of a recurrent myocardial infarction decreases with strict blood pressure. In the present study there was a significant increase in systolic and diastolic blood pressure activity in ISO intoxicated diabetic rats compared to control diabetic rats. Treatment with PIO, GLI and HES were significantly decrease in systolic and diastolic blood pressure activity in experimentally induced myocardial infarction in diabetic rats as compared to control diabetic rats.

Thiazolidinediones (also known as glitazones) represent a new class of oral antidiabetic agents. They activate the peroxisome proliferator-activated receptor (PPAR)-γ, thereby reducing insulin-resistance (Martens 2002). The thiazolidinedione pioglitazone targets vascular insulin resistance in particular by improving endothelial dysfunction and inflammatory processes in the arterial wall (Diamant 2003, Gilling 2002). Clinical data clearly demonstrate that thiazolidinediones have blood pressure-lowering effects in hypertensive diabetic and non-diabetic patients (Diamant 2003, Göke 2002, Gerber 2003, Füllert 2002).

Nobivolol and Valsartan treatment improved systolic and diastolic blood pressure as compared to diabetic control so cardioprotective without change of glucose, HbA1c because it may be direct or indirectly effect of receptor or any other effect.

Pioglitazone, Hesperidin and Glimepiride treatment reduced glucose, HbA1c and improve systolic and diastolic blood pressure in experimentally induced myocardial infarction in diabetic rats which suggest cardioprotective activity and control diabetes. But Nobivolol

and Valsartan treatment improved systolic and diastolic blood pressure as compared to diabetic control so cardioprotective without change of glucose This study concluded that PIO at 10 mg/kg, HES at 100 mg/kg and GLI at 0.5 mg/kg may show reduced systolic and diastolic blood pressure which suggest both drug better effects on cardiac complication in diabetic rats and Val at 8 mg/kg and NOB at 2mg/kg may show more reduced systolic and diastolic blood pressure same as control systolic and diastolic blood pressure without change in glucose, HbA1c.

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AUTHOR'S STATEMENTS

Competing interests: The authors declare no conflict of interest.

ANIMAL RIGHTS

The institutional and (inter) national guide for the care and use of laboratory animals was followed. See the experimental part for details.

REFERENCES

Abel ED 2005. Myocardial insulin resistance and cardiac complications of diabetes. Curr Drug Targets Immune Endocr Metabol Disord Jun; 5(2):219-26.

Diamant M, Heine RJ 2003. Thiazolidinediones in type II diabetes mellitus: current clinical evidence. Drugs; 63 (13): 1373-405.

Ferranini E 1999. Insulin resistance and blood pressure. In: Reaven G, Laws A, editors. Contemporary endocrinology: insulin resistance: the metabolic syndrome X. Totowa (NJ): Humana Press Inc., 281-308.

Füllert S, Schneider F, Haak E, et al 2002. Effects of pioglitazone in non-diabetic patients with arterial hypertension: a double-blind, placebo-controlled study. J Clin Endocrinol Metab; 87: 5503-6.

Gabbay, K.H 1976. Glycosylated hemoglobin and diabetic control. New England Journal Medicine 95: 443-454.

Gerber P, Lübben G, Heusler S, et al 2003. Effects of pioglitazone on metabolic control and blood pressure: a randomised study in patients with type 2 diabetes mellitus. Curr Med Res Opin; 19 (6): 532-9.

Gilling L, Suwattee P, DeSouza C, et al 2002. Effects of the thiazolidinediones on cardiovascular risk factors. Am J Cardiovasc Drugs; 2 (3): 149-56.

Göke B 2002. Improved glycemic control and lipid profile in a randomized study of pioglitazone compared with acarbose in patients with type 2 diabetes mellitus. Treat Endocrinol; 1: 329-36.

Grinsell JW, Lardinois CK, Swislocki A, Gonzalez R, Sare JS, Michaels JR, et al 2000. Pioglitazone attenuates basal and postprandial insulin concentrations and blood pressure in the spontaneously hypertensive rat. Am J Hypertens, 13:370-5.

Guidelines Subcommittee 1999. World Health Organization-International Society of Hypertension. Guidelines for the management of hypertension. J Hypertens; 17: 151-83.

Gumieniczek A 2003. Effect of the new thiazolidinedione-pioglitazone on the development of oxidative stress in liver and kidney of diabetic rabbits. Life Sci 74:553–62.

Haffner SM, Mykkanen L, Festa A, et al 2000. Insulin-resistant pre-diabetic subjects have more atherogenic risk factors than insulin-sensitive pre-diabetic subjects: implications for preventing coronary heart disease during pre-diabetic state. Circulation; 101: 975-80.

Kahn SE, Porte DJ. 1990. The pathophysiology of type II (noninsulin-dependent) diabetes mellitus: Implications for treatment. In: Rifkin H, Porte DJ, eds. Ellenberg and Rijkin's Diabetes Mellitus: Theory and Practice. New York: Elsevier Science 436-456.

Kemnitz JW, Elson DF, Roecker EB, Baum ST, Bergman RN, Meglasson MD 1994. Pioglitazone increases insulin sensitivity, reduces blood glucose, insulin, and lipid levels, and lowers blood pressure in obese, insulin-resistant Rhesus monkeys. Diabetes, 43:204– 11.

Koening, R.L., Peterson, C.M. Jones, R.L. Saudek, C. Lehrman, M. and Cerami, A 1976. Correlation of glucose regulation and hemoglobin A1C in diabetes mellitus. New England Journal of Medicine 295: 417-420.

Kotchen TA, Reddy S, Zhang HY 1997. Increasing insulin sensitivity lowers blood pressure in the fructose-fed rat. Am J Hypertens, 10:1020– 6.

Leibowitz HE. 1990. Oral hypoglycemic agents. In: Rifkin H, Porte DJ, eds. Ellenberg nd Rijkin's Diabetes Mellitus: Theory and Practice. New York: Elsevier Science 554-574.

Martens FMAC, Visseren FLJ, Lemay J, et al 2002. Metabolic and additional vascular effects of thiazolidinediones. Drugs; 62 (10): 1463-80.

Masiello, P., Broca, C., Gross, R., Roye, M., Manteghetti, M., Hillaire-Buys, D., Novelli, M., Ribes, G 1998. Experimental NIDDM: development of a new model in adult rats administered Streptozotocin and Nicotinamide. Diabetes 47, 224–229.

Paulsen, E.P.1993. Heamoglobin A1C in childhood of diabetes. Metabolism 22: 269- 271.

Perneger TV, Brancati FL, Whelton PK, et al 1994. End-stage renal disease attributable to diabetes mellitus. Ann Intern Med, 121: 912-8.

Senses V, Ozyazgan S, Ince E, Tuncdemir M, Kaya F, Ozturk M, et al 2001. Effect of 5-aminoimidazole-4-carboxamide riboside (AICA-r) on isolated thoracic aorta responses in streptozotocin-diabetic rats. J Basic Clin Physiol Pharmacol 12:227– 48.

Stamler J, Vaccaro O, Neaton JD, et al 1993. Diabetes, other risk factors, and 12 year cardiovascular mortality for men screened in the multiple risk factor intervention trial. Diabetes Care, 16: 434-44.

Uchida A, Nakata T, Hatta T, Kiyama M, Kawa T, Morimoto S, et al 2007. Reduction of insulin resistance attenuates the development of hypertension in sucrose-fed SHR. Life Sci, 61(4):455– 64.

Uemura S, Matsushita H, Li W, Glassford AJ, Asagami T, Lee KH, et al 2001. Diabetes mellitus enhances vascular matrix metalloproteinase activity: role of oxidative stress. Circ Res 88:1291-8.

United Kingdom Prospective Diabetes Study Group 1998. UK Prospective Diabetes Study 23: risk factors for coronary artery disease in non-insulin dependent diabetes. BMJ, 316: 823-8.