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# Effect of Combinations of Asparagus Racemosus and Eugenia Jambolana 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 on diabetic induced nephropathy of combinations of ethanolic root extract of *Asparagus racemosus* (REAR) and seed extract of *Eugenia jambolana*(SEEJ) by using *in-vivo* model. The *in-vivo* antidiabetic activity and the effect on diabetic nephropathy was evaluated on 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 urea, 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 combinations of REAR and SEEJ in diabetic nephropathy. The findings of this investigation concluded that combinations of REAR and SEEJ has significant antidiabetic activity and potential protective effect in diabetic nephropathy.

**Keywords:** Type-2 diabetes mellitus, Diabetic nephropathy, Streptozotocin, Nicotinamide, *Eugenia jambolana, Asparagus racemosus*.

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## 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 proteinuria, 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 phytochemicals that have antidiabetic potential, which are cost effective, potent and also safe without long-term side effects[1].

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[7].

The ethanolic extracts of seeds of *Eugenia jambolana* contain saponins which have reported to show the antidiabetic effect in previous studies, appear to be involved in stimulation of pancreatic β-cells and subsequent secretion of insulin[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 seeds of *Eugenia jambolana*, belonging to the family of Myrtaceaehas been indicated in Ayurveda for its use in diabetes mellitus[12]. It is reported to have hypoglycemic[2,11,13,14], hypolipidemic[13], antiulcer[14], antibacterial[15], anti-inflammatory[16],neuropsycho pharmacological[17], anti HIV[18] and antidiarrhoeal activity[19].

Although the roots of *Asparagus racemosus* and the seeds of *Eugenia jambolana* has been used in traditional medicine[13] yet scientific validation of their combination use in type-2 diabetes mellitus and effect on diabetic nephropathy needs to be studied. Hence this investigation was undertaken to evaluate the antidiabetic activity and protective effect on diabetic induced nephropathy of combinations of ethanolic root extract of *Asparagus racemosus* (REAR) and seed extract of *Eugenia jambolana* (SEEJ) 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 and the fruits of *Eugenia jambolana*was collected during January 2012, from Kaliakkavilai, Tamil Nadu. They were 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 and NMCC/69/2012).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%).Seed was separated and 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 SEEJ was 36.8 gm(1.84%).

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

**Animals**

Male albino *Wistar* strain rats weight about 180-220gm 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: 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**

A gift sample of Pioglitazone was obtained from Sun Pharmaceuticals Industries Ltd., Mumbai, India. The following drugs and chemicals were purchased from various companies: 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) and used for the study.

**Induction of type-2 diabetes mellitus**

Streptozotocin was freshly dissolved in (0.1M, $P^H$ 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 (4.5mg/kg, b.w), 15 min after the intraperitonial administration of nicotinamide (110mg/kg, b.w). The elevated plasma glucose level was determined on $3^{rd}$ 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 combinations of REAR and SEEJ was started on the third day after

streptozotocin 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 (n=6); a total of 36 rats (30 diabetic surviving rats, 6 normal control rats) were used. The combination of REAR and SEEJ were suspended in 1% w/v carboxy methyl cellulose (CMC) in water and administered orally using an intragastric tube.

Group I : Normal control rats treated with 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 gmb.w.

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

Group IV : Diabetic rats treated with combinations REAR and SEEJ each 50 mg/kg/day in 1 % W/V CMC in water, p.o, 1 ml/100 mg b.w.

Group V: Diabetic rats treated with combinations REAR and SEEJ each 100 mg/kg/day in 1 % CMC in water, p.o, 1 ml/100 mg b.w.

Group VI: Diabetic rats treated with combinations REAR and SEEJ each 200 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 was collected in metabolic cages(Instruments & Chemicals Pvt. Ltd, Ambalacity, 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 (24mg/kg, b.w, i.m injection) and used for the estimation of blood glucose, glycosylated haemoglobin($HbA_1C$), blood urea, 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, 4gm; sodium phosphate dibasic, 6.5 gm; 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 analyze the statistical significance for the effect of different doses of combinations of REAR and SEEJ 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 AND DISCUSSION

***In-vivo* anti-diabetic effect of combinations of REAR and SEEJ and their effect on diabetic nephropathy**

**The effect of combinations of REAR and SEEJ on body weight and kidney weight**

**Body weight:**

After 90 days of treatment, the final body weight of diabetic control group was significantly ($P<0.001$) decreased when compared to normal control group. In diabetic animals treated with the combinations of REAR 50 mg/kg and SEEJ 50 mg/kg ($P<0.001$), REAR 100 mg/kg and SEEJ 100 mg/kg ($P<0.001$) and REAR 200 mg/kg and SEEJ 200 mg/kg ($P<0.001$), the final body weight was significantly 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 diabetic control group (Table 1).The results of *in-vivo* antidiabetic effect of combinations of REAR and SEEJ 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[20]. But diabetic rats treated with combination of REAR and SEEJ 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[21].

**Kidney weight:**

The kidney weight of diabetic control group of rats increased significantly ($P<0.001$) when compared to normal control group. Diabetic rats treated with the combinations of REAR 50 mg/kg and SEEJ 50 mg/kg ($P<0.001$), REAR 100 mg/kg and SEEJ 100 mg/kg ($P<0.001$) and REAR 200 mg/kg and SEEJ 200 mg/kg ($P<0.001$) significantly decreased the kidney weights when compared to diabetic control group of rats(Table 1).Diabetic rats treated with combinations of REAR and SEEJ 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 occurring 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[22].

**Table 1.The effect of combinations of REAR and SEEJ on body weight and kidney weight**

| Groups | Treatment | Mean body weight (gms) | | Mean kidney weight (gms/100 gm body weight) |
|---|---|---|---|---|
| | | Initial | Final (After 90 days of treatment) | |
| I | Normal control | 199.17 ± 5.54 | 250.83 ± 4.36 | 0.70±0.03 |
| II | Diabetic control | 200.00 ± 6.19 | 175.83 ± 5.69 *** | 1.30±0.06*** |
| III | Diabetic+ PG 4.05 mg/kg | 203.33 ± 4.41 | 260.00 ± 4.28### | 1.28±0.05 |
| IV | Diabetic+ (REAR +SEEJ) 50 mg/kg | 200.00±4 .83 | 253.33 ± 3.80 ### | 0.90±0.04### |
| V | Diabetic+ (REAR +SEEJ) 100 mg/kg | 206.67 ±4.77 | 260.83 ± 4.36 ### | 0.74±0.03### |
| VI | Diabetic+(REAR +SEEJ) 200 mg/kg | 199.17 ±6.51 | 241.67 ±7.81### | 0.75±0.03### |

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

**The effect of combinations of REAR and SEEJ on blood parameters**

**Blood glucose levels:**

In diabetic control group of rats, 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 of rats. When compared to diabetic control group of rats, pioglitazone 4.05 mg/kg significantly (P<0.001) reduced the fasting blood glucose level from the 15th day to 90th day of treatment. In the diabetic rats treated with the combinations of REAR 50 mg/kg & SEEJ 50 mg/kg was significantly decreased (P<0.001) on 30 days to 90 days of treatment, REAR 100 mg/kg & SEEJ 100 mg/kg (P<0.01) on 15 days of treatment, (P<0.001) on 30 days to 90 days of treatment and REAR 200 mg/kg & SEEJ 200 mg/kg (P<0.01) on 15 days of treatment, (P<0.001) on 30 days to 90days of treatment(Table 1, Figure 1).In the diabetic rats treated with combinations of REAR and SEEJ the fasting blood sugar level was significantly decreased 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 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, at ranscription 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 combination drugs REAR and SEEJ exhibited significant reduction in blood glucose level and thus can be explored as glucose lowering agent to treat T2DM[23, 24].

**Table 2. The effect of combinations of REAR and SEEJ 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 day** | **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+PG 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+SEEJ) 50 mg/kg | 102.67± 05.47 | 383.92± 17.52 | 315.73± 06.77 | 259.47± 11.92 ### | 165.18± 05.67### | 131.33 ± 03.40 ### | 117.27± 05.50 ### | 118.23± 05.13### |
| V | Diabetic+ (REAR+SEEJ) 100 mg/kg | 99.75± 04.66 | 415.65± 13.51 | 319.38± 07.70 ## | 246.88± 10.20 ### | 184.87± 13.31 ### | 138.65± 03.79 ### | 126.67± 04.70 ### | 91.95± 04.40### |
| VI | Diabetic + (REAR+SEEJ) 200 mg/kg | 103.4 ±05.62 | 412.65±15 .69 | 328.12±15.1 9 ## | 234.88±08.0 1### | 187.23±06. 78### | 133.82±02. 97### | 113.05±05 .02### | 95.45 ±04.22### |

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

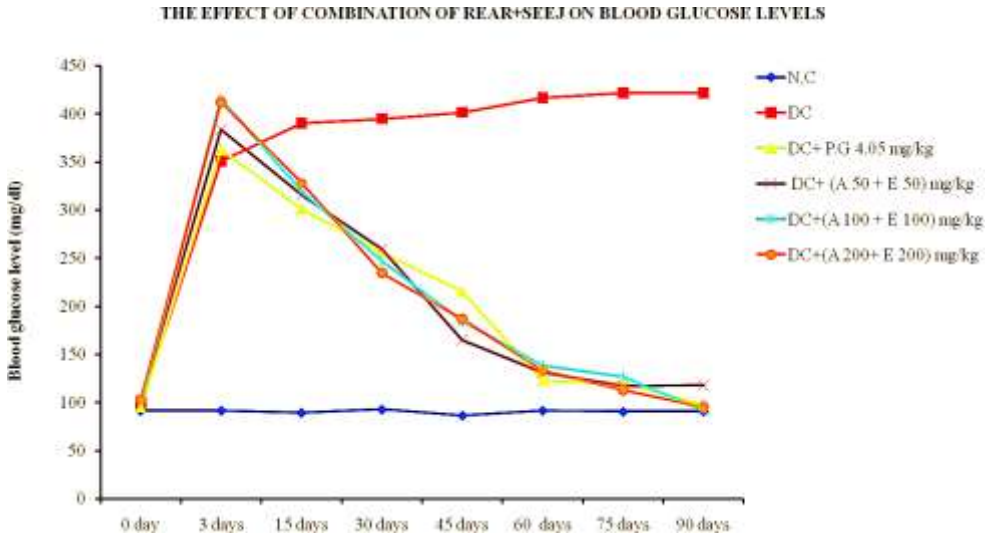


**Figure 1 : The effect of combinations of REAR and SEEJ on blood glucose level,**

**NC: Normal control, DC: Diabetic control, PG: Pioglitazone, A: *Asparagus racemosus*, E: *Eugenia jambolana***

**Glycosylated haemoglobin level:** The levels of glycosylated haemoglobin ($HbA_1C$) were significantly ($P<0.001$) increased in diabetic control group of rats. The pioglitazone 4.05mg/kg treated group showed significant ($P<0.001$) decrease in $HbA_1C$ when compared to diabetic control group of rats. In diabetic rats treated with combinations of REAR 50mg/kg and SEEJ 50mg/kg showed significant decrease ($P<0.001$), REAR 100mg/kg and SEEJ 100mg/kg ($P<0.001$) and REAR 200mg/kg and SEEJ 200mg/kg ($P<0.001$) in $HbA_1C$ when compared to diabetic control rats (Table 3).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 glycatedhaemoglobin 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 which serves as an indicator of metabolic control in diabetes[25]. Each 1% reduction in glycosylated haemoglobin is associated with a 37% reduction in microvascular complications, 18% myocardial infarction and 21% fewer diabetes-related

deaths[26]. In the present study also, $HbA_1C$ level increased in diabetic rats and administration of combinations of REAR andSEEJ controls the glycation of haemoglobin by an increase in glutathione peroxidase and thus decreases the level of $HbA_1C$ in experimental rats[1].

**Blood urea level:**

In diabetic control group of rats the blood urea level was significantly (P<0.001) increased when compared to normal control group. The diabetic group animals treated with the combinations of REAR 200 mg/kg and SEEJ 200 mg/kg significantly (P<0.05) decreased the blood urea level when compared to diabetic control group of animals (Table 3).The diabetic group rats treated with combinations of REAR and SEEJ significantly decreased the blood urea level when compared to diabetic control group. Elevated levels of urea occurs in diabetes mellitus due to increased protein breakdown and may also be seen in renal disorders like glomerular nephritis and chronic nephritis[2]. In the present investigation elevated level of blood urea in the diabetic group treated with combinations of REAR and SEEJ was restored to near normal level.

**Blood uric acid level:**

**Table 3. The effect of combinations of REAR and SEEJ on glycosylated haemoglobin, blood urea and blood uric acid levels**

| Groups | Treatment | Mean glycosylated haemoglobin levels(%) | Mean blood urealevels (mg/dl) | Mean blood uric acid levels (mg/dl) |
|---|---|---|---|---|
| I | Normal control | 5.08±0.30 | 35.60±1.32 | 0.95±0.12 |
| II | Diabetic control | 10.3±0.28$^{***}$ | 42.27±0.73$^{***}$ | 2.03±0.07$^{***}$ |
| III | Diabetic + PG(4.05mg/kg) | 6.6±0.14$^{\#\#\#}$ | 43.42±1.05 | 1.93±0.11 |
| IV | Diabetic+ (REAR+ SEEJ) 50 mg/kg | 7.65±0.30$^{\#\#\#}$ | 41.68±0.82 | 1.72±0.19 |
| V | Diabetic+ (REAR +SEEJ) 100 mg/kg | 5.85±0.41$^{\#\#\#}$ | 39.22±1.10 | 1.03±0.15$^{\#\#\#}$ |
| VI | Diabetic+ (REAR+SEEJ ) 200 mg/kg | 5.61±0.42$^{\#\#\#}$ | 37.23±1.01$^{\#}$ | 0.97±0.22$^{\#\#\#}$ |

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

The blood uric acid level of diabetic control group was significantly (P<0.001) increased when compared to normal control group. In diabetic rats treated with the combinations of REAR 100 mg/kg and SEEJ 100 mg/kg significantly (P<0.001) and REAR 200 mg/kg and SEEJ 200 mg/kg (P<0.001) significantly decreased the blood uric acid levels when compared to diabetic control group of rats(Table 3).In diabetic rats treated with combinations of REAR and SEEJ, 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 excreted in the proximal tubule through a voltage-sensitive urate

channel and a urateanion exchange mechanism. Hyperuricemia can be a result of either increased production or decreased excretion[27]. The combinations of REAR and SEEJ restored the elevated uric acid level in diabetic rats.

**Blood urea nitrogen level:**

In diabetic control group the blood urea nitrogen (BUN) was significantly (P<0.001) increased when compared to normal control group. In diabetic rats treated with the combinations of REAR 50 mg/kg and SEEJ 50 mg/kg(P<0.01), REAR 100 mg/kg and SEEJ 100 mg/kg (P<0.001), and REAR 200 mg/kg and SEEJ 200 mg/kg (P<0.001) significantly decreased the blood urea nitrogen level when compared to diabetic control group(Table 4).In diabetic rats treated with combinations of REAR and SEEJ the 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. The BUN of over 20mg/dl is an indicator of decreased kidney function. The combinations of REAR and SEEJ restored the elevated blood urea nitrogen in diabetic rats[28].

**Blood creatinine level:**

**Table 4. The effect of combinations of REAR and SEEJ on BUN and creatinine levels**

| Groups | Treatment | Mean BUN levels(mg/dl) | Mean creatinine levels(mg/dl) |
|---|---|---|---|
| I | Normal control | 15.48±0.79 | 0.45±0.04 |
| II | Diabetic control | 24.7±0.57*** | 0.68±0.06*** |
| III | Diabetic+ PG 4.05 mg/kg | 23.53±0.53 | 0.65±0.03 |
| IV | Diabetic+ (REAR+ SEEJ) 50 mg/kg | 21.25±0.41## | 0.50±0.02# |
| V | Diabetic+ (REAR +SEEJ) 100 mg/kg | 17.62±0.55### | 0.46±0.01### |
| VI | Diabetic+ (REAR+SEEJ ) 200 mg/kg | 16.63±0.47### | 0.44±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.

The blood creatinine levels of diabetic control group was significantly (P<0.001) increased when compared to normal control group. In diabetic rats treated with combinations of REAR 50 mg/kg & SEEJ 50 mg/kg (P<0.05), REAR 100 mg/kg and SEEJ 100 mg/kg significantly (P<0.001) and REAR 200 mg/kg and SEEJ 200 mg/kg showed significant decrease in the blood creatinine levels when compared to diabetic control group (Table 4).But diabetic rats treated with combinations of REAR and SEEJ 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[29]. If serum creatinine levels increased due to hyperglycemia that causes osmotic diuresis and depletion of extracellular fluid volume[30]. The combinations of REAR and SEEJ significantly reversed the elevated blood creatinine in diabetic rats.

**The effect of combination of REAR and SEEJ on urine parameters**

**Volume of urine:**

The diabetic control group of rats showed significant (P<0.001) increase in volume of urine when compared normal control group of 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 of rats. But in diabetic control group of rats treated with combinations of REAR 50 mg/kg and SEEJ 50 mg/kg (P<0.001), REAR 100 mg/kg and SEEJ 100 mg/kg significantly (P<0.001) and REAR 200 mg/kg and SEEJ 200 mg/kg (P<0.001), significantly decreased the volume of urine levels when compared to diabetic control group of rats(Table 5). 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 combinations of REAR and SEEJ,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[31]. The combinations of REAR and SEEJ significantly decreases the increased volume of urine output in diabetic rats.

**Urine microalbumin level:**

The urine microalbumin levels in the diabetic control group of rats were significantly (P<0.001) increased when compared to normal control group of rats. In diabetic control rats treated with the combinations of REAR 50 mg/kg & SEEJ 50 mg/kg (P<0.01), REAR 100 mg/kg and SEEJ 100 mg/kg significantly (P<0.001) and REAR 200 mg/kg and SEEJ 200 mg/kg (P<0.001) showed significant decrease in the urine microalbumin levels when compared to diabetic control group of rats (Table 5). The diabetic control rats treated with combinations of REAR and SEEJ 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 happends in diabetes[32]. The combinations of REAR and SEEJ restored the elevated levels of urine microalbumin in the diabetic rats.

**Table 5. The effect of combinations of REAR andSEEJ on volume of urine and urine microalbumin 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 | 19.6±1.02*** | 0.77±0.05*** |
| III | Diabetic+ PG 4.05 mg/kg | 3.05±0.54### | 0.58±0.06 |
| IV | Diabetic+ (REAR+ SEEJ) 50 mg/kg | 9.62±1.25### | 0.50±0.04## |
| V | Diabetic+ (REAR +SEEJ) 100 mg/kg | 3.12±0.39### | 0.35±0.04### |
| VI | Diabetic+ (REAR+SEEJ ) 200 mg/kg | 2.92±0.34### | 0.30±0.03### |

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

**Histopathalogical studies**

The histopathological observation of the rat kidneys revealed that the normal control group rats shows normal glomeruli and tubules with healthy epithelial cells. The kidneys of diabetic control rats shows thickening of vesicles, disrupted tubules, degeneration and necrosis of epithelial cells and inter tubular haemorrhage. But the kidneys of diabetic rats treated with the combinations of REAR 50 mg/kg and SEEJ 50 mg/kg, REAR 100 mg/kg and SEEJ 100 mg/kg and REAR 200 mg/kg and SEEJ 200 mg/kg, showed regeneration of tubular epithelium depicting normal tubules with intact epithelium and presence of few RBCs in between the tubules. The report of histopathological studies of rat kidneys strongly supports the outcome of the study by restoring the kidney damage.

## CONCLUSION

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 were significantly reversed by the combinations of REAR and SEEJ in streptozotocin- nicotinamide induced type- 2 diabetic nephropathyin male albino *Wistar* rats. Therefore, this investigation concluded that the combinations of REAR and SEEJ may be used as an antidiabetic agent and nephroprotective for chronic type-2 diabetes mellitus patients to prevent the nephropathy complications in diabetic populations, after confirming its efficacy and safety inwell-controlled clinical trials. If it is confirmed in humans, the

combinations of REAR and SEEJ may be a potent, safe and cost effective phytomedicine to prevent nephropathy-induced premature death in diabetic patients.

## REFERENCES


1. Pari L, Srinivasan S. Antihyperglycemic effect of diosmin on hepatic key enzymes of carbohydrate metabolism in streptozotocin-nicotinamide induced diabetic rats. Biomed Pharmacother 2010;64: 477- 481.
2. Srivastava B, Sinha AK, Sanjay G, Barshiliya Y. Study of hypoglycaemic and hypolipidemic activity of Eugenia jambolana pulp and seed extract in Streptozotocin induced diabetic albino rats. Asian J Pharma Life Sci 2012; 2: 10-19.
3. Ramachandran A, Das AK, Joshi SR, YajnikCS, Shah S, Prasannakumar KM. Current status of Diabetes in India and need for novel therapeutic agent. J Assoc physician India 2010; 58:7-9.
4. King H, Anbert RE, Herman, WH. Global burden of diabetes, 1995-2025. Prevalence, numerical estimates and projections. Diabet Care 1998; 21:1414-1431.
5. Kim OS, Kim YS, JangDS, Yoo NH, Kim JS. Cytoprotection against hydrogen peroxide-inducedcell death in cultured mouse mesangial cells by erigeron flavanone a novel compound from the flower of Erigerannuus. Chem Biol Interact 2009;180:414-420.
6. Chen KH, Hung CC, Hsu HH, Jing YH, Yang CW, Chen JK. Resveratrol ameliorates early diabetic nephropathy associated with suppression of augmented TGF-β/smad and ERK1/2 signaling in streptozotocin-induced diabetic. Rats. ChemBiol Interact 2011;190:45-53.
7. Vadivelan R, Dipanjan M, Umasankar P, Dhanabal SP, Satishkumar MN, Antony S, Elango K. Hypoglycemic, antioxidant and hypolipidemic activity of Asparagus racemosuson streptozotocin-induced diabetic in rats. Adv Applied Sci Res 2011; 2: 179-185.
8. Marles RJ, Farnsworth NR. Antidiabetic plants and their active constituents. Phytomed 1995; 2: 137-189.
9. De Sousa E, Zanatta L, Seifriz I, TB. Creczynski-Pasa TB, Pizzolatti MG, Szpoganiz B, SilvaFRMB. Hypoglycemic effect and antioxidant potential ofkaemferol-3,7-O-(a)-dirhamnoside from Bauhinia for cataleaves . J Nat Prod 2004;67:829-832.
10. Valiathan MS. Healing plants. CurrSci 1998; 75:1122-1126.

11. Grover JK, Yadav S, Vats V. Medicinal plants of India with antidiabetic potential. J EthnoPharmacol2002;81: 81-100.
12. Chopra RN, Chopra IC, Handa KL, Kapur LD. Indigenous drugs of India, 2nd ed., Calcutta: Dharand Sons; 1958: 686-89.
13. Sharma SB, Nasir A, Prabhu KM, Murthy PS, Dev G. Hypoglycemic and hypolipidemic effects ofethanolic extract of seeds of Eugenia jambolana inalloxan induced diabetic rabbits. JEthanoPharmacol2003;85:201-206.
14. Chaturvedi A, Bhawani G, Agarwal PK,Goel S, Singh A,Goel RK. Antidiabetic and antiulcer effects of extract of Eugenia jambolana seed in mild diabetic rats: Study on gastric mucosal offensive acid-pepsin secretion. Indian J PhysiolPharmacol 2009; 53: 137-146.
15. Bhuiyan MA, Mia MY, Rashid MA. Antibacterial principles of the seeds of Eugenia jambolana. Bangladesh J Biol 1996; 25: 239-241.
16. Chaudhuri N, Pal AK, Gomes S, Bhattacharya A. Anti-inflammatory and related action of Syzygium cumini seed extract. PhytotherRes 1990; 4:5-10.
17. Chakrabarty D, Mahapatra PK, ChaudhuriAKN. A Neuropsyco pharmacological study of Syzygium cumini. Planta Medico 1985; 2:139 -143.
18. Kusumoto IT, Nakabayashi T, Kida H, Miyashira H, Hattari H, Namba T, Shimotohno K. Screening ofvarious plant extracts used in Ayurvedic medicine for inhibitory effects on human immunodeficiency virus type I (HIV-I) protease. PhytotherRes 1995; 12: 488-493.
19. Indira G, Mohan R, Jamun Fruits. National Institute of Nutrition. Indian Council of Medical Research, Hyderabad 1993; 4: 34-37.
20. Swanston-Fiatt SK, Day C, Bailey CJ, FlattPR. Traditional plant treatments for diabetes: studies in normal and streptozotocin diabetic mice. Diabetologia 1990;33:462-464.
21. Shirwaikar A, Rajendran K, Dinesh kumar C, Bodla R. Anti-diabetic activity of aqueous leaf extract of Annona squamosa in streptozotocin- nicotinamide type 2 diabetic rats. J Ethnopharmacol 2004;91:171-175.
22. Kiran G, Nandini CD, Ramesh HP, Salimath PV. Progression of early phase diabetic nephropathy in streptozotocin-induced diabetic rats: Evaluation of various kidney related parameters. Indian J ExpBiol 2012;50:133-140.
23. Anandharajan R, Jaiganesh S, Shankeranarayanan NP, Viswakarma RA, Balakrishnan A. In-vitro glucose uptake activity of Aegles marmelos and Syzygium cumini by activation of Glut-4, PI3 Kinase and PPARγ in L6 myotubes. Phytomedi 2006; 13: 434-441.

24. Samidha K, Ashwini K, Renuka M, Swpriya, Bhalerao. Evaluation of the adipogenic potential and glucose uptake stimulatory activity of Phyllanthus emblica and Tinospora cordifolia: an in-vitro study. Inter J PharmaBiosci 2012; 3: 230-236.
25. Chen B, Bestetti G, Day RM, Turner AP. The synthesis and screening of a combinatorial peptidelibrary for affinity ligands for glycosylated haemoglobin. Biosens Bioelectron 1998;13:779-785.
26. Noriega CR, Avila O, Gutierrez E, Guerrero MC, Gerciglia R, Rojo C.Hypolipidemic activity of Eryngiumcarlinae on streptozotocin induced diabetic rats. Biochem Res Int 2012;10:60-65.
27. Siu YP, Leung KT, Tong MK, Kwan TH. Use of allopurinol in slowing the progression of renal disease through its ability to lower serum uric acid level. Am J Kidney Dis 2006;47:51-59.
28. Dabla KP. Renal function in diabetic nephropathy. World J Diabetes 2010;1:48-56.
29. Ramesh B, Viswanathan P, Pugalendi KV. Protective effect of umbelliferone on membranous fattyacid composition in streptozotocin-induced diabetic rats. Eur J Pharmacol 2007;566:231-239.
30. Patel S, Shan SR, Goyal KR. Antihyperglycemic antihyperlipidemic and antioxidant effects of a Dihar a polyherbal ayurvedic formulation in streptozotocin induced diabetic rats. Indian J ExpBiol 2009;47:564-570.
31. [Grover JK, Vats V, Rathi SS, Dawar R. Traditional Indian antidiabetic plants attenuate progression of renal damage in streptozotocin induced diabetic mice. J Ethano Pharmacol 2001;76:233-238.
32. Ramkumar KM, Ponmanickam P, Velayuthaprabhu S, Archunan G, Rajaguru P. Protective effect of Gymnemamontanum against renal damage in experimental diabetic rats. Food ChemToxicol 2009;47:2516-2525.