Research Article

www.ijrap.net

EFFICACY OF NATEGLINIDE AND PIOGLITAZONE COMBINATION THERAPY COMPARED WITH MONOTHERAPY ON DIABETIC NEPHROPATHY

Papiya Bigoniya *, Reetesh Malvi
Department of Pharmacology, Radharaman College of Pharmacy, Ratibad, Bhopal, India

*Corresponding author
E-mail: p_bigoniya2@hotmail.com

ABSTRACT

The objective of present work is to explore combined effect of nateglinide and pioglitazone on diabetic nephropathy. Nateglinide and pioglitazone were administered to the diabetic nephropathic animals as monotherapy and in median and high dose combination for 28 days. Body weight, relative kidney weight, serum and urine biochemical parameters were analyzed along with kidney tissue antioxidant level and histology. Loss in body weight was efficiently controlled by pioglitazone monotherapy and, nateglinide and pioglitazone high dose combination. Nateglinide treatment significantly ($P < 0.05$-0.001) reduced serum glucose, blood urea nitrogen (BUN), creatinine and insulin along with decrease in urine albumin and increase in creatinine. Both nateglinide and pioglitazone has significantly ($P < 0.05$-0.01) reduced glomerular filtration rate (GFR). Nateglinide has nonsignificant effect on kidney antioxidant level but pioglitazone has increased ($P < 0.05$) superoxide dismutase (SOD) level. Both median and high dose combination significantly ($P < 0.05$-0.001) reduced the elevated level of serum glucose, cholesterol, triglyceride, low density lipoprotein, BUN, creatinine, insulin and glycated haemoglobin. Combination doses were equally effective in decreasing albumin and increasing creatinine urinary excretion with increase in GFR. High dose combination significantly increased catalase, SOD and glutathione level in the kidney tissue. Monotherapy with nateglinide and pioglitazone reduce hyperglycemia and improve kidney functional ability. Both high and median dose combination of nateglinide and pioglitazone was found effective against disturbed serum and urine biochemical parameters. High dose combination was more effective and beneficial in ameliorating kidney pathological damage and oxidative stress in the treatment of diabetic nephropathy.

Keywords: Diabetic nephropathy, Nateglinide, Pioglitazone, antioxidant level, histopathology.

INTRODUCTION

Diabetic pandemic associated with metabolic complications is a major cause of morbidity and mortality worldwide making it a critical public health issue[1]. Diabetic nephropathy is clinically characterized as the progressive development of renal insufficiency in the setting of hyperglycemia coupled with rise in urine albumin excretion and blood pressure leading to decline in glomerular filtration and eventually end stage kidney failure[2]. Diabetic nephropathy is the most common cause of end stage renal disease associated with an increased cardiovascular risk[3]. Increased oxidative stress in diabetes plays important role in the development and progression of diabetic vascular complications, including nephropathy[4]. Diabetic nephropathy is characterized by hypertension and a relentless decline in kidney function, increased urinary excretion of protein and transforming growth factor-β[5]. Adequate control of blood glucose may slow the rate of diabetic nephropathy progression but in longer term it is difficult to achieve strict glycemic control on diabetic patient due to the limitations of available therapeutic approaches[6]. Despite the availability of treatments that lower blood glucose and blood pressure, many diabetic patients are still prone to development of kidney failure, indicating need for new therapeutic approaches based on combination drug therapy with different mechanisms of action. Nephropathy affects 30-40% long-term insulin dependent diabetes patients under the age of 31 years. Oxidative stress and inflammation are thought to be particularly important in mediating the events related to diabetic nephropathy.

Thiazolidinedione class, pioglitazone reduces urinary protein and growth factor-β excretion in patient of type 2 diabetes with microalbuminuric diabetic nephropathy[7]. Pioglitazone improves glycaemic control in people with type 2 diabetes by increasing insulin sensitivity through its action at peroxisome proliferator-activated receptor (PPAR) gamma 1 and PPAR gamma 2, and affects lipid metabolism through action at PPAR alpha[8]. Pioglitazone shows protective effect on obesity related diabetic nephropathy by reducing renal oxidative damage and advance glycation end-product deposition[9]. In patients with advanced diabetic nephropathy pioglitazone reduces proteinuria over 4 months[10]. Nateglinide is a short-acting pancreatic beta-cell-selective K(ATP) potassium channel blocker that binds rapidly to the sulfonylurea SUR1 receptor with a relatively low affinity and dissociates extreme rapidly with a unique "fast on-fast off" effect. Nateglinide stimulates and restores the normal physiology related first and early phase insulin secretion, consequently reducing postprandial hyperglycemia leading to improved glycemic control. In contrast to sulfonylureas, nateglinide increases pancreatic cell sensitivity to ambient glucose without increasing basal insulin secretion[11]. Nateglinide not only achieve improved overall glucose control, but also reduces the risk of vascular complications in the treatment of type 2 diabetes[12]. Nateglinide is well tolerated and suitable for use in diabetic patients with impaired renal function, undergoing dialysis due to comparable absorption and elimination profiles in renal impaired as well as in healthy subjects not requiring any dose adjustment[13].

Treatment of type 2 diabetes should ideally aim to restore and sustain the normal relationship between insulin sensitivity and secretion. Insulin sensitivity and insulin secretion are reciprocally related. Insulin resistance is adapted by increased insulin secretion to maintain normal glucose and lipid homeostasis. Nateglinide is an insulin-secretion enhancer that restores early-phase insulin secretion, making it evident that

nateglinide would be more effective when used in combination with a thiazolidinedione like insulin sensitizer. Pioglitazone do not stimulate insulin release, therefore, are potentially suitable candidates for combination therapy with an insulin-secretion enhancer, such as nateglinide[14]. The components of this combination therapy would function by different mechanisms targeting multiple pathophysiological targets. Metformin is very commonly used as combination therapy with pioglitazone and is a fixed dose combination (FDC) approved by USFDA for type 2 diabetes[15]. Patients previously failed on oral antidiabetic monotherapy, showed greater reductions of glycemic parameters with combination of repaglinide with pioglitazone having acceptable safety than therapy using either agent alone[16]. Antidiabetic drug combinations are associated with improved compliance and improved glycemic control. FDC of sulfonylurea and rosiglitazone showed improved antidiabetic compliance and HbA1c levels and similar data also exists with glibenclamide and metformin[17,18].

Literature survey showed unavailability of report regarding the effect of nateglinide monotherapy and its combination with pioglitazone on the diabetic nephropathy. Studies are needed to determine whether nateglinide in combination with pioglitazone can help to achieve treatment goal targeting type 2 diabetes. The study was designed to evaluate the effect of nateglinide and pioglitazone mono and combination therapy on renal functions of diabetic nephropathic rats. This article summarizes data concerning the efficacy of nateglinide and pioglitazone as monotherapy and combination therapy.

MATERIALS AND METHODS
Experimental Animals

Animal experiments were carried out with Wistar albino rats. Healthy albino rats of either sex weighing approximately 150-200 gm were procured from institutional animal house and provided standard laboratory diet with water ad libitum. They were kept in clean and dry polypropylene cages with paddy husk bedding and maintained at 65% ± 2 RH and 22 ± 2°C temperature with 12 hour light: dark cycle. Ethical committee approval was obtained from Institutional Animal Ethical Committee of Radharaman College of Pharmacy, (Reg. no. 1169/ac/08/CPCSEA), Bhopal, before carrying out the experiments. All the animal experiments were conducted according to guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi.

Induction of diabetes and diabetic nephropathy

Animals (45 rats) were kept on fasting for 24 hours and single dose of alloxon monohydrate (150 mg/kg) was given intraperitoneally (i.p) according to body weight. Animals were provided with 5% glucose solution immediately after alloxan administration to avoid excess hypoglycemia and provided with normal diet. Blood glucose level was checked after 72 hours using Glucocheck blood glucose strips (Rapid Diagnostic Pvt. Ltd., Delhi) collecting blood from tail vein. Animals with blood glucose level > 200 mg/dl were considered diabetic and selected for the further experiment. Hyperglycemia maintenance was confirmed by the elevated fasting glucose levels in blood determined 15 days after injection[19]. Diabetic animals were kept with free access to food and water *ad libitum* for one month.

After 30 days stabilization animals were kept in metabolic cage and 24 hr urine sample was collected to determine the total volume, content of albumin and creatinine. Blood urea nitrogen (BUN) and creatinine content was estimated in blood sample

drawn at some point during the 24-hour period. Creatinine clearance based glomerular filtration rate (GFR) and albumin: creatinine ratio was calculated. Content of serum BUN, and urine albumin, albumin: creatinine ratio and creatinine clearance based GFR was found to be on an average 22.45 ± 1.70 mg/dl, 69.75 ± 2.90 mg/dl, 7.02 and 0.16 ± 0.01 ml/min/100 gm respectively signifying initiation of kidney damage. Albumin and creatinine is the important marker of kidney damage. Animals having kidney damage were further proceeded for treatment with the drug under study[20].

Experimental Design

A total number of 36 animals inducted with diabetic nephropathy (with average blood glucose level of 220 ± 10.2 mg/dl) were divided in six groups as follows and were treated with experimental drugs for next 28 days.

Group A: Served as nondiabetic control (NC) group administered with vehicle.
Group B: Served as a control diabetic nephropathic (DN) group administered with vehicle.
Group C: DN + nateglinide (60 mg/kg).
Group D: DN + pioglitazone (15 mg/kg).
Group E: DN + median dose combination (nateglinide 60 mg/kg + pioglitazone 15 mg/kg).
Group F: DN + high dose combination (Nateglinide 120 mg/kg + pioglitazone 30 mg/kg).

Suspension of nateglinide and pioglitazone was prepared in 5% DMSO in double distilled water and administered orally at the given doses once daily.

Evaluation parameters

Body weight of all animals were recorded on day zero and drugs were administered as per protocol. On 28th day of drug administration body weight of rats were recorded and placed in metabolic cages for 24 hour. After 24 hour urine sample were collected to determine total urine volume, albumin and creatinine level. Creatinine clearance based GFR was calculated following Cockcroft and Gault formula developed in 1973. On 29th day rats were anesthetized, sacrificed by cardiac puncture and blood was collected in tubes[21]. Both the kidneys were isolated, kept in ice cold saline, blotted in filter paper and weighted. Relative weight of kidneys (kidney weight/100 gm of body weight) was calculated and recorded. A part of kidney was preserved in cold saline for estimation of free radical scavenging activity and reminder in 10% formalin for histopathology.

Blood was kept at room temperature for 30 mins and centrifuged at 3000 rpm for separating serum. Glucose level was determined with the help of Glucocheck glucometer. Serum biochemical parameters like cholesterol, triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein cholesterol (LDL), BUN, creatinine and albumin were determined with the help of diagnostic kits (Span Diagnostics Ltd, India) using auto analyzer Star 20 (Rapid Diagnostic Pvt. Ltd., Delhi). Serum insulin level was measured by solid-phase radioimmunoassay method (Merck Millipore, Germany) and glycated hemoglobin (HbA1c) by ion exchange resin method using the auto analyzer.

Kidney samples were fixed in 10% formalin and embedded with paraffin. Permanent slides were prepared by cutting tissue sections (10 μm) and staining with hematoxylin and eosin. Photomicrographs were taken at 40×magnification[22]. Liver homogenate (5%) in 10% TCA was prepared using a Teflon-glass tissue homogenizer (Remi, India) and centrifuged at 6000

rpm (4°C) for 10 min. The supernatant was diluted 10-fold in phosphate buffer, kept on ice and used for the estimation of catalase and glutathione. Homogenate was also prepared using 0.25% sucrose in phosphate buffer for estimation of superoxide dismutase. Catalase was determined as per the method of Aebi[23]. Decomposition of H_2O_2 by catalase is directly proportional to decrease in absorbance at 340 nm. The results were expressed as unit of H_2O_2 decomposed/min/mg of protein, using molar extinction coefficient of H_2O_2. Superoxide dismutase (SOD) was estimated as per the method of Misra and Fridovich[24]. One enzymatic unit of SOD is the amount of protein in the form of enzyme present in 100 μl of sample required to inhibit the reduction of 24 micromolar nitro-blue tetrazolium by 50% and is expressed as unit/mg of protein. Glutathione (GSH) is a sulfhydryl antioxidant, antitoxin and enzyme cofactor. GSH reduces H_2O_2 directly to water or react directly with the free radicals such as O_2*, $OH*$, O^{-*} by a radical transfer process, which yields thiol radicals. This thiol radical and the sulfhydryl group present in glutathione forms a colored complex with 5,5'-dithio-bis-2-nitrobenzoic acid, measured colorimetrically at 412 nm[25]. All these estimations were done using spectrophotometer UV-1800 (Shimadzu, Japan).

Statistical analysis

The results were expressed as mean ± SD for each parameter investigated. Differences among the groups were analyzed by one-way analysis of variance (ANOVA). Significant differences between treatment groups were determined by Dunnett t-test. P value > 0.05 were considered significant.

RESULTS
Body weight and relative kidney weight

Nondiabetic control group showed 19.21% rise in body weight after 28 days of treatment whereas diabetic nephropathic group showed 11.22% increase only. Nateglinide, pioglitazone, median dose and high dose combination showed respectively 4.52, 16.04, 7.30 and 13.52% weight gain. Diabetic nephropathic group showed significant (P < 0.001) increase in kidney weight. High dose combination group has significantly (P < 0.05) reduced kidney weight, all other treatment groups had nonsignificant effect on kidney weight (Table 1).

Serum biochemical parameters

Diabetic nephropathy induction in experimental animals has caused significant rise in serum glucose, cholesterol, TG, LDL, BUN, creatinine, insulin and HbA1c level and decrease in HDL. Nateglinide 60 mg/kg treatment has caused significant (P < 0.05-0.001) decrease in glucose, BUN, creatinine and insulin level where as pioglitazone 15 mg/kg has decreased glucose, cholesterol, BUN, creatinine, insulin and HbA1c level. Both median and high dose combinations have significantly (P < 0.05-0.001) reversed all the altered biochemical parameters with decrease in glucose, cholesterol, TG, LDL, BUN, creatinine, insulin and HbA1c level and increase in HDL (Table 2).

Urine biochemical parameters

Induction of diabetic nephropathy was confirmed with significant (P < 0.001) increase in total urine volume and albumin level, and decrease in creatinine and GFR. Pioglitazone has showed significant (P < 0.05-0.001) decrease in urine volume and albumin level, and increase in creatinine and GFR whereas nateglinide had less but similar profile except urine volume and albumin excretion. Median and high dose combination group had similar effect on urine biochemical parameters as significant (P < 0.01-0.001) decrease in urine volume and albumin level, and increase in creatinine and GFR. Albumin creatine ratio was respectively 0.15, 8.68, 0.90 and 0.66 in nondiabetic control, diabetic nephropathic, median and high dose combination groups (Table 3).

Kidney antioxidant level

Catalase, SOD and GSH content of diabetic nephropathic group was significantly (P < 0.001) reduced compared to nondiabetic control group. Nateglinide do not have any significant effect on kidney tissue antioxidant level where as pioglitazone and median dose combination has shown significant (P < 0.05-0.01) increase in SOD level only. High dose combination of nateglinide and pioglitazone has significantly (P < 0.05-0.001) elevated kidney tissue catalase, SOD and GSH content (Table 4).

Histopathology

Light microscopic examination of kidney from diabetic nephropathic group animal revealed glomerulopathy characterized by the thickening of glomerular basement membrane, mesangial matrix expansion and tubular interstitium damage. Treatment with nateglinide and pioglitazone showed slight reduction in GBM thickening and tubular interstitium damage. Median dose combination of nateglinide and pioglitazone showed reduced glomerular matrix expansion with presence of tubular damage but high dose combination has efficiently reduced glomerular matrix expansion and tubular dilation (Figure 1).

Table 1: Effect of nateglinide and pioglitazone treatment on body weight and kidney weight of diabetic nephropathic rats

Treatment (mg/kg, p.o)	Body weight in gm		Kidney weight in gm/100 gm body weight
	Zero day	28th day	
Nondiabetic control (NC)	230.66 ± 4.20	255.76 ± 5.10 (+ 19.21%)	0.36 ± 0.02
Diabetic nephropathic (DN)	223.33 ± 5.45	237.66 ± 5.14 (+ 11.22%)	0.62 ± 0.06c
DN + Nateglinide (60 mg/kg)	237.50 ± 6.29	243.55 ± 4.57 (+ 4.52%)	0.53 ± 0.04ns
DN + Pioglitazone (15 mg/kg)	221.02 ± 5.33	244.15 ± 4.68 (+ 16.04%)	0.58 ± 0.05ns
DN + Nateglinide + Pioglitazone (60+15)	255.22 ± 6.73	267.46 ± 6.04 (+ 7.30%)	0.49 ± 0.03ns
DN + Nateglinide + Pioglitazone (120+30)	242.33 ± 5.22	261.58 ± 5.62 (+ 13.52%)	0.44 ± 0.02*

All values are Mean ± SEM of 6 rats per group. cp < 0.001 when compared to nondiabetic control group. *p < 0.05 and ns = non significant when compared to diabetic nephropathic group. Values in parenthesis indicate percentage change in body weight in respect to zero day value per group.

Table 2: Effect of nateglinide and pioglitazone treatment on serum biochemical parameters of diabetic nephropathic rats

Treatment (mg/kg, p.o)	Glucose (mg/dl)	Cholesterol (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	BUN (mg/dl)	Creatinine (mg/dl)	Albumin (gm/dl)	Insulin (mU/ml)	HbA1c (ng/ml)
Nondiabetic control (NC)	94.44 ± 4.79	116.45 ± 5.68	76.90 ± 3.44	48.69 ± 2.13	65.14 ± 2.57	10.58 ± 1.10	0.68 ± 0.03	4.75 ± 0.42	2.58 ± 0.11	3.12 ± 0.28
Diabetic nephropathic (DN)	222.66 ± 11.46^C	249.95 ± 6.08^C	254.28 ± 10.47^C	28.05 ± 1.29^C	137.16 ± 6.78^C	46.36 ± 2.28^C	1.37 ± 0.13^C	3.03 ± 0.47^{ns}	19.52 ± 1.65^C	9.37 ± 0.93^C
DN + Nateglinide (60)	179.82 ± 7.36*	231.94 ± 7.05^{ns}	234.82 ± 8.42^{ns}	31.15 ± 1.70^{ns}	138.91 ± 7.76^{ns}	22.26 ± 1.05***	1.06 ± 0.07*	3.99 ± 0.66^{ns}	12.37 ± 1.02***	8.36 ± 0.60^{ns}
DN + Pioglitazone (15)	178.43 ± 5.45**	220.51 ± 8.00*	227.45 ± 9.11^{ns}	32.30 ± 1.82^{ns}	115.15 ± 5.82^{ns}	24.03 ± 2.04***	0.98 ± 0.05**	4.40 ± 0.35^{ns}	6.59 ± 0.82***	6.32 ± 0.88*
DN + Nateglinide + Pioglitazone (60+15)	164.45 ± 8.44***	207.30 ± 6.94**	209.35 ± 8.71*	36.85 ± 2.20*	100.23 ± 4.53***	12.82 ± 1.33***	0.88 ± 0.03**	4.60 ± 0.41^{ns}	5.96 ± 0.36***	6.20 ± 0.36*
DN + Nateglinide + Pioglitazone (120+30)	148.20 ± 7.60***	195.98 ± 5.64***	182.86 ± 7.08***	36.64 ± 1.97*	86.45 ± 3.70***	11.07 ± 1.05***	0.76 ± 0.04***	4.91 ± 0.69^{ns}	3.68 ± 0.97***	5.78 ± 0.49*

All values are Mean ± SEM of 6 rats per group. $^C p < 0.001$ when compared to nondiabetic control group. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and ns = non significant when compared to diabetic nephropathic control group. TG = Triglyceride, HDL = high density lipoprotein, LDL = low density lipoprotein, BUN = Blood urea nitrogen, HbA1c = glycated hemoglobin

Table 3: Effect of nateglinide and pioglitazone treatment on urine biochemical parameters of diabetic nephropathic rats

Treatment group (mg/kg, p.o)	Total urine volume (ml/100 gm)	Albumin (mg/dl)	Creatinine (mg/dl)	Albumin: Creatinine ratio	GFR (ml/min/100 gm)
Nondiabetic control (NC)	2.82 ± 0.16	17.80 ± 1.44	118.67 ± 6.83	0.15	0.23 ± 0.002
Diabetic nephropathic (DN)	7.06 ± 0.96^C	154.33 ± 8.02^C	17.76 ± 1.02^C	8.68	0.11 ± 0.008^C
DN + Nateglinide (60)	5.62 ± 0.88^{ns}	119.24 ± 6.91^{ns}	37.60 ± 1.89**	3.17	0.15 ± 0.004*
DN + Pioglitazone (15)	4.25 ± 0.57*	64.75 ± 4.09***	50.53 ± 2.44***	1.28	0.16 ± 0.003**
DN + Nateglinide + Pioglitazone (60+15)	3.81 ± 0.29**	52.90 ± 3.06***	58.55 ± 2.80***	0.90	0.17 ± 0.008***
DN + Nateglinide + Pioglitazone (120+30)	3.62 ± 0.32**	48.16 ± 2.04***	72.52 ± 2.13***	0.66	0.20 ± 0.005***

All values are M ± SEM of 6 rats per group. $^C p < 0.001$ when compared to nondiabetic control group. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ and ns = non significant when compared to diabetic nephropathic control group. GFR = Glomerular filtration rate.

Table 4: Effect of nateglinide and pioglitazone treatment on kidney antioxidant parameters of diabetic nephropathic rats

Treatment group (mg/kg, p.o)	Catalase (µg/min/mg protein)	Superoxide dismutase (µg/mg protein)	Glutathione (mmol/mg protein)
Nondiabetic control (NC)	26.58 ± 2.05	12.70 ± 1.22	48.34 ± 2.78
Diabetic nephropathic (DN)	9.26 ± 0.97^C	2.82 ± 0.54^C	7.92 ± 0.52^C
DN + Nateglinide (60)	9.83 ± 0.60^{ns}	3.37 ± 0.49^{ns}	8.19 ± 0.48^{ns}
DN + Pioglitazone (15)	10.76 ± 0.86^{ns}	4.81 ± 0.34*	9.08 ± 0.94^{ns}
DN + Nateglinide + Pioglitazone (60+15)	12.07 ± 1.20^{ns}	5.33 ± 0.44**	10.58 ± 1.40^{ns}
DN + Nateglinide + Pioglitazone (120+30)	14.86 ± 1.12*	6.20 ± 0.35***	12.23 ± 1.63*

All values are Mean ± SEM of 6 rats per group. $^C p < 0.001$ when compared to nondiabetic control group. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, ns = non significant when compared to diabetic nephropathic control group.
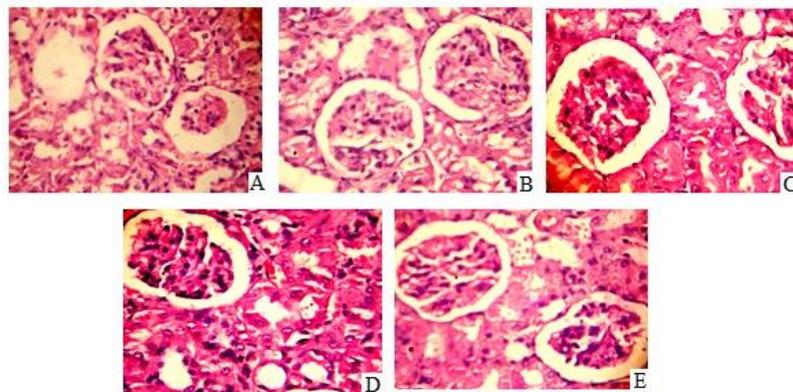

Figure 1: Effect of nateglinide and pioglitazone treatment on kidney histopathology of diabetic nephropathic rats
A: Diabetic glomerulopathy in diabetic control group showing thickening of glomerular basement membrane (GBM), expansion of mesangial cell and tubular interstitium damage. B: After treatment with nateglinide showing slight reduction in GBM thickening and tubular interstitium damage. C: Treatment with pioglitazone showing reduction in GBM thickening, normal tubules and reduced interstitial damage. D: Median dose combination of nateglinide and pioglitazone showed reduced glomerular matrix expansion with presence of tubular damage. E: High dose combination of nateglinide and pioglitazone reduced glomerular matrix expansion and tubular dilation.

DISCUSSION

The incidence of renal failure caused by diabetes is rising dramatically worldwide. Diabetic nephropathy occurs in 30-40% of patients with diabetes with high mortality and morbidity rates. Combination therapy of nateglinide and metformin was more effective than either treatment alone and appeared to be an excellent option for treating patients with type 2 diabetes not controlled with monotherapy[26]. Combination therapy of nateglinide and vildagliptin restored the decrease in pancreatic beta cell mass that can correct postprandial dysmetabolism associated with obese and insulin resistance[27,28].

Present study was designed to investigate the combined effect of nateglinide and pioglitazone on diabetic nephropathy compared to monotherapy. The result of this study revealed that high dose combination treated animal showed a significant reversal of body weight loss as compared to diabetic nephropathic group. Pioglitazone monotreatment produced a better control over reduction in body weight compared to the diabetic nephropathic group. There was a significant increase in kidney weight in the diabetic nephropathic control group which was normalized by high dose combination of nateglinide and pioglitazone. James et al.[29] reported that development of renal hypertrophy during both experimental and human diabetes. Obineche et al.[30] also reported renal hypertrophy evidenced by the increase in the weight of rat kidneys following long term diabetes. Data of the present study demonstrated better reduction in kidney weight compared to the diabetic rat by the high dose combination group.

The results demonstrated that diabetic nephropathy is associated with severe hyperglycemia, dyslipidemia, high BUN/creatinine ratio and insulin resistance. Nateglinide and pioglitazone reduced hyperglycemia both in mono and combination therapy groups but highly significant hypoglycemia effect was produced by combination therapy. Both the drugs are in therapy for diabetes and pioglitazone is reported to have beneficial effect in diabetic nephropathy. Nateglinide and pioglitazone monotherapy did not show corrective action on dyslipidemia but has significantly reduced BUN/creatinine ratio and insulin resistance. Pioglitazone improves glycaemic control in type 2 diabetes by improving insulin sensitivity through its action at PPAR gamma 1 and gamma 2[7,8]. Nateglinide ameliorates insulin resistance as well as insulin secretory defects in type 2 diabetic patients with improvement of insulin sensitivity and beta-cell function[31,32]. Treatment with nateglinide and pioglitazone combination has produced highly significant reduction in BUN.

Zhao et al.[33] showed hypolipidemic effect of pioglitazone as it affects lipid metabolism through action at PPAR alpha[8]. Thiazolidinedione can ameliorate risk of atherosclerosis improving lipid profiles, promoting improvement in adipokine level, and decreasing levels of biomarkers that are crucial in plaque development[34]. Pioglitazone has beneficial effect against low HDL cholesterol levels that is characterize as atherogenic dyslipidemia[35]. Data on HbA1c showed that nateglinide monotherapy had not but pioglitazone mono and combination therapy has significantly reduced the level of HbA1c in both median and high dose. Previous study stated HbA1c level decreasing effect of pioglitazone in the rats. Pioglitazone suppressed 8- hydroxydeoxyguanosine and malondialdehyde level and suppressed the expression of receptor for advance glycation product mRNA and transforming growth factor β mRNA in renal tissue[9]. The present work revealed that level of insulin was significantly decreased by monotherapy and combination therapy of the drugs. Pioglitazone improved insulin sensitivity thereby reducing the high glucose level in blood.

Pioglitazone lowered the high blood lipid level and as a result decreased the glycation reaction suppressing the generation of advance glycation end product[9]. Nateglinide significantly increases insulin secretion in response to high glucose and is known to function as an insulin secretagogue[31]. Nateglinide reduced daily insulin requirement by improving the early phase of insulin secretion thus helps in amelioration of insulin resistance[36].

The present data demonstrated that diabetic nephropathy produced a significant increase in serum creatinine. Good glycemic control was observed with monotherapy of nateglinide and pioglitazone as both decreased the elevated serum creatinine and the combination therapy in high dose decreased serum creatinine in greater extent. In long term diabetes, abnormal serum creatinine is always associated with measurable renal impairment signifying that more than half the filtering capacity of the kidneys has been lost. On the other hand, normal creatinine concentration can be obtained even when the glomerular filtration rate has dropped by 50% and so it is not a confirmed indicator of early phase renal insufficiency. The relationship between serum creatinine and glomerular filtration rate is subjected to several other non-renal influences i.e., lean body mass and presence of liver disease[37]. The results showed significant increase in creatinine clearance by mono and combination therapy. Pioglitazone monotherapy have showed high creatinine clearance along with the median and high dose combination.

Diabetes with nephropathy produced a significant increase in the 24 hour urine volume. Dhein et al.[38] reported marked increase in 24 hr urine volume as compared to normoglycaemic rats in experimentally induced diabetes. Treatment with pioglitazone alone and both median and high dose combination produced restoration of 24 hour urine volume towards normal range. In the early stages of diabetic nephropathy albuminuria results from altered processing of filtered albumin by the proximal tubule, which may be preceded by peptideuria[39]. The results showed that pioglitazone monotherapy is very effective in reducing urinary albuminuria. Median and high dose combinations produced similar effect compared to pioglitazone monotherapy. Pioglitazone, but not nateglinide, is reported to be effective in reducing urinary albumin excretion and the urinary liver-type fatty acid-binding protein level, suggesting role in ameliorating both glomerular and tubulointerstitial lesions associated with early diabetic nephropathy[40]. Pioglitazone play an important role in delaying the progression of tubulointerstitial injury in the patients with diabetic nephropathy. The decrease in urinary albumin excretion by pioglitazone might be related to improvement in glomerular enlargement and the resulting hyperfiltration in animal models[41]. Zafiriou et al.[42] showed that exposure of cortical fibroblast to high glucose condition induced an increase in the expression of collagen IV and that this increase was reversed by pioglitazone, may be by decreasing tubulointerstitial fibrosis under the hyperglycemic state.

Oxidative stress results from an imbalance between radical generating and radical-scavenging systems, i.e. increased free radical production or reduced antioxidant defense or both. Implication of oxidative stress in the pathogenesis of diabetes is associated with generation of oxygen free-radical due to nonenzymatic protein glycosylation and auto-oxidation of glucose[43]. Present study showed excellent increase in the level of SOD, GSH and CAT in high dose combination treated groups. Pioglitazone monotherapy and median dose combination has succeeded only to increase SOD, whereas high dose combination group most efficiently protect against oxidative stress in diabetic nephropathic animals. Pioglitazone is reported

to reduce renal marker and oxidative stress in ischemia/reperfusion induced renal marker in diabetic rats[44].

Huang et al.[45] reported glomerular hypertrophy with sporadic interstitial fibrosis and tubular atrophy without any alteration in mesangial morphology and overt glomerulosclerosis in diabetic mice. Examination of kidneys of diabetic nephropathic rats revealed thickening of basement membrane, expansion of mesangial cell and tubular interstitium damage. Treatment with nateglinide monotherapy did not reverse the histopathological changes though pioglitazone monotherapy had moderate improvements. High dose combination showed reduction in glomerular basement membrane thickening, reduced glomerular matrix expansion and tubular dilation. Studies suggested that pioglitazone can reduce the level of oxidative damage and ameliorate many of the physiological, cellular and molecular processes associated with diabetic nephropathy by the anti-inflammatory activity in patients with advance diabetic nephropathy[46-48].

CONCLUSION

Combination therapy of thiazolidinedione with nateglinide is effective, carries low risk of hypoglycemia and is suitable for patients with moderate renal impairment, although weight gain and edema are common side effects as reported by Voulgari and Tentolouris[14]. Present study revealed that pioglitazone more effectively reduced hyperglycemia, cholesterol, BUN/creatinine ratio and HbA1c level along with urinary albumin level compared to nateglinide. In concern to parameters like urine excretion volume and histopathological restoration efficacy, pioglitazone was found superior compared to nateglinide. Median and high dose combination therapy was very effective in reducing hyperglycemia, dyslipidemia, BUN/creatinine ratio, insulin resistance and HbA1c level as well as improving urinary excretion of creatinine and albuminuria. Median dose combination therapy showed little benefit over and high dose therapy regarding serum and urinary marker parameter in normalization of diabetic nephropathy except antioxidant defiance and histopathological restoration. In concern to protection against oxidative damage high dose combination treatment was very effective in reducing reactive oxygen species production during hyperglycemia. Combination therapy of pioglitazone and nateglinide does not show tendency of weight gain and edema, and significantly increases urine volume. Based on study outcome, it can be concluded that the combination therapy of antidiabetic drugs pioglitazone and nateglinide is highly effective and beneficial in ameliorating complications and free radical mediated kidney damage associated with diabetic nephropathy.

ACKNOWLEDGEMENT

REFERENCES

1. Kuhad A, Chopra K. Attenuation of diabetic nephropathy by tocotrienol: Involvement of NFkB signaling pathway. Life Sci 2009; 84: 296-301.
2. Greg HT, Terri JA. Rodent model of streptozotocin induced diabetic nephropathy. Nephrol 2007; 12: 261-66.
3. Enyioma ON, Abdu A. Update in diabetic nephropathy. Int J Diab Met 2005; 13: 1-19.
4. Soto C, Perez J, Garcia V, Uria E, Vadillo M, Raya L. Effect of silymarin on kidneys of rats suffering from alloxan induced diabetes mellitus. Phytomedicine 2010; 17: 1090-4.
5. Gong D, Lu J, Chen X, Reddy S, Crossman DJ, Glyn-Jones S. A copper(II)-selective chelator ameliorates diabetes-evoked renal fibrosis and albuminuria, and suppresses pathogenic TGFβ activation in the kidneys of rats used as a model of diabetes. Diabetologia 2008; 51: 1741-51.
6. Murali B. Goyal RK. Effect of chronic treatment with losartan on STZ induced diabetic nephropathy. Clin Exp Hyper 2001; 23: 513-20.
7. Katavetin P, Somchai E, Suwanwalaikorn S. Pioglitazone reduces urinary protein and urinary transforming growth factor- β excretion in patients with type 2 diabetes and overt nephropathy. J Med Assoc Thai 2006; 89: 170-7.
8. Smith U. Pioglitazone: mechanism of action. Int J Clin Suppl 2001; 121: 13-28.
9. Hirasawa Y, Matsui Y, Yamane K, Yabuki S, Kawasaki Y, Toyoshi T, et al. Pioglitazone improves obesity type diabetic Nephropathy: Relation to the mitigation of renal oxidative reaction. Exp Anim 2008; 57: 423-32.
10. Agarwal R, Saha C, Battiwala M, Vasavada N, Curley T, Chase SD, et al. A pilot randomized controlled trial of renal protection with pioglitazone in diabetic nephropathy. Kidney Int 2005; 68(1): 285-92.
11. Morimoto S, Mokuda O, Sakamoto Y. AY-4166 increases the sensitivity of insulin secretion to glucose in isolated perfused rat pancreas. Horm Metab Res 1998; 30: 77-9.
12. Norman P, Rabasseda X. Nateglinide: A structurally novel, short-acting, hypoglycemic agent. Drugs Today (Barc) 2001; 37(6): 411-26.
13. Devineni D, Walter YH, Smith HT, Lee JS, Prasad P, McLeod JF. Pharmacokinetics of nateglinide in renally impaired diabetic patients. J Clin Pharmacol 2003; 43(2): 163-70.
14. Voulgari C, Tentolouris N. Combination of nateglinide with thiazolidinediones in type 2 diabetes. Expert Rev Endocrinol Metab 2009; 4(6): 537-52.
15. John M, Gopinath D, Kalra S. Triple fixed drug combinations in type 2 diabetes. Ind J Endocrinol Metab 2015; 19(3): 311-3.
16. Jovanovic L, Hassman DR, Gooch B, Jain R, Greco S, Khutoryansky N, et al. Treatment of type 2 diabetes with a combination regimen of repaglinide plus pioglitazone. Diab Res Clin Pract 2004; 63(2): 127-34.
17. Thayer S, Arondekar B, Harley C, Darkow TE. Adherence to a fixed-dose combination of rosiglitazone/glimepiride in subjects switching from monotherapy or dual therapy with a thiazolidinedione and/or a sulfonylurea. Ann Pharmacother 2010; 44: 791-9.
18. Blonde L, Wogen J, Kreilick C, Seymour AA. Greater reductions in A1C in type 2 diabetic patients new to therapy with glyburide/metformin tablets as compared to glyburide co-administered with metformin. Diabetes Obes Metab 2003; 5: 424-31.
19. de Carvalho EN, de Carvalho NAS, Ferreira LM. Experimental model of induction of diabetes mellitus in rats. Acta Cirurgica Brasileira [serial on the Internet]. 2003 July [cited 2017 July 12];18: [about 2 p.]. Available from: http://dx.doi.org/10.1590/S0102-86502003001100009.
20. Kitada M, Ogura Y, Koya D. Rodent models of diabetic nephropathy: their utility and limitations. Int J Nephrol Renovasc Dis 2016; 9: 279-90.
21. Herck HV, Baumans V, Brandt CJWM, Boere HAG, Hesp APM, Lith HAV, et al. Blood sampling from the retro-orbital plexus, the saphenous vein and the tail vein in rats: comparative effects on selected behavioural and blood variables. Lab Anim 2001; 35: 1319

22. Nanji AA, Jakelainen K, Fotouhinia M, Rahemtulla A, Thomas P, Tipoe LG. Increased severity of alcoholic liver injury in female rats: Role of oxidative stress, endotoxin and chemotoxin. Am J Physiol Gastrointes Liver Physiol 2002; 281: G1348-56.

23. Aebi HU. Catalase. In: Bergmeyer HU, editors. Methods in enzymatic analysis. 3rd ed. New York; Academic Press; 1983. p. 276-86.

24. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1979; 247(10): 3170-75.

25. Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Anal Biochem 1969; 27(3): 502-22.

26. Hirschberg Y, Pietri AO, Karara AH, Mcleod JF. Improved control of mealtime glucose excursion with coadministration of nateglinide and metformin. Diabetes Care 2000; 22: 349-53.

27. Miura K, Kitahara Y, Yamagishi S. Combination therapy with nateglinide and vildagliptin improves postprandial metabolic derangements in Zucker fatty rats. Horm Metab Res 2010 42(10): 731-5.

28. Kudo-Fujimaki K, Hirose T, Yoshihara T, Sato F, Someya Y, Ohmura C, et al. Efficacy and safety of nateglinide plus vildagliptin combination therapy compared with switching to vildagliptin in type 2 diabetes patients inadequately controlled with nateglinide. J Diab Invest 2014; 5(4): 400-9.

29. James WL, Martha LB, Mary AP, McReynolds J, Acara M, Willsky GR. Vanadate on renal hypertrophy and sorbitol accumulation in streptozotocin induced diabetes in rats. Res Comm Chem Path Pharmacol 1991; 72: 180-9.

30. Obineche EN, Mensah-Brown E, Chandranath SI, Naseer O. Morphological changes in the rat kidney following long-term diabetes. Arch Physiol Biochem 2001; 109: 241-5.

31. Hazama Y, Matsuhisa M, Ohtoshi K, Gorogawa S, Kato K, Kawamori D, et al. Beneficial effects of nateglinide on insulin resistance in type 2 diabetes. Diabetes Res Clin Pract. 2006; 71(3): 251-5.

32. Li J, Tian H, Li Q, Wang N, Wu T, Liu Y, et al. Improvement of insulin sensitivity and beta-cell function by nateglinide and repaglinide in type 2 diabetic patients - a randomized controlled double-blind and double-dummy multicentre clinical trial. Diabetes Obes Metab 2007; 9(4): 558-65.

33. Zhao W, Thacker SG, Hodgin JB, Zhang H, Wang JH, Park JL, et al. The peroxisome proliferator-activated receptor gamma agonist pioglitazone improves cardiometabolic risk and renal inflammation in murine lupus. J Immunol 2009; 183(4): 2729-40.

34. Fujiwara T, Horikoshi H. Troglitazone and related compounds: therapeutic potential beyond diabetes. Life Sci 2000; 67: 2405-16.

35. Yki-Jarvinen H. Thiazolidinediones. New Eng J Med 2004; 351: 1106-18.

36. Bruice DG, Chisholm DJ, Storlein LH. Physiological importance of deficiency in early parandial insulin secretion in non insulin dependent diabetes. Diabetes 1988; 37: 736-44.

37. Mann JF. What are the short term and long term consequences of diabetic nephropathy? Nephrol Dial Trans 1999; 14: S29-36.

38. Dhein S, Hochreuther S, Aus Dem Spring C, Bollig K, Hufnagel C, Raschack M. Long term effect of the endothelin A receptor antagonist converting enzyme inhibitor trandopril on diabetic angiopathy and nephropathy in a chronic type diabetes mellitus rat model. J Pharmacol Exp Ther 2000; 293: 351-9.

39. Russo LM, Sandoval RM, Campos SB, Molitoris BA, Comper WD, Brown D. Impaired tubular uptake explains albuminuria in early diabetic nephropathy. J Am Soc Nephrol 2009; 20(3): 489-94.

40. Nakamura T, Sugaya S, Kawagoe Y, Ueda Y, Koide H. Effect of pioglitazone on urinary liver type fatty acid binding protein concentrations in diabetes patients with microalbuminuria. Diab Met Res Rev 2006; 22: 385-89.

41. Tanimoto M, Fan Q, Gohad T, Shike T, Makita Y, Tomino Y. Effect of pioglitazone on the early stage of type 2 diabetic nephropathy in KK/Ta mice. Metabolism 2004; 53: 1473-9.

42. Zafiriou S, Stanners SR, Saad S, Polhill TS, Poronnik P, Pollock CA. Pioglitazone inhibit cell growth and reduce matrix production in human kidney fibroblast. J Am Soc Nephro 2005; 16: 638-45.

43. Moussa SA. Oxidative stress in diabetes mellitus. Rom J Biophy 2008; 18: 225-36.

44. Kakadiya J, Shah N. Comparison effect of pioglitazone and glimepiride alone on renal function marker in experimentally induced renal damage in diabetic rats. J App Pharm Sci 2011; 1: 72-6.

45. Huang W, Yves G, Nadine B, Bruneval P, Heudes D, Belair MF, et al. Genetically increased angiotensin I-converting enzyme level and renal complications in the diabetic mouse. Proc Natl Acad Sci USA 2001; 98: 13330-4.

46. Agarwal R. Anti-inflammatory effect of short term pioglitazone therapy in men with advance diabetic nephropathy. American J Physiol 2006; 290: F600-05.

47. Ko GJ, Kang YS, Han SY, Lee MH, Song HK, Han KH, et al. Pioglitazone attenuates diabetic nephropathy through an anti-inflammatory mechanism in type 2 diabetic rats. Nephrol Dial Transplant 2008; 23(9): 2750-60.

48. Xu X, Chen P, Zheng Q, Wang Y, Chen W. Effect of pioglitazone on diabetic nephropathy and expression of HIF-1α and VEGF in the renal tissues of type 2 diabetic rats. Diab Res Clin Pract 2011; 93(1): 63-9.

Cite this article as: