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International Journal of Pharmaceutical and Biological Science Archive 1 (3) 2013, 01-05

RESEARCH ARTICLE

A COMPARATIVE PRE CLINICAL TRIAL ON THE IMPACT OF NEBIVOLOL AND ACE INHIBITOR TREATMENT ON THE RENAL BIO CHEMICAL PARAMETERS IN THE FRUCTOSE INDUCED DIABETIC HYPERTENSIVE RAT

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Received 10 November 2013; Revised 13 November 2013; Accepted 14 November 2013

ABSTRACT

The objective of the present study is to evaluate and compare the impact of Nebivolol and ACE (Angiotensin Converting Enzyme) inhibitor treatment on the renal biochemical parameters in the fructose induced diabetic hypertensive rats. This preclinical study was carried out with 32 *Sprague dawley* rats .They were divided into four groups (A,B,C and D)with each group containing eight rats. Disease was induced in each group using different duration and concentrations of fructose. Blood pressure was regularly assessed at periodic intervals by tail cuff method. Biochemical parameters were assessed with respective kits and CPCC 3000 version auto analyser. The biochemical parameters like glucose, total cholesterol, urea, creatinine and blood pressure were compared between, rats treated with ACE inhibitors and rats treated with Nebivolol, using SPSS software & probability values. This study clearly indicates that Nebivolol has better metabolic control, showed improvement in renal biochemical parameters and good control on blood pressure, compared to ACE inhibitor treated diabetic hypertensive rats with statistical significance($p<0.05$)(except serum creatinine)

Keywords: *Sprague dawley,* fructose, SPSS, tail cuff, Nebivolol

INTRODUCTION:

Diabetes is a global health problem. More than 62% diabetics resided in developing countries in 1995. In 2025 it may be more than 75%.[1] Type 2 diabetes mellitus is a significant condition that can lead to microvascular and macrovascular complications affecting various organs if blood glucose is not adequately controlled. Uncontrolled diabetes can lead to myocardial infarction, stroke, retinal damage, renal failure, pregnancy complications, toe, foot or leg infections requiring amputation

Currently ACE inhibitors are the first choice of drug in diabetics preventing end organ damage like diabetic nephropathy. ACE inhibitors work by reducing, proteinuria, systemic blood pressure, hydraulic glomerular pressure, filtration fraction, dilating efferent glomerular arterioles and by inhibition of growth factors[2]. But ACE inhibitors carry poor patient compliance in asthmatics, elderly & cardiac patients. Has adverse impact on metabolic parameters and absolutely contraindicated in pregnancy.ACE inhibitors have no role in reducing oxidative stress or balancing endothelial dysfunction or regulating advanced glycosylated end product formation. Rather nebivolol which is a second generation beta blocker lacks intrinsic sympathomimetic activity, has better compliance in asthmatics[3] , elderly[4] & cardiac patients[5]. Nebivolol also causes peripheral vasodilation[6] (as it releases nitric oxide) an added benefit for patients with

Cardiovascular diseases associated with peripheral vascular diseases. These advantages of nebivolol can be utilized in preventing end organ damage in diabetics. Hence this study is planned as a pre-clinical trial in diabetic hypertensive rats

Diabetes and hypertension are two most important diseases contributed by life style changes and westernization. They are closely related to the pathogenesis of chronic kidney disease, and is mostly unrecognized in earlier stages.

CURES study in India, conducted in 50,000 subjects revealed that 16% of diabetics were above 20 years old and 23% of subjects had hypertension[7].

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There is no preclinical study available to validate my objective. To know the renal protectivity of Nebivolol in diabetic nephropathy, the diseases like diabetes hypertension alone or in combination were induced in rat model using fructose. Fructose induced diabetes & hypertension are concentration and duration dependent. These diseases in rat model are very closely similar to the disease in humans.

METHODOLOGY:

Animals:

Adult male *Sprague dawley* rats (PSG animal house), initially weighing 250-300g were used for all experiments. Male animals were particularly selected because, fructose induced changes in the metabolic parameters and the blood pressure, do not manifest in female rats due to the presence of ovarian sex hormones. Animals are procured from National Institute of Nutrition, Hyderabad, and was quarantized for a week.

Prior to dietary manipulation all rats were fed standard rat chow and drinking water *ad libitum*, and maintained on a 12-hr light/ dark cycle. As per the guidelines for ethical conduct in animal research, four animals were kept in a rectangular cage with proper floor area and height of the wall. The cages were well ventilated with mesh top and the temperature was maintained at 25 c. care taken to meet the physiological and psychological needs of rats as closely as possible. Cage cleaning regimen was scheduled for every three days, so as to maintain ammonia levels within a cage below 25ppm.

All cages had labels attached to them providing information on strain, sex, number, age of rats and chief investigators name and contact numbers.

Study design:

32 rats were divided into four groups (A,B,C and D)with each group containing eight rats. Disease was induced in each group using different concentrations and duration of fructose[8] (LOBA CHEMIE) as follows:

A group – Diabetes mellitus II model- 60% fructose in drinking water for 2 weeks.

B group – Hypertension model – 10% fructose in drinking water for 2 weeks

C & D groups – Diabetes mellitus II+ Hypertension model – 20% fructose in drinking water for 8 weeks.

Induction was followed by blood collection. Blood was collected from rat tail vein after removing scales on the particular location. Cleansed with spirit. Lancet is used to prick the surface of vein. Blood is sucked out under pressure using needle and syringe and by giving gravitational flow of bood to the tail tip. After the procedure sufficient pressure was given to arrest bleeding. Povidone iodine is applied and replaced into cages after sometime.

Blood was collected in EDTA tubes before disease induction and before treatment and after treatment. Totally 0.5ml of blood were withdrawn[9]. Sufficient pressure was given with gloved fingers to stop bleeding and antibiotic cream was applied after blood collection. The collected blood was centrifuged to seperate serum and was stored at -20 degree c for further analysis. Freezer had 24 hour power supply to prevent intermittent thaw.

The blood collection was followed by treatment. The treatment regime was as follows:

A group –DM model: Metformin (GLYCOMET, Glaxosmithkline) 120 mg/kg, once a day, for 4 weeks by oral gavage[10]

B group –HT model**:** Nebivolol (NEBISTAR) 8 mg/kg once a day, for 4 weeks by oral gavage[11]. C group – DM + HT model I-Metformin 120 mg/kg + Nebivolol 8 mg/kg once a day, for 4 weeks by oral gavage

D group –DM +HT model II -Metformin 120 mg/kg + Lisinopril (LISORIL, Lupin)15 mg/kg once a day, for 4 weeks by oral gavage[12]

(DM-Diabetes Mellitus ;2 HT- Hypertension ;DM +HT-Diabetes Mellitus2 &Hypertension)

After treatment, blood was collected by the same procedure as `described above.

Parameters studied:

Parameters studied are blood pressure, glucose, total cholesterol, urea, creatinine.

Blood pressure and blood glucose were assessed at periodic intervals routinely. Blood pressure was assessed by non Invasive Blood Pressure measurement using tail cuff method (MEDI ANALYTICA, Spain) at PSG department of pharmacology, CNS experiment unit. **Procedure**: A small inflatable cuff with distally positioned pulse detector is placed around the base of the tail, and systolic and diastolic were recorded as displayed in the monitor and the principle is similar to sphygmomanometer. The animals were handled with minimal discomfort. The restrainer used is made of perplex glass, well ventilated with a tail outlet. Not too tight which minimized discomfort and allowed licking of paws. Care was taken to prevent damage to the testes. Animals were acclamatized in the restrainer before taking readings. Three readings were taken three times in a day for each rat and mean blood pressure was calculated Blood glucose was measured using ACUCHECK glucose strips. Total cholesterol, urea, creatinine were assessed with the stored serum, using auto analyser (CPC 3000 version) at

PSG ANIMAL HOUSE with respective kits at the end of the study.

Statistical analysis:

All statistical analysis were done out with SPSS version 17 software. Nebivolol and ACE inhibitors in diabetic hypertensive rat model are compared by paired student t' test. Blood pressure, glucose, total cholesterol, urea, creatinine are compared between groups by ANOVA multivariate method. Reversal of disease conditions after treatment are analysed by chi square method.

RESULTS:

- In the present study, Nebivolol shows a significant ($p<0.05$) antihypertensive effect in both hypertension and diabetic with hypertension rat model(table I& chart I)
- The effect of Nebivolol in lowering of blood pressure is more consistent, stable and higher with significance ($p<0.05$) ,than ACE inhibitor treated group
- Nebivolol also showed hypoglycaemic response with metformin, while, ACE inhibitors didn't showed this response (table I& chart II)
- In the diabetic with hypertensive rats, Nebivolol showed a significant ($p<0.05$) reduction in blood urea level but not statistically significant reduction in serum creatinine level(table I& chart IV, V)
- Total cholesterol brought to near normal with Nebivolol treated group and comparatively statistically significant($P<0.05$) with ACE inhibitors(table I &chart III)
- **Illustrations of results:**
- **TABLE- I Blood pressure & biochemical changes in normal, disease model before and after treatment** (BP- Blood Pressure; TC- Total Cholesterol

GROUP	BP (mm Hg)	GLUCOSE (mg/dl)	TC (mg/dl)	UREA (mg/dl)	CREATININE (mg/dl)
NORMAL	117.00±2.4	130.00 ±2	100.00±1.3	25.00±1.2	0.98±0.03
DM	118.00±2.2	190.00 ±4.1	135.55±1.2	90.00±2.1	1.02±0.26
DM+MET	115.00±2.3	160.00 ±3.9	120.32±2.3	62.00±2.6	1.02±0.06
HT	136.00±3.2	133.00 ± 2.0	125.00±2.3	26.00±2.2	1.00±0.02
HT+NEB	106.00±3.1	133.00 ± 2.1	125.00±2.6	25.00±2.6	1.00±0.12
DM+HT	170.00±7.0	196.00 ± 3.2	140.00±3.4	80.00±2.4	1.06±0.24
DM+HT+MET+ACEI	125.00±3.0	180.00±4.10	140.00±3.9	67.00±3.1	1.02±0.30
DM+HT+MET+NEB	119.00±4.0	162.00 ±3.1	135.00±4.2	50.00±2.1	1.00±0.10

Table 1: Blood pressure in the normal, disease control and treatment groups (chart1)

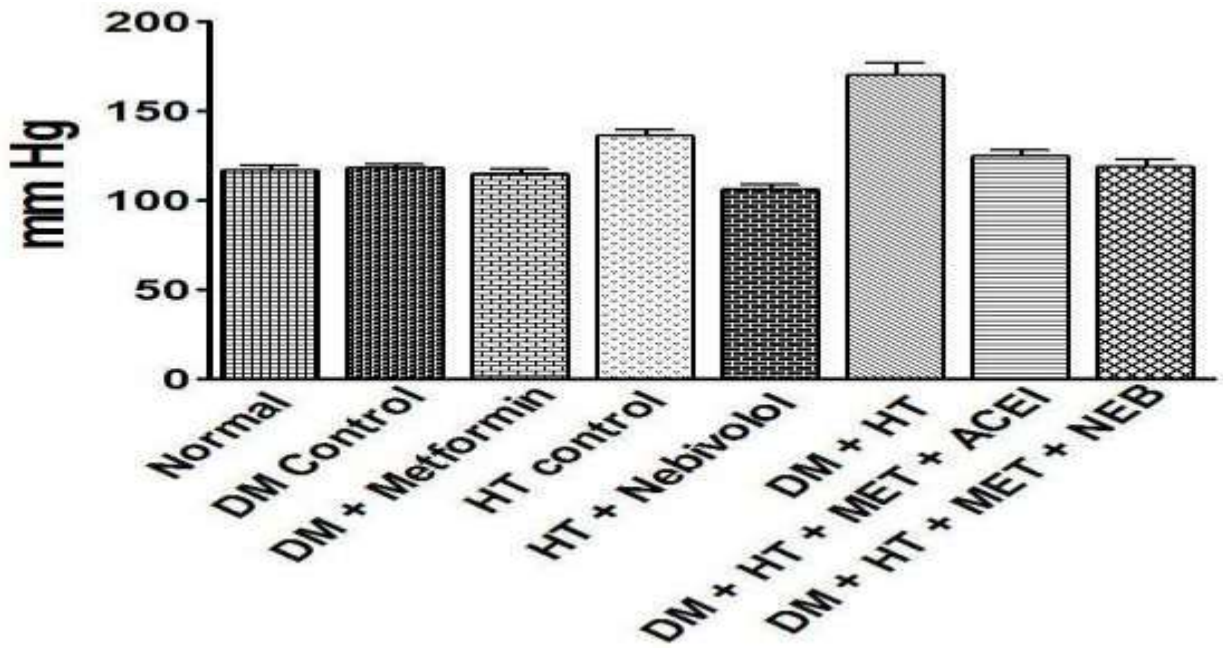


Figure 1: Blood glucose in the normal, disease control and treatment groups (chart 2)

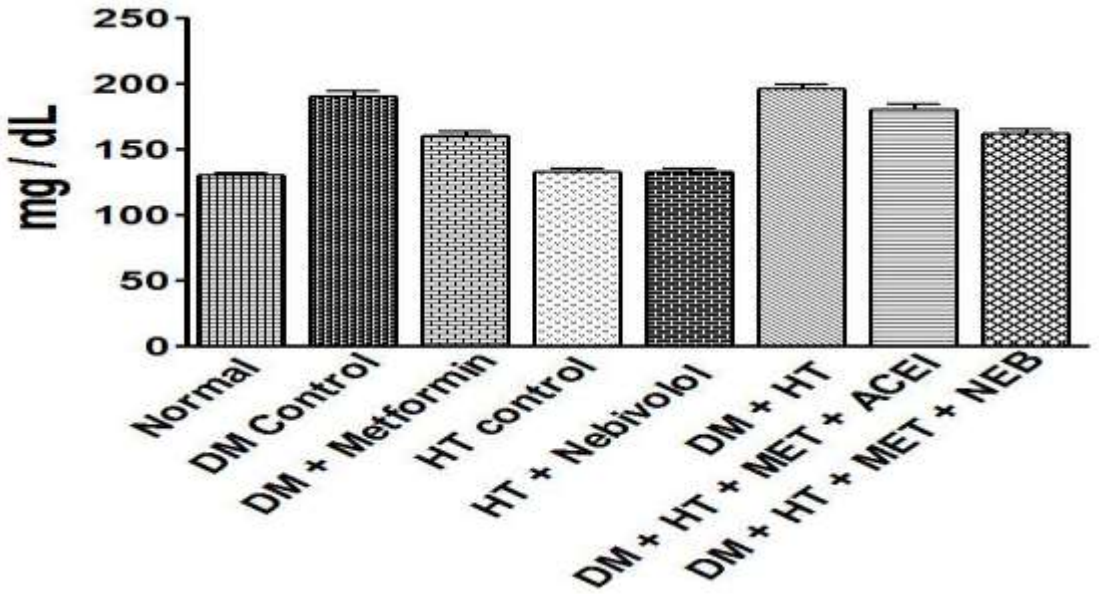


Figure 2: Serum total cholesterol in the normal, disease control and treatment groups (chart 3)

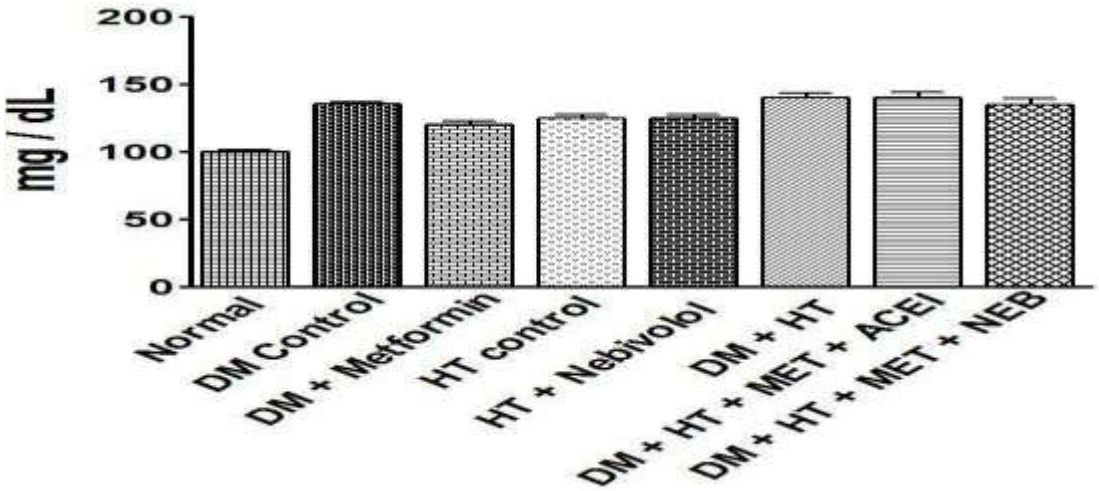


Figure 3: Blood urea in the normal, disease control and treatment groups (chart 4)

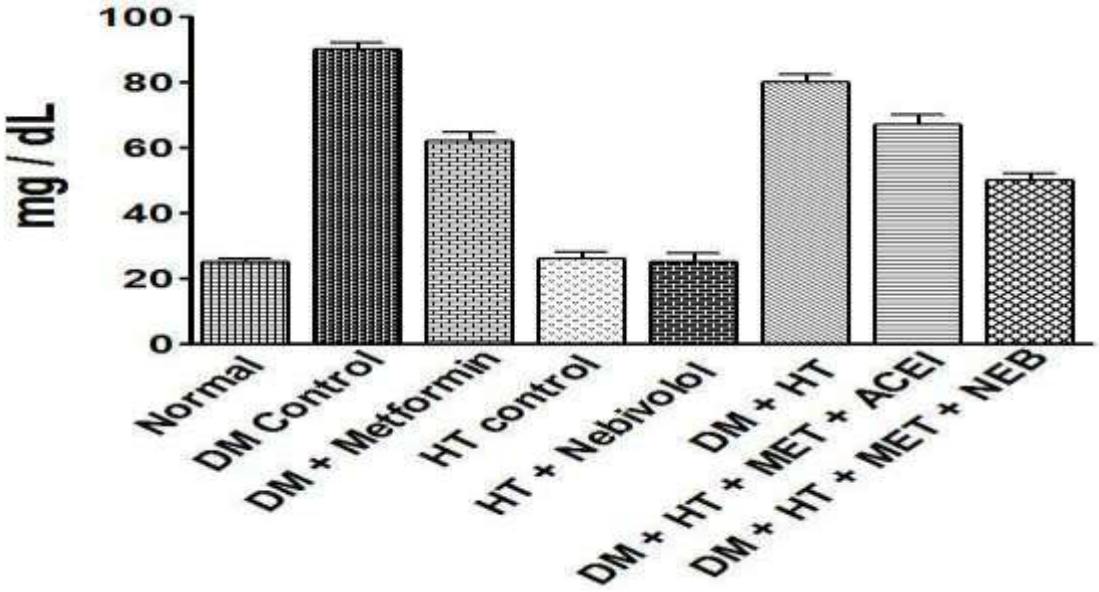
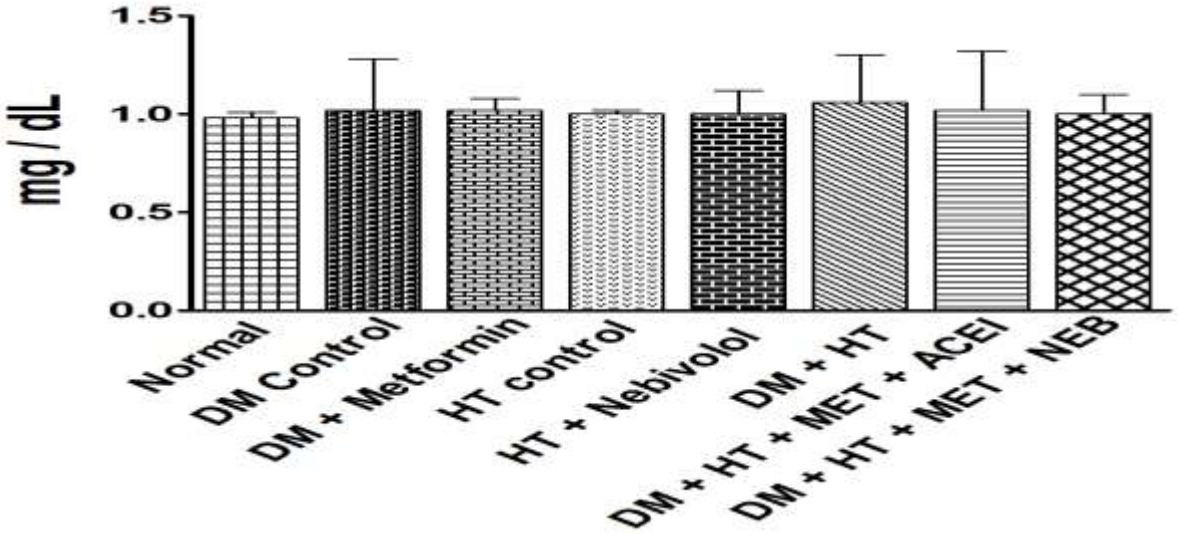


Figure 4: Blood creatinine in the normal, disease control and treatment groups (chart 5)



DISCUSSION:

Nebivolol clinical significance is evidenced by its pharmacological mechanism of action on the pathogenesis reversal, at the primary stage of diabetes hypertension ie, endothelial dysfunction, oxidative stress, advanced end glycosylate (AEG) product formation, autooxidation of lipids by caveole activation pathway. In addition, it has more benefits in the prevention of end organ damage (kidney) as evidenced by this study. Reversal of renal bio chemical parameters

favourable with Nebivolol and it is statistically significant. Serum creatinine changes is not drastic, in terms of statistical significance, because of short term treatment. But has drastic importance at clinical level, in terms of, the short period of treatment(4 weeks) This preclinical study paves the way for, the new indication of Nebivolol, in diabetic hypertensives,, in the prevention of diabetic nephropathy. After clinical trial in human, Nebivolol has the potential to replace the status of, ACE inhibitor as the first choice drug in diabetics, in the view of prevention of end organ damage.

ACKNOWLEDGEMENTS:

My sincere thanks to, my parents, for their moral support and finanical help, my project guides, Dr kothai (PSG animal house secretary),Dr Anil mathew (bio statistician)

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