Research Article

Effect of Asparagus Racemosus Against Streptozotocin-Nicotinamide Induced Type-2 Diabetes Mellitus With Special Reference To Diabetic Nephropathy in Rats

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ABSTRACT
The chronic type-2 diabetes mellitus leads to diabetic nephropathy, which is one of the major microvascular complication of end stage renal disease worldwide and causes premature death in diabetic patients. The objective of the present investigation was to evaluate the antidiabetic activity and protective effect of diabetic induced nephropathy of ethanolic extract of roots of *Asparagus racemosus* (REAR) against streptozotocin-nicotinamide induced type-2 diabetes mellitus in male albino *Wistar* rats. The *in-vivo* study showed that blood glucose level was significantly reduced in dose dependent manner when compared to the diabetic control group. In addition, it significantly restored the body weight loss, increased kidney weight, glycosylated haemoglobin, blood uric acid, blood urea nitrogen, blood creatinine, urine volume and urine microalbumin levels when compared to diabetic control groups. The report of histopathological study of rat kidney tissues strongly supported the protective effect of REAR in diabetic nephropathy. The findings of this investigation concluded that REAR has significant antidiabetic activity and potential protective effect in diabetic nephropathy.

Keywords: Type-2 diabetes mellitus, Diabetic nephropathy, streptozotocin, nicotinamide.

INTRODUCTION

The world is facing an explosive increase in the incidence of type-2 diabetes mellitus (T2DM), which is a chronic metabolic disease with highest rates of prevalence and mortality affects more than 100 million people worldwide. It is caused by an absolute or relative lack of insulin and/or reduced insulin activity (insulin resistance)[1]. The prevalence rate of diabetes is estimated to be 1-5% in India. The number of people with diabetes in India is currently around 50.8 million and expected to rise to 87 million by 2030 unless urgent preventive steps are taken[2,3]. Hence India leads the world with largest number of diabetic patients earning the dubious distinction of being the "diabetic capital of the world"[4]. Diabetes is now considered to be a vascular disease. Diabetic nephropathy is one of the major microvascular complication of type-2 diabetes mellitus and is the major cause of end-stage renal disease (ESRD). The early changes in diabetic nephropathy are characterized by an increase in kidney size, glomerular volume and kidney function followed by the accumulation of glomerular extracellular matrix, increased urinary microalbumin excretion, glomerular sclerosis and tubular fibrosis. Last stage overt diabetic nephropathy is clinically characterized by proteinurea, hypertension and progressive renal insufficiency[5]. Diabetic nephropathy has been a growing threat in the world and Eastern countries are not an exception. In Australia type-2 diabetes mellitus patients starting dialysis increased 5-fold between 1993 and 2007 and in India diabetic nephropathy, is expected to rise to 6.6 million of the more than 100 million patients suffering diabetes. So it is a major cause of morbidity in diabetic patients[5,6]. All of the pharmacological modalities show limited efficacy and certain adverse effects such as hepatotoxicity, lactic acidosis, diarrhoea, obesity or weight loss and attenuation of response after prolonged use, dry cough and are expensive particularly for developing countries like India and China. Comparatively very less side effects and low cost of phytopharmaceuticals from natural resources open new avenues for the treatment

of various diseases including diabetes. Therefore there is a need for phytochemical that have antidiabetic potential, which are cost effective, potent and also safe without long-term side effects[1].

The ethanolic extracts of routes of *Asparagus racemosus* contain saponins which have reported to show the antidiabetic effect in previous studies[7,8]. Despite the availability of many antidiabetic medicines in the market, diabetes and its microvascular and macrovascular complications continues to be a major medical problem. Plant derivatives with purported antidiabetic activity are used in folk medicine and traditional healing systems around the world[9]. Herbal drugs are prescribed widely even when their biologically active ingredients are unknown[10]. Substantial efforts have been made in recent years to identify new natural and synthetic antidiabetic drugs. There is flood of scientific data about medicinal plants including those with antidiabetic potential[11].

The roots of *Asparagus racemosus* belonging to the family of Liliaceae has been recommended in Ayurvedic texts for prevention and treatment of gastric ulcers, dyspepsia, galactogogue, aphrodisiac, nervous disorders, nervine tonic, liver diseases, inflammation, antioxytocic, anticancer, diuretic, nutritive, rejuvenating, constipating, diarrhoea, tuberculosis, cough, bronchitis, gonorrhoea, leprosy, epilepsy, fatigues, threatened abortion, diabetes mellitus and burning sensation[8,12,13].

Although the roots of *Asparagus racemosus* has been used in traditional medicine yet scientific validation of its use in type-2 diabetes mellitus and its effect on diabetic nephropathy needs to be studied. Hence this investigation was undertaken to evaluate the antidiabetic activity and protective effect of diabetic induced nephropathy of ethanolic extract of roots of *Asparagus racemosus* against streptozotocin-nicotinamide induced type-2 diabetes mellitus in male albino *Wistar* rats.

MATERIALS AND METHODS

Plant material and extract preparation

The roots of *Asparagus racemosus* was collected during May 2011, from Kaliakkavilai, Tamil Nadu. It was identified and authenticated by botanist Dr. K. Paul Raj and voucher specimen was deposited in the Herbarium, department of botany, Nesamony Memorial Christian College, Marthandam (NMCC/47/2011). The roots were washed, cut into small pieces, dried in shade and coarse powdered (2000 gm) in a mixer grinder. It was extracted with soxhlet using 95% ethanol for 72 hours, concentrated on water bath (70^0 C), kept in oven (30^0 C) for drying and stored in desiccator. The yield of ethanolic extract of REAR was 26.4 gm (1.37%).

In-vivo antidiabetic effect of REAR against streptozotocin-nicotinamide induced type 2 diabetes mellitus and their effect on diabetic nephropathy

Animals

Male albino *Wistar* strain rats weight about 180-220 gm were procured from the central animal house of Swamy Vivekanandha College of Pharmacy were used for the study. They were maintained in temperature 21 ± 2^0 C, standard laboratory conditions and the relative humidity of 55-60% with a 12 hour light cycle and 12-hour dark cycle. They were allowed access to food with standard pellet diet and water ad libitum. The study protocol was approved by the institutional animal ethical committee of Swamy Vivekanandha College of Pharmacy, Elayampalayam, Tiruchengode, Tamil Nadu. (Protocol no: SVCP/IAEC/Ph.D /019/Feb/2012) and studies were carried out in accordance with the guidelines of Committee for the purpose of control and supervision of experiments on animals (CPCSEA), India.

Drugs and chemicals

The following drugs and chemicals were purchased from Streptozotocin (Sisco Research Laboratories Pvt. Ltd., Mumbai, India), Nicotinamide (Ranbaxy Chemicals Ltd., Mumbai, India), Carboxyl methyl cellulose (Loba Chemicals Pvt. Ltd., Mumbai, India), Formaldehyde (Nine chemicals Pvt. Ltd., Mumbai, India), Sodium citrate (Loba chemicals Pvt. Ltd., Mumbai, India), Citric acid (Loba chemicals Pvt. Ltd., Mumbai, India), Sodium phosphate monobasic (Loba chemicals Pvt. Ltd., Mumbai, India), Sodium phosphate dibasic (Loba chemicals Pvt. Ltd., Mumbai, India); Pioglitazone (Gift sample from Sun Pharmaceuticals Industries Ltd., Mumbai, India).

Induction of type-2 diabetes mellitus

Streptozotocin was freshly dissolved in (0.1 M, P^H 4.5) citrate buffer and nicotinamide was dissolved in normal physiological saline and maintained on ice prior to use. Non-insulin dependent diabetes mellitus (T2DM) was induced in overnight fasted rats by a single intraperitonial (i.p) injection of streptozotocin (4.5 mg/kg, b.w), 15 min after the intraperitonial administration of nicotinamide (110 mg/kg, b.w). The elevated plasma glucose level was determined on 3rd day of

streptozotocin and nicotinamide administration and those rats with fasting glucose levels greater than 250 mg/dl were served as diabetic rats and used in the study. Treatment with REAR was started on the third day after streptozocin and nicotinamide induction and continued for 90 days. Nephropathy was noted in diabetic rats between 4-8 weeks after the administration of streptozotocin and nicotinamide[1].

Treatment protocol

The animals were separated into 6 groups, containing 6 animals each groups (n=6); a total of 36 rats (30 diabetic surviving rats, 6 normal control rats) were used. REAR was suspended in 1% w/v carboxy methyl cellulose (CMC) in water and administered orally using an intra gastric tube.

Group I : Normal control (1 % W/V CMC in water, p.o, 1 ml/100 gm b.w.)

Group II : Diabetic rats treated with 1 % W/V CMC in water, p.o, 1 ml/100 gm b.w.

Group III : Diabetic + Pioglitazone (4.05 mg/kg/day in 1 % W/V CMC in water, p.o, 1 ml/100gm b.w.)

Group IV : Diabetic + REAR (100 mg/kg/day in 1 % W/V CMC in water, p.o, 1ml/100 mg b.w.)

Group V : Diabetic + REAR (200 mg/kg/day in 1 % W/V CMC in water, p.o, 1 ml/100 mg b.w.)

Group VI : Diabetic + REAR (400 mg/kg/day in 1 % W/V CMC in water, p.o, 1 ml/100 mg b.w.)

The initial and final body weight of various groups were recorded. At the end of 90 days treatment, the 24 hours urine were collected in metabolic cages (Instruments & Chemicals Pvt. Ltd, Ambala city, India) and the volume of urine was noted in all the groups. Then, it was used for the estimation of urine microalbumin. After that, in overnight fasted animals, blood samples were collected in tubes containing EDTA by cardiac puncture after anaesthetizing them using ketamine hydrochloride (24 mg/kg, b.w, i.m injection) and used for the estimation of blood glucose, glycosylated haemoglobin (HbA_1C), blood uric acid, blood urea nitrogen (BUN) and blood creatinine levels. Blood samples were collected from retro orbital sinus and blood sugar levels were estimated in every 15 days throughout the 90 days study. The urine and blood parameters were evaluated using semi auto analyzer - Mind Rays Ba-88a., (Mind Rays Medical India Pvt. Ltd., Mumbai, India) following suitable methods.

Histopathological study of kidneys

After 90 days of treatment, the anaesthetized rats were sacrificed by cervical decapitation and kidneys were excised quickly and stored in 10% buffered formalin solution (formaldehyde, 100 ml; sodium phosphate monobasic, 4 g; sodium phosphate dibasic, 6.5 g; and water, 900 ml) and subjected to further processing for histopathological studies.

Statistics

All the data were expressed as mean ± SEM. The One-way analysis of Variance (ANOVA) followed by Tukey's multiple comparison test was used to analyse the statistical significance for the effect of different doses of REAR when compared to control with the help of Graph pad Instat software, version 3.01; values are considered statistically significant when $P<0.05$.

RESULTS

***In-vivo* antidiabetic effect of REAR and its effect on diabetic nephropathy**

The effect of REAR on body weight and kidney weight

Body weight: After 90 days of treatment, the final body weight of diabetic control group significantly ($P<0.001$) decreased when compared to final body weight of normal control group. In diabetic animals treated with REAR 200 mg/kg, the final body weight was significantly ($P<0.05$) increased when compared to diabetic control. Also the final body weight of REAR 400 mg/kg treated group, significantly ($P<0.01$) increased when compared to diabetic control. In animals treated with pioglitazone 4.05 mg/kg, the final body weight was significantly ($P<0.001$) increased when compared to the final body weight of diabetic control group (Table 1).

Kidney weight

The Kidney weight of diabetic control rats increased significantly ($P<0.001$) when compared to normal control rats. Diabetic rats treated with REAR 400 mg/kg, the Kidney weights were significantly ($P<0.05$) decreased when compared to diabetic rats (Table 1).

The effect of REAR on blood parameters

Blood glucose levels

In diabetic control group, the fasting blood glucose level was significantly ($P<0.001$) increased on the 3rd day after streptozotocin-nicotinamide administration and was maintained same in every fifteen days of blood glucose analysis till the 90th day of treatment schedule when compared to normal control group. When compared to diabetic control group, Pioglitazone at the dose of 4.05 mg/kg, significantly ($P<0.001$) reduced the fasting blood glucose level, from the fifteenth day onwards, until the completion of 90 days

treatment. In the diabetic rats treated with REAR 400 mg/kg, significantly decreased (p<0.05), (P<0.01), (P<0.01) the fasting blood glucose level on 60th day, 75th day and 90th day treatment when compared to diabetic control rats (Table 2, Figure 1).

Glycosylated haemoglobin level

The levels of glycosylated haemoglobin (HbA_1C) were significantly (P<0.001) increased in diabetic group, when compared to normal control group. The pioglitazone 4.05 mg/kg treated group showed significant (P<0.001) decrease in HbA_1C when compared to diabetic control group. In REAR 400 mg/kg treated group, showed significant (P<0.05) decrease in glycosylated haemoglobin when compared to diabetic control group (Table 3).

Blood uric acid level

The study showed that the blood uric acid levels of diabetic control group was significantly (P<0.001) increased when compared to normal control group. In diabetic rats treated with REAR 400 mg/kg showed significant (P<0.01) decrease in blood uric acid level when compared to diabetic control group (Table 3).

Blood urea nitrogen level

In diabetic control group, blood urea nitrogen (BUN) was significantly increased (P<0.001) when compared to normal control group. In diabetic rats treated with REAR 400 mg/kg significantly (P<0.05) decreased the blood urea nitrogen level when compared to diabetic control group (Table 3).

Blood creatinine level

The blood creatinine levels of diabetic control group was increased significantly (P<0.001) when compared to normal control group. But diabetic rats treated with REAR 200 mg/kg (P<0.05) and REAR 400 mg/kg (P<0.01) showed significant decrease in blood creatinine level when compared to diabetic control group (Table 3).

The effect of REAR on urine parameters

Volume of urine

In diabetic control rats volume of urine was significantly increased (P<0.001) when compared to normal control rats. In diabetic rats treated with pioglitazone 4.05 mg/kg, significantly (P<0.001) decreased the volume of urine when compared to diabetic control group. But in diabetic rats treated with REAR 200 mg/kg (P<0.05) and REAR 400 mg/kg (P<0.01) significantly decreased the volume of urine when compared to diabetic control group (Table 4).

Urine microalbumin level

The urine microalbumin levels in the diabetic control group were significantly (P<0.001) increased when compared to normal control group. In diabetic control rats treated with REAR 400 mg/kg significantly decreased (P<0.05) the urine microalbumin levels when compared to diabetic control rats (Table 4).

Histopathalogical studies

The histopathological observations of the rat kidneys revealed that the normal group rats showed normal glomeruli and kidney tubules with healthy epithelial cells. The kidney of diabetic control group rats shows thickening of vesicles, disrupted tubules, degeneration and necrosis of epithelial cells and intertubular haemorrhage. But the kidneys of diabetic rats treated with REAR 400 mg/kg showed regeneration of tubular epithelium and moderate intertubular haemorrhage.

Table 1: The effect of REAR on body weight and kidney weight

Groups	Treatment	Mean body weight (gms)		Mean kidney weight (gms/100gm body weight)
		Initial	Final (After 90 days of treatment)	
I	Normal control	199.17 ±5.54	250.83 ± 4.36	0.701±0.03
II	Diabetic control	200.00 ±6.19	175.83 ± 5.69***	1.303±0.06***
III	Diabetic+ PG 4.05mg/kg	203.33 ±4.41	260.00 ± 4.28$^{\#\#\#}$	1.280±0.06
IV	Diabetic+ REAR(100mg/kg)	205.83 ±4.55	185.00 ± 6.19	1.257±0.05
V	Diabetic+ REAR(200mg/kg)	204.17 ±5.23	212.50 ± 13.71$^{\#}$	1.236±0.04
VI	Diabetic+ REAR(400mg/kg)	210.00 ±3.42	224.17 ± 8.70$^{\#\#}$	1.062±0.05$^{\#}$

n=6, The values are expressed as mean ±SEM; ***P<0.001 when compared to normal control group $^{\#}$P<0.05, $^{\#\#}$P<0.01 $^{\#\#\#}$P<0.001 when compared to diabetic control group.

Table 2: The effect of REAR on blood glucose levels

Groups	Treatment	Mean blood glucose levels (mg/dl)							
		0 day	3rd day	15th day	30th day	45th day	60th day	75th days	90th Day
I	Normal Control	91.63 ± 05.73	91.76±03.97	89.48± 04.66	93.05± 06.25	86.35± 04.42	91.63± 05.62	90.50± 04.26	90.77±05.61
II	Diabetic control	99.15± 06.57	351.10±27.31***	390.37±19.47***	395.27±15.36***	401.82±17.93***	416.9±16.41***	421.82±18.17***	422.17±21.27***
III	Diabetic+ P.G 4.05 mg/kg	95.02± 04.76	363.43±19.09	301.38±05.04###	256.27±08.84###	215.45±11.49###	122.80±05.80###	120.42±03.59###	98.17 ±01.82###
IV	Diabetic + REAR (100mg/kg)	100.65±04.70	359.67±9.44	380.53±10.98	377.08±22.65	377.68±15.27	395.72±15.4	405.05±17.39	409.77±14.99
V	Diabetic + REAR (200mg/kg)	102.73±05.11	362.50±22.59	381.08±18.99	385.68±16.53	381.01±18.43	383.52±17.46	383.28±17.85	380.12±19.60
VI	Diabetic+ REAR (400mg/kg)	103.68±5.63	382.93±29.37	365.60±12.23	362.12±13.67	378.05±11.04	351.35±11.38#	345.38±12.65 ##	340.12±11.38##

n=6, The values are expressed as mean ±SEM; ***P<0.001 when compared to normal control group, #P<0.05, ##P<0.01 ###P<0.001 when compared to diabetic control group.

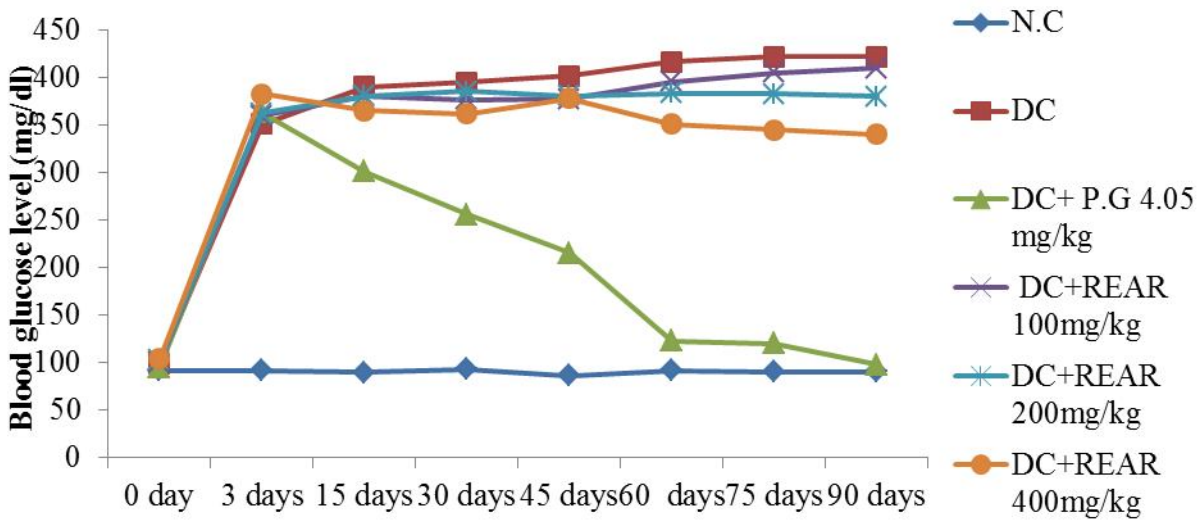

Fig. 1: The effect of REAR on blood glucose level (mg/dl), NC: Normal control, DC: Diabetic control, PG: Pioglitazone

Table 3: The effect of REAR on glycosylated haemoglobin, blood uric acid, BUN and creatinine levels

Groups	Treatment	Mean glycosylated haemoglobin level (%)	Mean blood uric acid levels (mg/dl)	Mean BUN levels (mg/dl)	Mean creatinine levels (mg/dl)
I	Normal control	5.08 ± 0.30	0.95±0.12	5.48±0.79	0.45±0.04
II	Diabetic control	10.3± 0.28***	2.03±0.06***	24.70±0.57***	0.67±0.06***
III	Diabetic + P.G(4.05mg/kg)	6.60 ± 0.14###	1.93±0.11	23.53±0.53	0.65±0.03
IV	Diabetic + REAR(100mg/kg)	9.45 ± 0.45	1.87±0.07	23.02±0.54	0.64±0.02
V	Diabetic + REAR (200mg/kg) Diabetic	9.27 ± 0.42	1.55±0.18	23.08±0.38	0.51±0.02#
VI	+REAR(400mg/kg)	8.65 ± 0.40#	1.25±0.18##	21.87±0.48#	0.48±0.01##

n=6, The values are expressed as mean ±SEM; ***P<0.001 when compared to normal control group, #P<0.05, ##P<0.01 ###P<0.001 when compared to diabetic control group.

Table 4: The effect of REAR on volume of urine and urine micro albumin levels

Groups	Treatment	Mean volume of urine (ml)	Mean urine micro albumin levels (mg/dl)
I	Normal control	2.18±0.30	0.33±0.05
II	Diabetic control	19.60±1.02***	0.77±0.05***
III	Diabetic + P.G(4.05mg/kg)	3.05±0.54###	0.58±0.06
IV	Diabetic +REAR (100mg/kg)	16.40±1.67	0.60±0.05
V	Diabetic +REAR (200mg/kg)	15.55±0.40#	0.57±0.04
VI	Diabetic +REAR(400mg/kg)	14.23±0.46##	0.50±0.03#

n=6, The values are expressed as mean ±SEM; ***P<0.001 when compared to normal control group, #P<0.05, ##P<0.01 ###P<0.001 when compared to diabetic control group.

DISCUSSION

Chronic diabetes mellitus causes multiple complications like diabetic nephropathy and premature mortality, accounting for atleast 10 % of total health care expenditure in many countries[1]. The results of *in-vivo* antidiabetic effect of REAR by streptozotocin-nicotinamide induced type-2 diabetes mellitus revealed that the reduction of final body weight of diabetic control group is due to increased muscle wasting in diabetes[14]. But diabetic rats treated with REAR showed an increase in body weight as compared to the diabetic control which may be due to its protective effect in controlling muscle wasting i.e reversal of gluconeogenesis. This observation is consistence with the results of previous researchers, as they reported that any drug possessing antidiabetic activity protects the muscle wasting in diabetic animals[15].

Diabetic rats treated with REAR significantly decreased the kidney weights when compared to diabetic control rats. The increase in kidney weight was due to renal enlargement, which is one of the key features occuring during nephropathy, a hypertrophy and hyperfunction of the kidneys with typical increase in kidney size and glomerular filtration rate can be observed. This is due to the factors such as glomerular hypertrophy and nephromegaly (whole kidney enlargement), an early feature of both experimental and human diabetes occurs due to combination of tubular hypertrophy hyperplasia and interstitial expansion[16].

In the diabetic rats treated with REAR, the fasting blood glucose level was significantly decreased when compared to diabetic control rats. Diabetic rats treated with pioglitazone also significantly decreased the fasting blood glucose level when compared to diabetic control rats. Liver is mainly responsible for maintaining normal concentrations of blood glucose by its ability to store glucose as glycogen and to produce glucose from glycogen breakdown or from gluconeogenic precursors. Selective destruction of pancreatic β–cells by streptozotocin using experimental diabetes results in the decreased plasma insulin levels. This in turn leads to the defective glucose oxidation and causes hyperglycemia in diabetes involves over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues[1]. The activation of PPARγ by PPARγ agonist (insulin sensitisers) which are currently being used in the treatment of insulin resistance associated with type- 2 diabetes mellitus and thus influenced the peripheral glucose uptake. PPARγ, a transcription factor belonging to the nuclear receptor family. Drugs like thiazolidinediones and insulin cause differentiation of pre-adipocytes into adipocytes. The adipocytes then directly enhances insulin signaling and stimulate glucose uptake in muscle on binding with PPARγ agonists and thus aid in reducing the blood glucose levels. Therefore, drugs which exhibit glucose uptake activity would be desirable for patients with T2DM. The drug REAR exhibited significant reduction in blood glucose level and thus can be explored as glucose lowering agent to treat T2DM[17,18]. The reports of the present study was consistence with the results of previous researchers who reported the REAR showed significant decrease in fasting blood glucose level in drug treated group when compared to diabetic control group[8]. This indicates that the REAR has possessing significant antidiabetic effect at 400 mg/kg dose level.

The REAR treated diabetic rats showed significant decrease in HbA_1C when compared to diabetic control rats. When compared to pioglitazone the REAR was also effective in controlling HbA_1C level in diabetic rats. In an uncontrolled or poorly controlled diabetes, there is an increased glycosylation of a number of proteins, including haemoglobin. HbA_1C is 3.4-4.8 % of total haemoglobins in normal human red blood cells and it would found to increase in diabetic patients upto 16 %. The level of HbA_1C is the indicator of the degree of control of diabetes in patients and its level reflects the average blood glucose concentration over the past three months[1]. HbA_1C is the most abundant glycated

haemoglobin product, which initiates and participates in multiple organ damage in diabetes patients. The reaction between glucose and haemoglobin forming HbA_1C is a type of a nonenzymatic condensation of glucose with the free amino groups of the N-terminals of the b-chain of the haemoglobin molecules. The process is slow, continuous and irreversible. It serves as an indicator of metabolic control in diabetes[19]. Each 1 % reduction in glycosylated haemoglobin is associated with a 37 % reduction in microvascular complications, 18 % myocardial infarction and 21 % fewer diabetes-related deaths[20]. In the present study also, HbA_1C level increased in diabetic rats and administration of REAR controls the glycation of haemoglobin by an increase in glutathione peroxidase and thus decreases the level of HbA_1C in experimental rats[1].

In diabetic rats treated with REAR, the blood uric acid level was significantly decreased when compared to diabetic control group. Uric acid is a product of purine metabolism. The increase in uric acid could be due to the fact that filtered uric acid is both reabsorbed and excreated in the proximal tubule through a voltage-sensitive urate channel and a urate-anion exchange mechanism. Hyperuricemia can be a result of either increased production or decreased excretion[21]. The REAR restored the elevated uric acid level in diabetic rats.

In diabetic rats treated with the REAR blood urea nitrogen level was significantly decreased when compared to diabetic control group. Blood urea nitrogen is formed when protein breaks down, which is another marker of kidney function. When blood flows through the body, protein circulates to cells. Cells use the protein and excrete the waste products urea which is filtered out of the blood by kidneys. Urea also contain nitrogen. In diabetic nephropathy urea and nitrogen stay in the blood. A BUN of over 20 mg/dl is an indicator of decreased kidney function. The REAR restored the elevated blood urea nitrogen in diabetic rats[22].

But diabetic rats treated with REAR showed significant decrease in blood creatinine level when compared to diabetic control group. Creatinine is endogenously produced and released into body fluids and its clearance measured as an indicator of glomerular filtration rate[23]. If serum creatinine levels increased due to hyperglycemia that causes osmotic diuresis and depletion of extracellular fluid volume[24]. The REAR significantly reversed the elevated blood creatinine in diabetic rats.

In diabetic rats treated with pioglitazone, the volume of urine significantly decreased when compared to diabetic control group of rats. In diabetic control group of rats treated with REAR, the volume of urine level was significantly decreased when compared to diabetic control group. Polyuria is the symptom of diabetes, the volume of urine levels increased in diabetic rats, since the renal tubules are unable to absorb all of the glucose filtered in the glomeruli. The renal excretion of glucose requires excretion of water and produces an osmotic diuresis which is called polyuria or excessive urination. It can cause dehydration, resulting in dry skin and blurred vision, which is due to fluctuation in the amount of glucose and water in the lenses of the eye during dehydration. Glucose needs water to flow from the body. Loss of water causes an increase in the serum polarity that stimulates the thirst centre in the hypothalamus[25]. The REAR significantly decreases the increased volume of urine output in diabetic rats.

The diabetic control rats treated with REAR showed significant decrease in the urine microalbumin levels when compared to diabetic control rats. The increase in urine microalbumin was due to proteins from the kidney, appear in the urine as a consequence of normal process of cell turn over and metabolism. The release of the protein is increased during kidney's functional impairment as happens in diabetes[26]. The REAR restored the elevated levels of urine microalbumin in the diabetic rats. The report of histopathological studies of rat kidneys strongly support the outcome of the study by moderately restoring the kidney damage that occurred in the diabetic rats treated with higher doses of REAR 400mg/kg.

CONCLUSIONS

In this investigation the abnormalities in body weight, kidney weight, blood glucose, glycosylated haemoglobin, blood urea, blood uric acid, blood creatinine and urine microalbumin levels in the diabetic control group was significantly reversed by REAR in streptozotocin-nicotinamide induced type-2 diabetic nephropathy in male albino *Wistar* rats. Therefore, this investigation concluded that REAR may be used as an antidiabetic agent and renal protective for chronic type-2 diabetes mellitus patients to prevent the nephropathy complications in diabetic populations, after confirming its efficacy and safety in well-controlled clinical trials. If it is confirmed in humans, REAR may be a potent, safe and cost effective phytomedicine to

prevent nephropathy-induced premature death in diabetic patients.

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