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OLMESARTAN & PIOGLITAZONE COMBINATION IN EFFECTIVE TREATMENT OF DIABETIC NEPHROPATHY

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ABSTRACT

Diabetes mellitus is a major global life-threatening disease with profound public health consequences. Evidence has accumulated indicating that hyperglycaemia associated with generation of free radicals and subsequent enhanced oxidative stress may play an important role in the pathogenesis of diabetic nephropathy. Olmesartan, Pioglitazone are two antidiabetic drugs acting by two different mechanism. So the present study was carried out to evaluate the effect of Olmesartan, Pioglitazone and their combination on Diabetic Nephropathy by evaluating the various biochemicals, oxidative and histopathological parameters. Wistar albino rats (150-200g) purchased from Kolkata were used in this study. Alloxan induced diabetes followed by estimation of albumin, blood glucose level and total cholesterol were measured for assurance of kidney damage. Depending on the results obtained in the study we conclude that the combination of antidiabetic drug Pioglitazone hydrochloride & antihypertensive drug Olmesartan medoxomil at high dose was found to be more effective & beneficial than low dose combination, or either of the drugs given individually.

Key words: *Olmesartan, Pioglitazone, Total Cholesterol, Diabetic nephropathy, Albumin*

INTRODUCTION

Diabetes mellitus is a major global life-threatening disease with profound public health consequences. Although it has been centuries since diabetes was first recognised, it is still poorly understood and generally poorly managed. Consequently, the global prevalence of diabetes is predicted to rise from 135 million in 1995 to 300 million by 2025[1] Diabetes mellitus is defined as a metabolic disorder of multiple aetiology characterised by chronic hyperglycaemia with disturbances of carbohydrate; lipid and protein metabolism resulting from defects in insulin secretion, insulin action or a combination of both. It may present with characteristic symptoms such as polydipsia, polyuria, polyphagia, weight loss and the long-term effects of diabetes include progressive development of microvascular complications, particularly in the eye and kidney, and an increased frequency of macrovascular disease such as peripheral vascular and coronary heart disease [2, 3].

The commonest of the systemic disease involving the kidney is diabetes mellitus. Since the advent of insulin therapy and improve survival of subjects with diabetes nephropathy has proved to be an important consequence of mortality. Diabetic nephropathy plays a significant role as one cause of end stage renal failure in the western world. Diabetic nephropathy is a clinical syndrome characterized by persistent albuminuria, arterial blood pressure elevation, a relentless decline in glomerular filtration rate (GFR), and an associated high risk of cardiovascular morbidity and mortality [4]. This major life-threatening complication develops in approximately 35% of subjects with type 1 diabetes [5]. The prevalence in type 2 diabetes is higher than type 1; this form of diabetes now contributes to at least 50% [6] of those with diabetic nephropathy who develops end stage renal disease (ESRD) and requires dialysis or transplantation for survival. Evidence has accumulated indicating that hyperglycaemia associated with generation of free radicals and subsequent enhanced oxidative stress may play an important role in the pathogenesis of diabetic

nephropathy. These processes are believed to reflect biochemical/pathophysiological changes in the diabetic kidney, resulting in both glomerular and tubular insult that may ultimately lead to the development of early diabetic nephropathy. However, the exact pathogenic processes, and the time needed to develop renal dysfunction involved in the development of diabetic nephropathy have not yet been clearly defined. Diabetic nephropathy is one of the most serious micro vascular complications of diabetes. The pathologic changes of diabetic nephropathy are characterized by early glomerular hypertrophy and later glomerulosclerosis and tubulointerstitial fibrosis, which are caused by the accumulation of extracellular matrix (ECM) components in the glomerular mesangium and tubulointerstitium, variety of clinical syndromes are associated with diabetic nephropathy that includes the asymptomatic proteinuria, nephritic syndrome, progressive renal failure and hypertension. Pioglitazone effects may be mediated by a reduction of insulin resistance. Pioglitazone appears to act via activation of specific nuclear receptors (peroxisome proliferator activated receptor gamma) leading to increased insulin sensitivity of liver, fat and skeletal muscle cells; Thiazolidinediones (Pioglitazone) could reduce the urinary protein excretion in patient with Diabetic nephropathy, HbA1c can be reduced during the early stage, weight gain may be observed with higher dose, pioglitazone have been accompanied by an improvement in insulin sensitivity, reduced total plasma triglycerides and free fatty acids, and increased HDL-cholesterol levels, where as olmesartan has two primary actions: it reduces inflammation by blocking the Nuclear Factor-kappaB cytokine pathway and it is an agonist of the Vitamin D Receptor (VDR). As a VDR agonist, olmesartan activates the innate immune response which helps in maintaining the immune system to prior bacterial infections, one of the adverse effects include hyperglycemia which will be a plus point, The antihypertensive effect is dose-dependent and has a long duration of action, From hemodynamic studies it appears that the antihypertensive effect is due to dilation of blood vessels throughout the body; however, regional blood flow in major organs is unaffected except for the kidney, where blood flow is markedly increased. So the present study is carried out to evaluate the effect of Olmesartan, Pioglitazone and their combination on Diabetic Nephropathy by evaluating the various biochemicals, oxidative and histopathological parameters.

MATERIAL AND METHOD

Chemicals and Diognostic Kits

Standard drug Pioglitazone and Olmesartan were obtained from INTAS Pharmaceuticals Ltd., (Dehradun) U.K as a gift sample. Alloxan monohydrate obtained from Lobachem Pvt. Ltd, Creatinine kit obtained from Span Diagnostic Ltd, BUN obtained from Span Diagnostic Ltd, Albumin kit, HDL-C kit and Triglycerides kit obtained from Span Diagnostic Ltd.

Animals

Wistar albino rats (150-200g) purchased from Kolkata was maintained in the Department animal house for experimental purpose. Then all the animals were acclimatized for seven days under standard husbandry conditions, i.e.; room temperature of 25 ± 1^0 C; relative humidity 45-55% and a 12:12h light/ dark cycle. The animals had free access to standard rat pellet (Vinod Agro Industries Ltd, Vadodara, India), with water supplied *ad libitum* under strict hygienic conditions. Each experimental group had separate set of animals and care was taken to ensure that animals used for one response were not employed elsewhere. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of non-specific stress. The approval of the Institutional Animal Ethical Committee (IAEC) of SLT Institute of Pharmaceutical Sciences, Bilaspur (Chhattisgarh) was taken prior to the experiments. All the protocols and the experiments were conducted in strict compliance according to ethical

principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Approval No. IEAC/ Pharmacy/ 2012/ 37).

Induction of Diabetes Mellitus

Animals were kept on fasting for 24 hours and single dose of alloxan monohydrate (150 mg/kg) was given (ip) to the animals according to their body weight. Animal were provided 5% glucose solution after alloxan administration to avoid excess hypoglycemia. Blood glucose level was checked after 72 hours using blood glucose strips collecting blood from tail vein. Animals with blood glucose level 280 mg/dl were selected for the further experiment and provided one month stabilization period provided for induction of diabetic nephropathy.

Induction of Diabetic Nephropathy

After one month animals were kept in metabolic cage for 24 hour urine collection. Parameters like albumin, creatinine clearance and urine volume were measured for assurance of kidney damage which was average 220±11.2 mg/dl, 35±9.8 ml/ min, 9.7± 10.9 ml/day respectively. Albumin and creatinine is the good marker of kidney damage. Animals having kidney damage further proceed for the treatment.

Experimental Design and Treatment Schedule

Wistar rats, weighing (150-200 gm) were divided into 5 groups consisting of 6 animals in each group. After induction of diabetic nephropathy animals with blood glucose level 220±10.2 mg/dl were used for the further studies. The treatment of drug is as follows for next one month. Group A: Served as **Normal control** which received normal saline (6 ml/kg, P.O.).Group B: Served as **Standard 1** group which received Olmesartan (20 mg/kg, P.O.). Group C: Served as **Standard 2** which received Pioglitazone (15 mg/kg, P.O.). Group D: Served as **Test 1** this received Low dose combination group [Olmesartan (20 mg/kg) + Pioglitazone (15mg/ kg, P.O.)].Group E: Served as **Test 2** this received High dose combination group [Olmesartan (40 mg/kg) + Pioglitazone (30mg/ kg, P.O.)]. Treatment schedule was planned for one month and the route of drug administration was by oral route before meal. GroupA (normal diabetic group) was given normal saline for a month. Group B was given suspension of standard drug Olmesartan orally at a dose of 20 mg/kg, body weight once daily before meal. Group C was given suspension of standard drug Pioglitazone at a dose of 15 mg/kg, body weight. Group D was given suspension in combination of Olmesartan and Pioglitazone at dose of 20 mg/kg and 15 mg/kg respectively. Group E was given same combination at high dose of Olmesartan (40 mg/kg) + Pioglitazone (30 mg/kg).

Collection of blood sample

After 28 days rats were anesthetized with diethyl ether and blood was withdrawn from cardiac puncture and collected in tube. Blood was centrifuged for separating serum from blood and biochemical parameters like Glucose, HDL-C, LDL, Insulin, Glycated HbA1c, BUN, creatinine and albumin were analyzed as describe by Herck et al., 2001.

Collection of urine sample:

Animals were accommodates in metabolic cage for 24 hours. Urine sample were collected from all groups of the animals to determine urine volume, albumin and Creatinine.

Estimation of Biochemical Parameters:

Blood glucose level: Blood sample was collected from rats and glucose level was checked by the One Touch Horizon glucometer using glucose strip and levels were expressed as mg/dl (One Touch Horizon test strip).

Albumin content

Three solutions were prepared such as Blank (reagent 1), Test (Serum (10µL) + Reagent 1 (1000µL)), Standard (Reagent 1(1000µL) + Reagent 2 (10µL)). Solutions were mixed well and incubated at room temperature (15-30) ° C for 1 minute. Analyzer was programmed and then aspirated the reagent Blank in Analyser. Measured absorbance of standard followed by test. The content of albumin determined by formula Albumin (g/dL) = (Abs. of test / Abs. of Standard) × 4

HDL cholesterol

Decreased levels of HDL Cholesterol lead to increased chance of coronary heart disease while increased levels of HDL Cholesterol reduce these chances. Lower values of HDL Cholesterol & increased ratio of total cholesterol to HDL Cholesterol are taken as risk factor for CHD. High density lipoproteins (HDL) are obtained in the supernatant after centrifugation and determined by the method of Wybenga et al., 1970[7] by using formula.

Serum/Plasma HDL- Cholesterol (mg/dl) = $\frac{O.D.Test}{O.D.Standard} \times 50$

RESULTS

Blood glucose concentration was 225.33±6.74 (mg/dL) in diabetic control group, 177±14.27 in olmesartan (Standard1) treated group, 178.8±10.94 in pioglitazone (Standard2) treated group, 144.16±17.33 in low dose (Test1) combination & 119.83±8.90 in high dose (Test2) combination group. As seen in (Table 1) and illustrated in (Figure 4.3.1), Alloxan-induced diabetic rats treated with test2, showed a significant ($P<0.05$, ANOVA) decrease in blood glucose level when compared to diabetic control group. The decrease in the glucose level is dose dependent.

Table-1: Blood glucose level (mg/dL) at various stages during study with Mean± SD.

Time (Days)	Normal Control	Standard 1	Standard 2	Test 1	Test 2
1st day	221	180	179	150	112
7th day	215	162	180	136	132
13th day	230	176	196	169	120
19th day	233	195	186	135	118
22st day	225	180	168	155	109
28th day	230	175	155	120	128
Mean± SD	**225.33±6.74**	**177±14.27****	**178.8±10.94****	**144.16±17.33****	**119.83±8.90***

N= 6 albino rats per groups, tabular value represents- Mean± SD

*: significantly different with $p<0.05$,

**: significantly different with $p<0.01$, p is significant when compared with normal control.

The mean value of albumin was 4.17±0.25 in diabetic group, 4.20±0.30 in olmesartan group, 4.29±0.42 in pioglitazone group, 4.0.±0.17 in low dose combination group, & 3.98±0.39 in high dose treated group. As shown in table-2, Alloxan-induced diabetic rats treated with test2, showed a significant (P<0.05, ANOVA) decrease in albumin level when compared to their diabetic control group. The decrease is dose dependent.

Table 2. Blood Albumin level (mg/dL) at various stages during study with Mean± SD.

Group Time (Days)	**Standard Diabetic**	**Std Dia+ OM**	**Std Dia+PGZ**	**Std Dia+ OM+ PGZ Low dose (15+30)mg**	**Std Dia+ OM+PGZ High dose (30+60)mg**
1st day	4.24	3.79	3.72	3.88	3.35
7th day	3.49	3.95	4.36	4.26	4.46
13th day	3	4.32	4.22	4.19	4.2
19th day	4.79	4.12	4.56	4.12	3.84
22st day	3.36	4.68	4.37	3.91	4.22
30th day	4.16	4.89	3.98	3.86	3.86
Mean± SD	**4.17±0.25**	**4.20±0.30****	**4.29±0.42*****	**4.03±0.17****	**3.98±0.39***

N= 6 albino rats per groups, tabular value represents- Mean± SD

*: significantly different with p<0.05, **: significantly different with p<0.01,

***: significantly different with p<0.001, ns: non-significant with p>0.05

The mean value of HDL-C was 27.16±2.92 in normal control group, 30.16±3.92 in standard1 group, 36.33±2.73 in standard2 group, 34.0.±4.05 in test1 group, & 39.83±4.99 in test2 group. As shown in table-3, Alloxan-induced diabetic rats treated with test2, showed a significant (P<0.05, ANOVA) increase in blood HDL-C level when compared to their diabetic control group, which is in dose dependent manner.

Table-3 Blood HDL-C (mg/dL) level at various stages during study with Mean± SD.

Group Time (Days)	Normal Control	Standard 1	Standard 2	Test 1	Test 2
1st day	25	32	30	31	36
7th day	29	38	28	28	30
13th day	22	36	37	24	27
19th day	27	40	32	29	22
22st day	26	37	28	32	30
30th day	24	35	26	36	34
Mean± SD	27.16±2.92	30.16±3.92**	36.33±2.73*	34.0±4.05**	39.83±4.99*

N= 6 albino rats per groups, tabular value represents- Mean± SD

*: significantly different with p<0.05, **: significantly different with p<0.01,

***: significantly different with p<0.001, ns: non-significant with p>0.05

DISCUSSION

The present investigation revealed that induction of diabetes using alloxan resulted in hyperglycemia that was accompanied by loss of body weight, increase in kidney weight to body weight ratio, lipid abnormalities and progressive renal damage as indicated by polyuria, elevation in serum creatinine level, increase in urinary albumin excretion, proteinuria and reduction in creatinine clearance as indicator of GFR. Our results are similar to previous studies [8]. In the present study, hyperglycaemia resulted in increased kidney to body weight ratio reflecting renal hypertrophy. The precise mechanism of renal hypertrophy in diabetes is still unclear. Several growth factors have been proposed in mediating the development of renal hypertrophy including angiotensin II, TGF-β_1, insulin like growth factor-1, platelet derived growth factor and hepatocyte growth factor. Among them, TGF-β_1 is regarded as one of the most important factors contributing to renal hypertrophy and subsequent renal fibrosis. TGF-β_1 can arrest cells in G_1-phase of the cell cycle through induction of cyclin-dependent kinase (CdK) inhibitors such as p27 and p21 leading to the development of renal hypertrophy. In the current investigation, induction of diabetes was accompanied by impaired renal function revealed by elevated albumin excretion rate and reduced GFR. These data are similar to those described by other researchers. Microalbuminuria can occur early in the development of diabetic nephropathy. Microalbuminuria is associated with poor glycemic control in diabetic patients and glycemic control is considered to be a predictor of survival for the diabetic patients on hemodialysis with end-stage renal disease. In the current investigation, hyperglycaemic animals have lipid profile abnormalities manifested by an increase in serum levels of total cholesterol. There is growing recognition of the importance of hyperlipidaemia and dyslipidaemia in the progression of microvascular disease in diabetes and the development of chronic renal disease [9]. Studies in humans have supported the relationship between dyslipidaemia and progression of chronic kidney disease [10, 11]. Recently, researchers reported the association of dyslipidemia and inflammatory markers with decreased renal function among middle-aged and older type 2 diabetes, a population at high risk for chronic kidney disease. Treating dyslipidaemia can slow the progression of chronic renal disease.

CONCLUSION

Depending on the results obtained in the study we conclude that the combination of antidiabetic drug Pioglitazone hydrochloride & antihypertensive drug Olmesartan medoxomil at high dose was found to be more effective & beneficial than low dose combination, or either of the drugs given individually. These studies suggest that AT1 receptor blockade by OM may be an effective mechanism for the prevention and treatment of nephropathy in type II diabetic patients and potentially in those patients that develop end-stage renal disease (ESRD), while TZDs are also potential therapeutic agents for diabetic nephropathy that may prevent glomerular dysfunction independent of their insulin-sensitizing action through the inhibition of the DAG-PKC-ERK pathway. The combination would be more effective for the treatment of the diabetes & hypertension occurring in Diabetic Nephropathy as it would help in curing of both the symptoms simultaneously, which would be actually a greater benefit for the patients.

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