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Research Article

Evaluation of Antidiabetic Plants used by Tribes of Telangana State on Diabetic Complications like Neuropathy, Nephropathy and Cardiomyopathy in Rats

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Abstract

Background: India is "diabetes capital of the world". Diabetes Atlas 2006 published by International Diabetes Federation, India currently around 40.9 million is expected to rise to 69.9 million by 2025 unless urgent preventive steps are taken. Over the past 30 yr, the status of diabetes has changed from being considered as a mild disorder to major causes of morbidity and mortality.

Methods: Rats treated with Alloxan (150 mg/kg) i.p. results diabetic rats given ethanol extract of *Senna auriculata* leaf, *Syzygium cumini (L.)* Skeels seeds and *Syzygium cumini (L.)* Skeels seeds (150 mg/kg) p.o., respectively for 42 days. Biochemical parameters of diabetic neuropathy, nephropathy and cardiomyopathy and histopathology of sciatic nerve, kidney and heart was done at the end of study.

Results: In Diabetic Group found Blood Glucose Level (BGL) (84.42±6.384 to 369.36±7.784mg/dl); Muscle Grip Strength (MGS) (59.32±1.052 to 13.52±0.883seconds); Thermal Pain Response (TPR) (5.55±0.621 to 13.67±1.164seconds). blood protein (7.48±0.051 to 25.18±0.046mg/dl); urine protein (0.692±0.061 to 2.68±0.056mg/dl); blood albumin (1.94±0.043 to 0.248±0.007mg/dl); urine albumin (0.082±0.009 to 2.68±0.056mg/dl); blood myoglobin (0.042±0.00274 to 0.056±0.00207ng/dl); urine myoglobin (0.0048±0.00142 to 0.0098±0.00107mg/dl); Blood Urea Nitrogen (BUN) (23.04±1.093 to 124.81±1.238 mg/dl); Serum Creatinine (84.06±6.723 to 218.56±7.586 (µMol/dl). Etholic extract of *Senna auriculata leaf*, *Phyllanthus emblica.L.* fruits and *Syzygium cumini (L.)* Skeels seeds & combination treated groups found BGL124.42±7.042, 112.07±6.942, 126.25±7.051 & 98.83±6.932mg/dl; MGS 49.06±0.962, 52.05±1.247, 54.06±1.268 & 56.79±1.125 seconds; TPR 6.54±0.841, 7.38±0.802, 6.45±1.062 & 6.14±0.837 seconds; blood protein 7.98±0.039, 8.02±0.053, 8.06±0.039 & 7.48±0.045mg/dl; urine protein 1.22±0.058, 0.94±0.049, 0.96±0.056 & 0.82±0.062mg/dl; blood albumin 1.64±0.033, 1.82±0.036, 1.87±0.044 & 1.96±0.039mg/dl; urine albumin 0.122±0.008, 0.098±0.007, 0.132±0.009 & 0.108±0.011mg/dl; blood myoglobin 0.045±0.00189, 0.036±0.00177, 0.041±0.00223 & 0.043±0.00175ng/dl; urine myoglobin 0.0042±0.00129, 0.0052±0.00119, 0.0064±0.00126 & 0.0036±0.00125mg/dl; BUN 35.81±1.186, 36.06±1.123, 34.53±1.177 & 29.03±1.229mg/dl; Serum Creatinine 98.42±5.526, 99.73±6.064, 101.97±6.052 & 94.83±6.678µMol/dl.

Conclusion: Ethanol extract of Senna auriculata leaf, *Phyllanthus emblica L.* fruit and *Syzygium cumini (L.)* Skeels seeds (150mg/kg) and its combination normalizes biochemical parameters & Morphological changes in sciatic nerve, myocardium & kidney and improvement of the general behavioral parameters. Combination was found to be more effective in these diabetic complications.

Introduction

Diabetes mellitus, often simply referred to as diabetes is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced [1]. India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the "diabetes capital of the world". According to the Diabetes Atlas 2006 published by the International Diabetes Federation, the number of people with diabetes in India currently around 40.9 million is expected to rise to 69.9 million by 2025 unless urgent preventive steps are taken. Even though the prevalence of microvascular complications of diabetes like retinopathy and nephropathy are comparatively lower in Indians, the prevalence of premature coronary artery disease is much higher in Indians compared to other ethnic groups. The most disturbing trend is the shift in age of onset of diabetes to a younger age in the recent years. This could have long lasting adverse effects on nation's health and economy. The prevalence of diabetes is rapidly rising all over the globe at an alarming rate. Over the past 30 yr, the status of diabetes has changed from being considered as a mild disorder of the elderly to one of the major causes of morbidity and mortality affecting the youth and middle aged people [2].

Although there is an increase in the prevalence of type 1diabetes also, the major driver of the epidemic is the 217 more common form of diabetes, namely type 2 diabetes, which accounts for more than 90 per cent of all diabetes cases. Nowhere is the diabetes epidemic more pronounced than in India as the World Health Organization (WHO) reports show that 32 million people had

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diabetes in the year 2002. The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025. The prevalence was 2.1 per cent in urban population and 1.5 per cent in the rural population while in those above 40 yr of age, the prevalence was 5 per cent in urban and 2.8 per cent in rural areas7. Prevalence of diabetes in adults worldwide was estimated to be 4.0% in 1995 and to rise to 5.4% by the year 2025. It is higher in developed than in developing countries. The number of adults with diabetes in the world will rise from 135 million in 1995 to 300 million in the year 2025. The association between diabetes and adverse cardiovascular outcomes, such as Heart Failure (HF) with or without preserved systolic ventricular function, is well known8. Overall, 36-47% of all patients with clinical HF and 32-33% of those with HF and a normal Ejection Fraction (EF) have diabetes. Although they are frequently associated, the cardiomyopathy of diabetes seems to develop independently of coronary artery disease, valvular heart disease, or hypertension. Diabetic cardiomyopathy progresses from impaired ventricular relaxation to diastolic dysfunction, with high left ventricular filling pressures, and finally to overt HF [2-4].

- Diabetic Cardiomyopathy (DCM) is a clinical condition diagnosed when ventricular dysfunction develops in patients with diabetes in the absence of coronary atherosclerosis and hypertension [5].
- DCM may be characterized functionally by ventricular dilation, myocyte hypertrophy, prominent interstitial fibrosis and decreased or preserved systolic function in the presence of a diastolic dysfunction [5].

Symptoms

- Signs and symptoms of diabetic cardiomyopathy may be absent. Congestive heart failure symptoms include shortness of breath, swelling in the abdomen or lower extremities and intolerance to exercise. Fluid that accumulates in the chest because of the decreased ability of the heart to pump effectively may lead to chest congestion and cough, in addition to feelings of pressure in the chest [6-8].
- Decreased diastolic compliance
- Systolic dysfunction
- Left ventricular hypertrophy
- Higher LV wall thickness and mass in diabetic hearts.
- Ventricular hypertrophy and dysfunction
- Diabetic neuropathies are a family of nerve disorders caused by diabetes. People with diabetes can, over time, develop nerve damage throughout the body. Some people with nerve damage have no symptoms. Others may have symptoms such as pain, tingling, or numbness-loss of feeling-in the hands, arms, feet, and legs. Nerve problems can occur in every organ system, including the digestive tract, heart, and sex organs.
- Diabetic neuropathies are neuropathic disorders that are associated with diabetes mellitus. These conditions are thought to result from diabetic microvascular injury involving small blood vessels that supply nerves (vasa nervorum) in addition to macrovascular conditions that can culminate in diabetic neuropathy. Relatively common conditions which may be associated with diabetic neuropathy include third nerve palsy; mononeuropathy; mononeuropathy multiplex; diabetic amyotrophy; a painful polyneuropathy; autonomic neuropathy; and thoracoabdominal neuropathy [9].

Diabetic nephropathy is the leading cause of renal failure. It is defined by proteinuria > 500 mg in 24 hours in the setting of diabetes, but this is preceded by lower degrees of proteinuria, or "microalbuminuria." Microalbuminuria is defined as albumin excretion of 30-299 mg/24 hours. Without intervention, diabetic patients with microalbuminuria typically progress to proteinuria and overt diabetic nephropathy. This progression occurs in both type 1 and type 2 diabetes.

The pathological changes to the kidney include increased glomerular basement membrane thickness, microaneurysm formation, mesangial nodule formation (Kimmelsteil-Wilson bodies), and other changes. As many as 7% of patients with type 2 diabetes may already have microalbuminuria at the time they are diagnosed with diabetes [10].

Therapeutics agents like insulin, sulfonylureas, biguanides and thiazolidinedione derivatives and α glucosidase inhibitors are preferred [11]. To reduce the hyperglycemic condition. The drugs that are preferred for treatment such as sulfonylureas which stimulates pancreatic islets to secrete insulin. Biguanides which are responsible for the reduction of hepatic glucose output. Thiazolidinedione derivatives exert their peripheral action by lowering insulin resistance in peripheral tissue. α - glucosidase inhibitors augment glucose utilisation and responsible for suppression of glucose production [12]. Apart from the therapeutic option for diabetes like oral hypoglycemic and insulin have some adverse effects [13]. Hence the current therapy is focused on herbal medicines [14]. And they are used for current therapy due to presumed effectiveness, relatively low cost, presumed fewer side effects and low toxicity [15]. The medicinal plants might provide a useful source of new oral hypoglycemic compounds, and this may lead to the development of pharmaceutical entities, and this may act as a dietary ~ 32 ~. The Pharma Innovation Journal adjunct to existing therapies [16]. Worldwide there are more than 1200 plant species, some of the medicinal plants that are used to control blood glucose levels such as *Azadirachta Indica*, *Catharanthus roseus*, *Allium Sativum*, *Memordica judaica*, *Aloe Vera*, *Trigonella foenum graecum*. Due to the presence of active principles in medicinal plants they have been reported to possess some characteristic properties like pancreatic β cell regenerating, insulin-releasing and fighting the problem of insulin resistance. India is well known for its great heritage of herbal medicinal knowledge. Large number of tribals and ethnic people living in the remote forest areas depend on plants to a great extent for foods, medicine, pharmaceuticals and agrochemicals. From the decades studies on ethnobotany have gained importance. Diabetes is an important chronic disorder afflicting many from various walks of life around the world. Though they are various allopathic drugs used to treat the worse effects of diabetes, herbal formulations are preferred to minimize the risk of side effects and due to low cost [17-19].

According to WHO's estimation 80% of the world's population use herbal medicine. Now a days traditional medicine with good clinical practice is showing a lively future in treating diabetes and its

complications. From the decades vigorous research on ethnobotany shows that plant and its derivatives are useful in the treatment of diabetes mellitus. Though there are numerous approaches to treat diabetes but traditional medicine is preferred due to its lesser side effects and low cost. In Indian systems of herbal medicine most traditional practitioners formulate and give out their own recipes. India is the largest producer of medicinal plants and approx. 2,500 species of plants are used for medicinal purposes [20-22]. The current study was undertaken in the tribal region of Telangana state in order to list out the plant species having antidiabetic activity used by the traditional practitioners.

Materials and Methods

Plant material

The *Senna auriculata (L.) Roxb*. Leaves, The *Phyllanthus emblica L.* Fruits and The *Syzygium cumini (L.)* Skeels seeds were freshly collected from the rural areas of Hyderabad, telangana state, India. The plant were identified and authenticated by Dr. A. Manohar Rao; Professor & Head; Department of Horticulture, College of Agriculture, Professor Jayashankar Telangana Agriculture University, Rajendranagar, Hyderbad, Telagana-500030.; India. A voucher specimens was deposited in Ethnopharmacology unit, Department of Horticulture, College of Agriculture, Professor Jayashankar Telangana Agriculture University, Rajendranagar, Hyderbad, Telagana-500030.

Preparation of plant extract for antidiabetic studies [23,24]: (A) The Senna auriculata leaves were shade dried at room temperature and the dried leaves were powdered in a Wiley mill. Hundred grams of powdered Senna auriculata leaves was packed in a Soxhlet apparatus and extracted with ethanol the extract were subjected to qualitative test for the identification of various phytochemical constituents as per the standard procedures. The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract were used for antidiabetic studies.

(B) The *Phyllanthus emblica L.* fruits were shade dried at room temperature and the dried leaves were powdered in a Wiley mill. Hundred grams of powdered *Phyllanthus emblica L.* fruits was packed in a Soxhlet apparatus and extracted with ethanol the extract were subjected to qualitative test for the identification of various phytochemical constituents as per the standard procedures. The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract were used for antidiabetic studies.

(C) The *Syzygium cumini (L.)* Skeels seeds were shade dried at room temperature and the dried leaves were powdered in a Wiley mill. Hundred grams of powdered *Syzygium cumini (L.)* Skeels seeds was packed in a Soxhlet apparatus and extracted with ethanol The extract were subjected to qualitative test for the identification of various phytochemical constituents as per the standard procedures. The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract were used for antidiabetic studies.

Animals

Normal healthy male Wistar albino rats (180-240g) were housed under standard environmental conditions at temperature (25±2°C) and light and dark (12:12 h). Rats were fed with standard pellet diet (Kisan Feeds, New Delhi, India) and water ad libitum. The Experimental Protocol has been approved by Institutional Animal Ethical Commeettee, Arya College of Pharmacy S-40, RIICO Industrial Area, Delhi Road Kukas, Jaipur, Rajasthan. India. CPCSEA No. 1013/PO/c/06/CPCSEA.

Acute toxicity study

Acute oral toxicity study was performed as per OECD- 423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study (Acute oral Toxicity- Acute Toxic Class method. OECD. Paris. 2002). The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50,100, and 1000 mg/kg body weight.

Induction of experimental diabetes

Rats were induced diabetes by the administration of simple intraperitioneal dose of alloxan monohydrate (150 mg/kg). Two days after alloxan injection, rats screened for diabetes having glycosuria and hypoglycemia with blood glucose level of 200-260 mg/100 ml were taken for the study. All animals were allowed free access to water and pellet diet and maintained at room temperature in plastic cages [25].

Experimental design: In the investigation, a total of 42 rats (36 diabetic surviving rats and 6 normal rats) were taken and divided into seven groups of 6 rats each.

Group I: Normal, untreated rats.

Group II: Diabetic control rats

Group III: Diabetic rats given standard drug glimepiride (10mg/kg of body weight).

Group IV: Diabetic rats given ethanol extract of *Senna auriculata leaf* (150 mg/kg of body weight).

Group V: Diabetic rats given ethanol extract of *Phyllanthus emblica L.* fruit. (150 mg/kg of body weight).

Group VI: Diabetic rats given ethanol extract of *Syzygium cumini (L.)* Skeels seeds (150 mg/kg of body weight).

Group VI: Diabetic rats given combination of ethanol extract of *Senna auriculata leaf*, *Phyllanthus emblica L.* fruit and *Syzygium cumini (L.)* Skeels seeds (150 mg/kg of body weight).

Drug treatment was carried for 6 weeks with the help of oral catheter on every day morning. At the end of drug treatment duration, all the animals were fasted overnight but allowed free access to water. Following morning, the following parameters were analyzed in blood and urine:

Collection of blood sample and urine

At the end of drug treatment, all the animals were kept in metabolic cages for 24 hrs. All the animals were fasted overnight but allowed free access to water. Next day morning, blood sample was withdrawn by retro-orbital puncture under mild ether anesthesia.

Serum: Blood sample was collected into an eppendorf tube. The sample was allowed to clot completely (20 minutes) before centrifugation. It was centrifuged at 4000 rpm for 30 minutes in a refrigerated centrifuge at 4°C. The serum separated as straw colored supernatant was analyzed for above stated biochemical parameters and markers. Serum was stored at -20°C until the completion of analysis.

Collection of urine sample: At the end of drug treatment, all the animals were kept in metabolic cages for 24 hrs. Animals were fasted but allowed free access to water. Urine sample were collected after 24 hrs in urine collecting bottles.

Biochemical analysis

The animals were sacrificed at the end of experimental period of 14 days by decapitation. Blood was collected, sera separated by centrifugation at 3000 g for 10 minutes. Following parameters of diabetic complications (cardiomyopathy, neuropathy & nephropathy) analyzed in the normal, diabetic induced and drug treated rats.

Biochemical estimation of parameters of diabetic nephropathy: Measurement of renal function and biochemical parameters

1. Blood glucose was measured by Accu-Chek Active glucose strips. The blood glucose estimation was done weekly after administration of test compound.
2. Protein Estimation in Urine and serum: The rat's urine was collected through activity cage. The protein was precipitated with trichloroacetic acid (final concentration was 0.33 mol/liter). After mixtures had stood for 30 min at room temperature, the precipitates were centrifuged for 20 min at 110xg. The precipitate was processed and, after reaction with biuret reagent, absorbency was measured by colorimeter.

   The total protein concentration was determined by……

   Absorbance of Test

   Total Protein Concentration (g/dl) = ---------- × 6.5

   Absorbance of Standard

   The formula was used for both determination of protein in serum as well as in urine samples.

3. **Serum and urine Albumin Levels**

Bromo Creso Green (BCG) method: (using Span and Ranbaxy diagnostic kits by autoanalyser (Echo, Logotech Pvt. Ltd, India) [26].

Principle: Albumin binds with the dye Bromocresol Green in a buffered medium to form a green coloured complex. The intensity of the colour formed is directly proportional to the amount of albumin present in the sample.

Wavelength / filter : 630 nm (Hg 623 nm) / Red

Temperature : R.T.

Light path : 1 cm

Reagents: All chemicals must be Analar grade

Sodium hydroxide 1 M: Weigh out 4.0 g of sodium hydroxide (NaOH), dissolve and make up to 100ml with distilled water. This solution is stable for several months at room temperature (25-35^{0}C) in a polypropylene container.

Brij - 35..... 30 g/dl

Readily available at the above concentration from S.D Fine chemicals or Loba Chemical Company, in India.

Solid Brij can also be obtained from Sigma Co. In this case, warm 30 g solid Brij in a beaker in a small volume of distilled water to dissolve and make up to 100ml with distilled water.

Bromo Cresol Green (BCG) dye solution: Transfer 25 ml of I M NaOH into a one-litre volumetric flask containing 600ml distilled water. Add 5.6g succinic acid and then add 56 mg of BCG powder. Mix and then make up to 1 litre with distilled water. Check the pH. If it is less than 4.15, adjust to 4.15 + 0.05 by the dropwise addition of 1 M NaOH.

Add 100 mg sodium azide and 3.5 ml 30 g/dl Brij-35 to the reagent. Check the absorbance of the reagent at 630 nm/ red filter against distilled water. It should be less than 0.2. If it is greater than 0.2, add some more Brij to bring down the absorbance. Store ina polyethlyene container. Stable for 6 months at room temperature (25-35°C).

Standard: Bovine Serum Albumin: 4 g/dl.

Procedure: The protocol of the procedure is described below.

Mix all tubes well. Incubate at room temperature (25-35°C) for 10 minutes. Set the spectrophotometer /filter photometer to zero using blank at 630 nm/ red filter and measure the absorbance of standards, test.

4. BUN value were measured by BUN GLDH kit (Bhat Bio-tech Pvt.Ltd,Bangalore,India) technique as per instructions of manufacturers provided in BUN kits.

A Blood Urea Nitrogen (BUN) test measures the amount of nitrogen in your blood that comes from the waste product urea. Urea is made when protein is broken down in your body. Urea is made in the liver and passed out of your body in the urine.

A BUN test is done to see how well your kidneys are working. If your kidneys are not able to remove urea from the blood normally, your BUN level rises. Heart failure, dehydration, or a diet high in protein can also make your BUN level higher. Liver disease or damage can lower your BUN level. A low BUN level can occur normally in the second or third trimester of pregnancy.

5. Serum Creatinine rate was measured using CREATININE KIT by Mod. Jaffe's Kinetic Method (Coral Clinical System, Goa, India).

Creatinine is the catabolic product of creatinine phosphate, which is used by the skeletal muscle. The daily production depends on muscular mass and it is excreted out of the body entirely by the kidneys. Elevated levels are found in renal dysfunction, reduced renal blood flow (shock, dehydration, congestive heart failure) diabetes acromegaly. Decreased levels are found in muscular dystrophy.

Principle: Picric acid in an alkaline medium reacts with creatinine to form an orange coloured complex with the alkaline picrate. Intensity of the colour formed during the fixed time is directly proportional to the amount of creatinine present in the sample.

Creatinine + Alkaline Picrate- Orange Coloured Complex

6. Serum and urine Myoglobin Estimation**:** In the clinical methods for the quantitative estimation of serum proteins, filtration, through filter paper, is the usual procedure for the separation of the globulin precipitated from albumin by a 1.50 M sodium sulfate solution. On account of the nature of the precipitate, a highly retentive paper is needed, and also, with most sera, the filtrate must be refiltered many times before it is clear. Paper adsorbs a definite amount of the soluble protein. Therefore, it is necessary to discard the first portion of the filtrate, because there is a loss of albumin. Later portions are uniform in nitrogen concentration and contain the protein that is soluble in this salt concentration [27].

Histopathological studies were also carried out of heart, to evaluate degree of beneficiary effect of new pharmacological interventions by seeing morphometric changes. Tissues were also analyzed for degree of deterioration by loss of contractile protein, necrosis, inflammation, myocytolysis, and contracture bands.

Methodology for diabetic neuropathy group

(A) Body weight: Diabetic animals show reduction in body weight hence body weight of all the animals measured every week till the completion of study [28]; (B) Grip strength: (By using Rota-rod apparatus) It is used for evaluation of muscle strength during Diabetes. The test was being used to assess muscle strength or neuromuscular function in rodent which can be influenced not only by sedative drugs and muscle relaxant compound but also by toxic agents. The apparatus consist of a horizontal wooden rod or metal rod coated with rubber with 3cm diameter attached to a motor with the aped adjusted to 25 rpm. The rod is 23 cm in length and is divided into 3 sections discs, thereby allowing the simultaneous testing of 3 rats. Cages below the section serve to restrict the movement of the animal when they from the roller. Wister Albino Rats with a weight between 150-200 gm under a pretested on the apparatus. Only those animal which have demonstrated their ability to remain on the revolving rod for, at least 1minute were used for the test. The compounds were administered orally. Every week the rats were placed on the rotating rod. The fall of time was measured [29,30].

(C) Pain sensitivity: (By using Eddy's hot plat) The evaluation of pain threshold was done to evaluate sensory function. The hot plate test was carried out according to the method of Eddy's et al. Animal were placed on the hot plate maintained at 55±1°C and the reaction time was recorded as response latency. The response latencies were measured before treatment and after treatment. The cut off time for hot plate latency was at 10 seconds [29,30].

Histopathological examination

At the end of the experiments, all rats were sacrificed and pathological analysis of the heart, sciatic nerve, kidney was performed. The kidney tissues were preserved in buffered neutral formalin and stored at -20°C until processed for histopathology. Tissues were preserved in 1% w/v glutaraldehyde 4% w/v formaldehyde in phosphate buffer, pH-7.2 at 4°C until processed for electron microscopy. Tissues were processed for histopathology at room temperature and involved following steps: (a) Fixation, (b) Processing of tissues-dehydrating, clearing and embedding, (c) Preparation and cutting of sections, (d) Attaching sections to slides. After processing, sections were stained using hematoxylineosin stain using Harris's alum hematoxylin and Stock 1% w/v alcohol eosin solution. The stained sections were finally mounted in D.P.X.

Statistical analysis

Statistical Analysis was performed with Graph SYSTAT 12 software. Quantitative data were expressed as Mean ±Standard Error Mean (SEM), all data were statistically analyzed by student's t-test. Statistically significant difference between groups was set at $P<0.05$.

Results

Effect of drugs in diabetic rats on body weight

The body weight decreased rapidly in Alloxan induced diabetes in rats. Measurement of body weight of the rats of all experimental groups are shown in table 1 and figure 1. The body weight increased normally in control rats, while Alloxan induced diabetic rats (negative control) showed a significant decrease in body weight as soon as one week post Alloxan injection (Pre: 167.41±4.958 to 162.62±5.78, $p<0.01$). A progressive loss of body weight was noted after 14-days in negative control group (Pre: 167.41±4.958 to 156.89±6.203, $P<0.001$). The maximum decrease in body weight was observed after 6 weeks of Alloxan injection (Pre: 167.41±4.958 to 129.03±5.932, $P<0.001$). The weight of the animals of other groups was also decreased significantly till day 21 as compared to negative control group (Table 5a). The individual extracts (*Senna (cassia) auriculata*; *Phyllanthus emblica L.*; *Syzygium cumini (L.)* Skeels) & Standard drug treated diabetic rats showed non-significant decrease in body weight. Whereas combination of extracts group showed non-significant increase in body weight (no weight gain) (Table 1).

Table 1: Effect of Senna (cassia) auriculata; Phyllanthus emblica L.; Syzygium cumini (L.) Skeels and its combination on body weight in rats (in grams).

Groups	0 Days	7 Days	14 Days	21 Days	28 Days	35 Days	42 Days
Control	160.62±6.412	160.32±6.354	160.24±6.38	161.48±5.833	161.62±4.655	162.81±4.83	163.64±5.832
Diabetic	167.41±4.958	162.62± 5.78	156.89±6.203	153.44±6.662	140.69±5.894	134.64±6.365	129.03±5.932
Standard	153.83±5.132	149.47±6.283	146.61±6.051	138.68±6.452	140.84±5.966	143.23±5.734	141.27±6.057
Senna (cassia) auriculata	162.47±5.334	157.83±6.125	153.89±4.894	148.23±5.483	150.69±5.746	153.95±6.127	150.67±4.893
Phyllanthus emblica L.	171.93±6.052	166.79±5.943	161.42±6.423	154.46±5.651	157.03±6.198	159.28±6.235	157.81±5.846
Syzygium cumini (L.) Skeels	159.06±6.429	154.21±5.841	150.71±6.236	145.25±6.374	148.26±5.784	151.22±6.063	148.62±5.734
Combination	160.56±6.274	162.69±6.458	163.18±5.732	162.36±5.841	164.47±6.588	162.54±5.852	164.38±5.942

Note: Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, b = ns when compared to normal control group, a*** = P<0.001 when compared to negative control group, c = ns when compared to standard group. Standard = Glimipiride.

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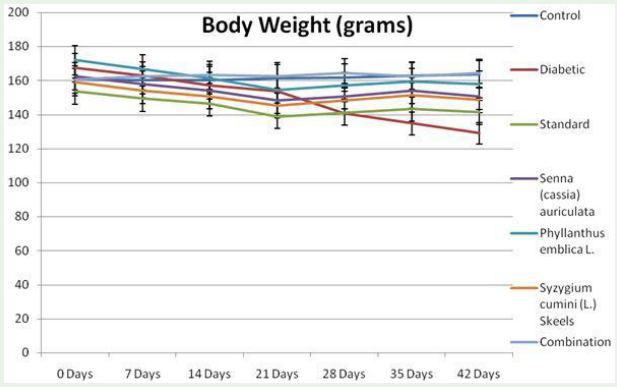

Figure 1: The Effect of *Senna (cassia) auriculata*; *Phyllanthus emblica L.*; Syzygium cumini (L.) Skeels and its combination on body weight in Alloxan induced diabetic in rats.

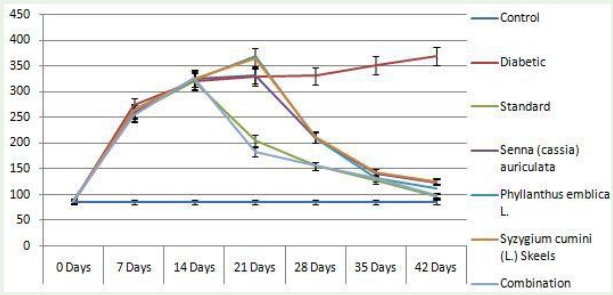

Figure 2: The Effect of *Senna (cassia) auriculata*; *Phyllanthus emblica L.*; Syzygium cumini (L.) Skeels and its combination on Blood Glucose level (mg/dl) in Alloxan induced diabetic in rats.

Effect of drugs in diabetic rats on blood glucose level

The blood glucose level of all experimental groups, except normal control group, was increased significantly after the Alloxan injection till day 21 (Table 2 and Figure 2). On the day 28 to 42 of diabetes induction, the Diabetic group observed with significant increased in blood glucose level from normal control animals (P<0.001). In the diabetic group (Negative control) the blood glucose level increased to the maximum measurable value of 369.36±7.784 mg/dl on day 42 and found to be significant increased (P<0.001) compared to the value of day 0 was 84.42±6.384 mg/dl. In control animals remain normoglycaemic during the entire testing period of 42 days (Table 5b). The animals treated on the day 21st with different groups of drug therapy like standard & Extracts of *Senna (cassia) auriculata*; *Phyllanthus emblica L.*; *Syzygium cumini (L.)* Skeels and its were observed that significant decreased in blood glucose level (P<0.001) compared to normal control group on the day 28, 35 and 42 days.

Effect of drugs in diabetic rats on muscle grip strength

Measurement of muscle grip strength was used to diagnose the diabetic neuropathy after 14 days of Alloxan injection. The muscle grip strength reduced significantly in all Alloxan treated groups that showed the induction of diabetic neuropathy. In the normal control group the muscle grip strength was normal (60.42±1.274 to 64.07±1.036 min.), so there was not a statistically significant difference found in control group (P=ns) but in the diabetic group there was significant difference was found in the muscle grip strength (59.32±1.052 to 13.52±0.883; P<0.001). The grip strength of standard and all test animals were increased significantly (P<0.001) compared to positive control group on the day 28, 35 and 42 (Table 3 and Figure 3). The grip strength of standard drug, *Senna (cassia) auriculata*; *Phyllanthus emblica L.*; *Syzygium cumini (L.)* Skeels and its combination were more significant on day 28, 35 and 42 as compared to negative control (Diabetic) group (Table 3).

Effect of drugs in diabetic rats on pain sensation (thermal pain)

In rats, a single systemic injection of Alloxan induced a hyperalgesic reaction observed for 42 days after the onset of diabetic neuropathy. In present study, hyperalgesic reaction was evaluated for a period of 42

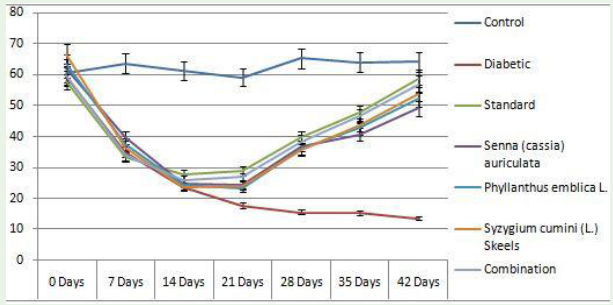

Figure 3: Effect of *Senna (cassia) auriculata*; *Phyllanthus emblica L.*; Syzygium cumini (L.) Skeels and its combination on muscle grip strength in rats (in seconds).

Table 2: Effect of Senna (cassia) auriculata; Phyllanthus emblica L.; Syzygium cumini (L.) Skeels and its combination on blood glucose level in rats (in mg/dl).

Groups	0 Days	7 Days	14 Days	21 Days	28 Days	35 Days	42 Days
Control	85.83±6.428	85.82±7.042	85.68±6.893	85.46±7.631	85.24±7.924	84.81±6.725	85.87±7.146
Diabetic	84.42±6.384	272.79±8.034	321.45±7.782	328.22±6.883	331.08±7.864	350.52±8.031	369.36±7.784
Standard	87.07±6.836	256.52±7.428	319.56±6.942	204.97±7.147	154.72±6.842	127.94±7.253	96.86±6.631
Senna (cassia) auriculata	88.72±7.035	259.98±6.931	325.92±6.739	332.04±6.853	211.05±7.326	141.81±7.736	124.42±7.042
Phyllanthus emblica L.	86.41±7.482	264.63±7.156	323.17±7.035	368.98±7.452	209.43±6.883	133.06±6.903	112.07±6.942
Syzygium cumini (L.) Skeels	87.69±6.832	262.44±6.582	324.85±6.832	365.37±6.894	212.08±7.237	142.87±7.287	126.25±7.051
Combination	88.21±6.674	253.94±6.745	326.63±8.052	182.49±7.312	156.27±6.548	132.83±8.126	98.83±6.932

Note: Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, b = ns when compared to normal control group, a*** = P<0.001 when compared to negative control group, c = ns when compared to standard group. Standard = Glimipiride.

Citation: Hussain SA and Sharma AK. Evaluation of Antidiabetic Plants used by Tribes of Telangana State on Diabetic Complications like Neuropathy, Nephropathy and Cardiomyopathy in Rats. J Nephrol Kidney Dis. 2017; 1(2): 1005.
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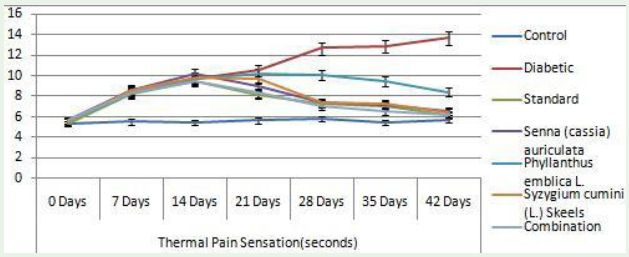

Figure 4: The Effect of *Senna (cassia) auriculata*; *Phyllanthus emblica L.*; Syzygium cumini (L.) Skeels and its combination on hot plate induced thermal pain in Alloxan induced Diabetic Neuropathy in rats.

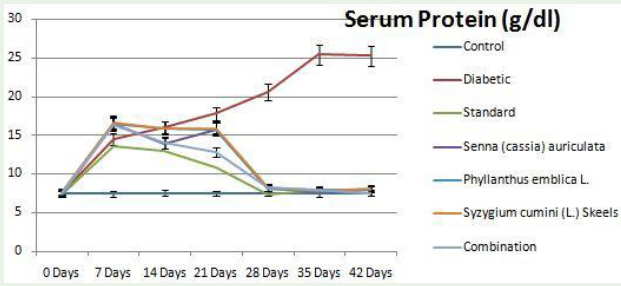

Figure 5(a): The Effect of *Senna (cassia) auriculata*; *Phyllanthus emblica L.*; Syzygium cumini (L.) Skeels and its combination on Blood Protein Level in rats (in mg/dl) in Alloxan induced Diabetic rats.

days post Alloxan treatment. The paw jumping response was measured by Eddy's hot plate. There was significant difference was found in paw jumping response after 14 days in diabetic neuropathy induce rats; but there was no significant difference was found in control group in which diabetes was not induced (5.33±0.618 to 5.68±0.647). In diabetic induce rats (negative control) there was significant increase found in paw jumping response (5.55±0.621 to 13.67±1.164). The paw jumping response of all standard ant test groups on day 21, 28 and 35 were reduced significantly to the negative control (Diabetic) group. On the day 35 the paw jumping response of standard group, *Senna (cassia) auriculata*; *Phyllanthus emblica L.*; *Syzygium cumini (L.)* Skeels and its combination were more significant on day 28th, 35th and 42nd as compared to negative control (Diabetic) group & was found to be comparable with normal control group. Treatment with combination on 15th day to 35th day produced significant effect in pain threshold when compared to negative control (Diabetic) group (Table 4 and Figure 4).

Effect of drugs in diabetic rats on protein level in blood and in urine

The protein level in blood in all experimental groups, except normal control group, was significantly increased and in urine, protein excretion rate is increased after Aloxan injection (Tables 5a & 5b and Figure 5a & 5b). On the 21st, 28th and 35th of diabetes induction, the negative control (Diabetic) group with statistical significant increased in blood protein level and increased in urine protein level from control group (P<0.001). In diabetic group (negative control group) the blood protein level increased to the maximum value of 7.48±0.051 to 25.18±0.046 mg/dl and urine protein level increased 0.692±0.061 to 2.68±0.056 and found to be statistical significance (P<0.001). In contrast, control group shows normal protein level in blood (7.42±0.044 to 7.46±0.043) during the entire testing period of 42-days tables 5a & 5b and figures 5a & 5b. The animal treated with *Senna (cassia) auriculata*; *Phyllanthus emblica L.*; *Syzygium cumini (L.)* Skeels and its combination were observed with significant decrease in blood protein level and urine protein level (P<0.001) compared to negative control group on day 21st, 28th, 35th and 42nd day. The blood protein level in Combination therapy on day 42nd was 7.48±0.045 which was significant compared with negative control (Diabetic) group.

Effect of drugs in diabetic rats on albumin (mg/dl) level in blood and in urine

The Albumin level in blood in all experimental groups, except normal control group, was significantly increased and in urine, albumin excretion rate is increased after Alloxan injection (Tables

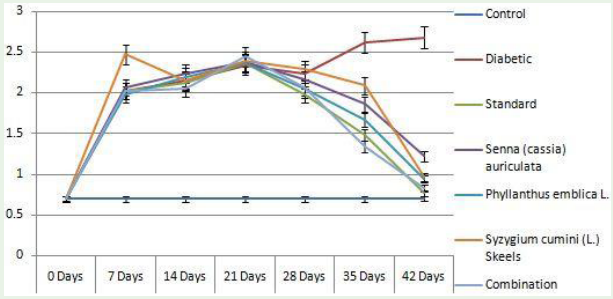

Figure 5(b): The Effect of *Senna (cassia) auriculata*; *Phyllanthus emblica L.*; Syzygium cumini (L.) Skeels and its combination on Protein Level in Urine in Alloxan induced Diabetic rats.

Table 3: Effect of Senna (cassia) auriculata; Phyllanthus emblica L.; Syzygium cumini (L.) Skeels and its combination on muscle grip strength in rats (in sec.).

Groups	0 Days	7 Days	14 Days	21 Days	28 Days	35 Days	42 Days
Control	60.42±1.274	63.57±0.893	61.38±1.237	59.14±1.042	65.32±0.831	63.94±0.925	64.07±1.036
Diabetic	59.32±1.052	34.94±0.931	23.53±1.042	17.67±1.126	15.53±1.036	15.22±0.942	13.52±0.883
Standard	58.17±1.216	33.47±0.942	27.67±0.873	28.83±1.014	39.83±0.936	47.62±1.204	58.76±1.051
Senna (cassia) auriculata	61.94±1.102	39.65±0.938	24.82±1.139	24.46±0.994	36.94±0.893	40.74±1.182	49.06±0.962
Phyllanthus emblica L.	63.43±0.932	37.38±0.869	24.01±0.942	23.04±0.877	36.06±1.235	42.71±1.037	52.05±1.247
Syzygium cumini (L.) Skeels	66.71±1.204	36.72±1.045	23.74±0.894	24.12±1.203	35.63±0.931	43.69±1.206	54.06±1.268
Combination	59.83±1.138	34.08±1.206	25.89±1.248	26.93±1.249	38.44±1.236	46.46±1.179	56.79±1.125

Note: Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, b = ns when compared to normal control group, a*** = P<0.001 when compared to negative control group, c = ns when compared to standard group. Standard = Glimipiride.

Citation: Hussain SA and Sharma AK. Evaluation of Antidiabetic Plants used by Tribes of Telangana State on Diabetic Complications like Neuropathy, Nephropathy and Cardiomyopathy in Rats. J Nephrol Kidney Dis. 2017; 1(2): 1005.
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Table 4: Effect of Senna (cassia) auriculata; Phyllanthus emblica L.; Syzygium cumini (L.) Skeels and its combination on thermal pain in rats (in sec.).

Thermal Pain Sensation(seconds)							
Groups	**0 Days**	**7 Days**	**14 Days**	**21 Days**	**28 Days**	**35 Days**	**42 Days**
Control	5.33± 0.618	5.53±0.636	5.45±0.572	5.66±0.821	5.83±0.938	5.46±0.619	5.68±0.647
Diabetic	5.55±0.621	8.56±0.719	9.65±1.038	10.5±0.735	12.66±0.693	12.87±0.942	13.67±1.164
Standard	5.33±8.643	8.23±7.784	9.45±0.726	8.16±0.847	7.28±1.0553	7.02±0.831	6.23±0.736
Senna (cassia) auriculata	5.65±1.028	8.66±0.783	10.23±0.952	8.95±0.895	7.33±1.126	7.14±0.956	6.54±0.841
Phyllanthus emblica L.	5.52±1.093	8.33±0.847	9.66±0.852	10.16±0.963	10.03±0.683	9.47±0.745	7.38±0.802
Syzygium cumini (L.) Skeels	5.57±1.043	8.56±1.058	9.83±0.842	9.73±0.938	7.43±0.793	7.26±0.928	6.45±1.062
Combination	5.66±0.629	8.39±0.736	9.52±0.842	8.36±0.741	7.06±1.152	6.48±0.835	6.14±0.837

Note: Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, b = ns when compared to normal control group, a*** = P<0.001 when compared to negative control group, c = ns when compared to standard group. Standard = Glimipiride.

6a & 6b and Figures 6a & 6b). On the 21st, 28th and 35th of diabetes induction, the negative control (Diabetic) group with statistical significant decreased in blood albumin level and increased in urine albumin level from control group (P<0.001). In diabetic group (negative control group) the blood albumin level decreased to the maximum value of 1.94±0.043 to 0.248±0.007 mg/dl and urine albumin level increased 0.082±0.009 to 2.68±0.056 and found to be statistical significance (P<0.001). In contrast, control group shows normal albumin level in blood (1.98±0.037 to 1.94±0.042) during the entire testing period of 42-days Tables 5a & 5b and Figures 5a & 5b). The animal treated with *Senna (cassia) auriculata*; *Phyllanthus emblica L.*; *Syzygium cumini (L.)* Skeels and its combination were observed with significant normal blood albumin level and urine albumin level (P<0.001) compared to negative control group on day 21st, 28th, 35th and 42nd day. The blood albumin level in Combination therapy on day 42nd was 1.96±0.039 which was significant compared with negative control (Diabetic) group.

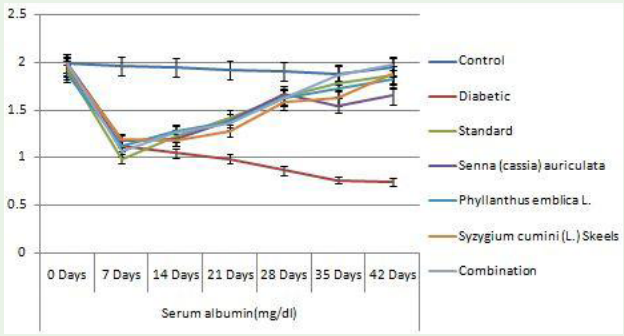

Figure 6a: The Effect of *Senna (cassia) auriculata*; *Phyllanthus emblica L.*; Syzygium cumini (L.) Skeels and its combination on Serum Albumin (mg/dl) in Alloxan induced Diabetic rats.

Effect of drugs in diabetic rats on myoglobin level in blood (ng/dl) and in urine (mg/dl)

Myoglobin levels are indication in diabetic cardiomyopathy. The Myoglobin level in blood in all experimental groups, except normal

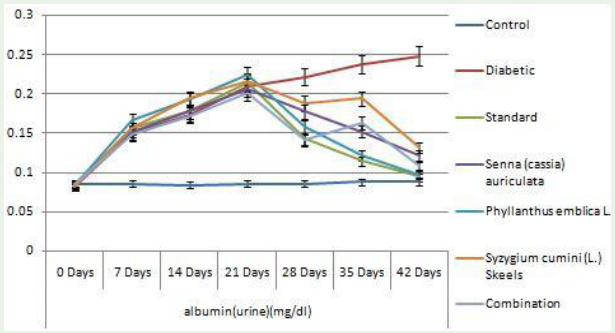

Figure 6b: Effect of *Senna (cassia) auriculata*; *Phyllanthus emblica L.*; Syzygium cumini (L.) Skeels and its combination on Albumin Urine (mg/dl). Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, b = ns when compared to normal control group, a*** = P<0.001 when compared to negative control group, c = ns when compared to standard group. Standard = Glimipiride.

Table 5a: Effect of Senna (cassia) auriculata; Phyllanthus emblica L.; Syzygium cumini (L.) Skeels and its combination on Blood Protein Level in rats (in mg/dl).

Serum Protein (g/dl)							
Groups	**0 Days**	**7 Days**	**14 Days**	**21 Days**	**28 Days**	**35 Days**	**42 Days**
Control	7.42±0.044	7.42±0.039	7.48±0.052	7.46±0.037	7.46±0.051	7.44±0.037	7.46±0.043
Diabetic	7.48±0.051	14.48±0.062	15.98±0.043	17.76±0.038	20.58±0.043	25.38±0.052	25.18±0.046
Standard	7.32±0.052	13.53±0.058	12.98±0.041	10.86±0.047	7.38±0.052	7.92±0.056	7.42±0.039
Senna (cassia) auriculata	7.34±0.038	16.52±0.052	13.98±0.044	15.82±0.041	8.16±0.047	7.85±0.053	7.98±0.039
Phyllanthus emblica L.	7.36±0.045	16.48±0.048	15.92±0.036	15.76±0.052	8.18±0.043	7.94±0.044	8.02±0.053
Syzygium cumini (L.) Skeels	7.67±0.052	16.68±0.043	15.98±0.038	15.98±0.051	8.23±0.037	7.87±0.046	8.06±0.039
Combination	7.43±0.051	16.35±0.036	14.02±0.042	12.83±0.035	8.21±0.046	7.85±0.042	7.48±0.045

Note: Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, b = ns when compared to normal control group, a*** = P<0.001 when compared to negative control group, c = ns when compared to standard group. Standard = Glimipiride.

Table 5b: Effect of Senna (cassia) auriculata; Phyllanthus emblica L.; Syzygium cumini (L.) Skeels and its combination on Protein Level in Urine in rats (mg/dl).

Protein(Urine)(mg/dl)							
Groups	0 Days	7 Days	14 Days	21 Days	28 Days	35 Days	42 Days
Control	0.694±0.052	0.696±0.047	0.696±0.039	0.694±0.042	0.696±0.036	0.694±0.051	0.698±0.055
Diabetic	0.692±0.061	2.02±0.052	2.15±0.041	2.34±0.053	2.234±0.046	2.62±0.061	2.68±0.056
Standard	0.692±0.064	2.02±0.063	2.12±0.058	2.36±0.049	1.98±0.052	1.48±0.048	0.76±0.063
Senna (cassia) auriculata	0.692±0.052	2.06±0.063	2.23±0.061	2.38±0.059	2.16±0.049	1.86±0.057	1.22±0.058
Phyllanthus emblica L.	0.692±0.045	1.98±0.062	2.19±0.056	2.36±0.064	2.04±0.054	1.663±0.062	0.94±0.049
Syzygium cumini (L.) Skeels	0.694±0.049	2.47±0.053	2.14±0.048	2.38±0.069	2.28±0.043	2.08±0.052	0.96±0.056
Combination	0.697±0.043	2.02±0.054	2.05±0.049	2.44±0.058	2.06±0.055	1.34±0.051	0.82±0.062

Note: Values are expressed MEAN±SEM, n=6, * = P<0.05 when compared to negative control group, c = ns when compared to standard group. Standard = Glimipiride.

Table 6a: Effect of Senna (cassia) auriculata; Phyllanthus emblica L.; Syzygium cumini (L.) Skeels and its combination on Serum Albumin (g/dl).

Serum albumin(mg/dl)							
Groups	**0 Days**	**7 Days**	**14 Days**	**21 Days**	**28 Days**	**35 Days**	**42 Days**
Control	1.98±0.037	1.96±0.043	1.94±0.031	1.92±0.042	1.9±0.036	1.88±0.038	1.94±0.042
Diabetic	1.94±0.043	1.12±0.033	1.04±0.035	0.98±0.042	0.86±0.038	0.76±0.032	0.74±0.043
Standard	1.92±0.032	0.98±0.041	1.22±0.046	1.42±0.031	1.64±0.035	1.78±0.035	1.86±0.042
Senna (cassia) auriculata	1.98±0.043	1.17±0.038	1.18±0.037	1.38±0.038	1.66±0.039	1.54±0.037	1.64±0.033
Phyllanthus emblica L.	1.88±0.039	1.12±0.039	1.28±0.033	1.38±0.041	1.62±0.038	1.72±0.041	1.82±0.036
Syzygium cumini (L.) Skeels	1.96±0.042	1.19±0.043	1.18±0.034	1.28±0.036	1.58±0.045	1.62±0.043	1.87±0.044
Combination	1.98±0.038	1.06±0.038	1.26±0.045	1.35±0.043	1.62±0.038	1.86±0.039	1.96±0.039

Note: Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, b = ns when compared to normal control group, a*** = P<0.001 when compared to negative control group, c = ns when compared to standard group. Standard = Glimipiride.

control group, was significantly increased and in urine, myoglobin excretion rate is increased after Alloxan injection (Tables 7a & 7b and Figures7a & 7b). On the 21, 28 and 35 of diabetes induction, the negative control (Diabetic) group with statistical significant increased in blood myoglobin level and increased in urine myoglobin level from control group (P<0.001). In diabetic group (negative control group) the blood myoglobin level increased to the maximum value of 0.042±0.00274to 0.056±0.00207 ng/dl and urine myoglobin level increased 0.0048±0.00142 to 0.0098±0.00107 mg/dl and found to be statistical significance (P<0.001). In contrast, control group shows normal myoglobin level in blood (0.038±0.00238 to 0.042±0.00276) during the entire testing period of 42-days Tables 7a & 7b and Figures 7a & 7b). The animal treated with *Senna (cassia) auriculata*; *Phyllanthus emblica L.*; *Syzygium cumini (L.)* Skeels and its combination were observed with significant normal blood myoglobin level and urine myoglobin level (P<0.001) compared to negative control group on day 21st, 28th, 35th and 42nd day. The blood myoglobin level in Combination therapy on day 42nd was 0.043±0.00175 which was significant compared with negative control (Diabetic) group.

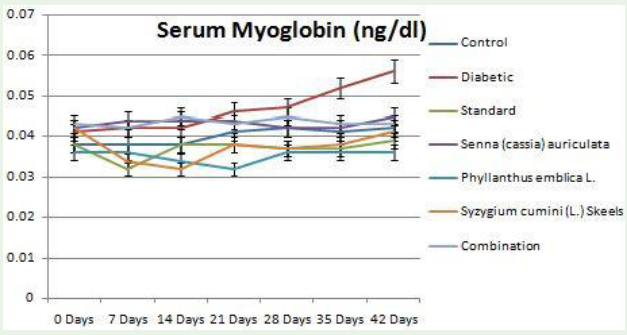

Figure 7a: Effect of *Senna (cassia) auriculata*; *Phyllanthus emblica L.*; Syzygium cumini (L.) Skeels and its combination on Myoglobin serum (ng/dl). Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, b = ns when compared to normal control group, a*** = P<0.001 when compared to negative control group, c = ns when compared to standard group. Standard = Glimipiride.

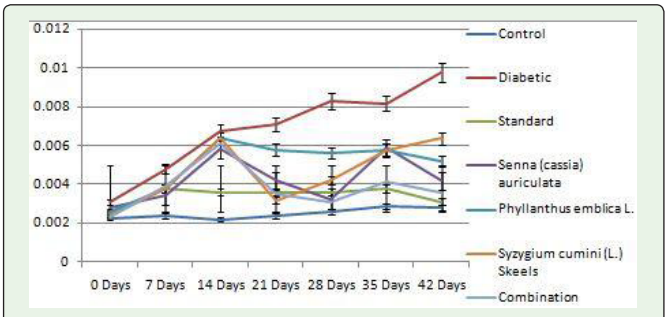

Figure 7b: Effect of *Senna (cassia) auriculata*; *Phyllanthus emblica L.*; Syzygium cumini (L.) Skeels and its combination on Myoglobin serum (ng/dl). Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, b = ns when compared to normal control group, a*** = P<0.001 when compared to negative control group, c = ns when compared to standard group. Standard = Glimipiride.

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https://dx.doi.org/10.36876/smjnkd.1005

Table 6b: Effect of Senna (cassia) auriculata; Phyllanthus emblica L.; Syzygium cumini (L.) Skeels and its combination on Albumin Urine (mg/dl).

Albumin(urine)(mg/dl)							
Groups	**0 Days**	**7 Days**	**14 Days**	**21 Days**	**28 Days**	**35 Days**	**42 Days**
Control	0.086±0.008	0.086±0.009	0.084±0.007	0.086±0.004	0.086±0.013	0.088±0.013	0.088±0.012
Diabetic	0.082±0.009	0.152±0.012	0.174±0.013	0.213±0.009	0.222±0.008	0.238±0.012	0.248±0.007
Standard	0.083±0.012	0.156±0.008	0.178±0.007	0.214±0.012	0.142±0.009	0.114±0.011	0.094±0.012
Senna auriculata	0.086±0.008	0.152±0.007	0.178±0.009	0.206±0.011	0.178±0.010	0.152±0.007	0.122±0.008
Phyllanthus emblica L.	0.084±0.012	0.167±0.014	0.192±0.006	0.224±0.007	0.158±0.0011	0.122±0.006	0.098±0.007
Syzygium cumini (L.) Skeels	0.082±0.013	0.156±0.009	0.194±0.012	0.216±0.008	0.188±0.013	0.194±0.011	0.132±0.009
Combination	0.085±0.010	0.148±0.008	0.172±0.012	0.202±0.009	0.141±0.011	0.164±0.007	0.108±0.011

Note: Values are expressed MEAN±SEM, n=6.

Effect of drugs in diabetic rats on blood urea nitrogen (bun) (mg/dl)

Blood Urea Nitrogen (BUN) (mg/dl) level in blood is indicated in diabetic nephropathy. The Blood Urea Nitrogen (BUN) (mg/dl) level in blood in all experimental groups, except normal control group, was significantly increased after Alloxan injection (Table 8 and Figure 8). On the 21st, 28th and 35th of diabetes induction, the negative control (Diabetic) group with statistical significant increased in Blood Urea Nitrogen (BUN) ($P<0.001$). In diabetic group (negative control group) the Blood Urea Nitrogen (BUN) increased to the maximum value of 23.04±1.093 to 124.81±1.238 mg/dl and found to be statistical significance ($P<0.001$). In contrast, control group shows normal Blood Urea Nitrogen (BUN) level in blood (22.76±1.352 to 24.04±1.246) during the entire testing period of 42-days Table 5 and Figure 5). The animal treated with *Senna (cassia) auriculata*; *Phyllanthus emblica L.*; *Syzygium cumini (L.)* Skeels and its combination were observed with significant normal Blood Urea Nitrogen (BUN) level ($P<0.001$) compared to negative control group on day 21st, 28th, 35th and 42nd day. The BUN level in Combination therapy on day 42nd was 29.03±1.229 which was significant compared with negative control (Diabetic) group.

Effect of drugs in diabetic rats on serum creatinine (µMol/dl)

Table 7a: Effect of Senna (cassia) auriculata; Phyllanthus emblica L.; Syzygium cumini (L.) Skeels and its combination on Myoglobin serum (ng/dl).

Serum MYOGLOBIN (ng/dl)							
Groups	**0 Days**	**7 Days**	**14 Days**	**21 Days**	**28 Days**	**35 Days**	**42 Days**
Control	0.038±0.00238	0.038±0.00219	0.038±0.00249	0.041±0.00310	0.042±0.00238	0.041±0.00192	0.042±0.00276
Diabetic	0.041±0.00210	0.042±0.00274	0.042±0.00182	0.046±0.00241	0.047±0.00239	0.052±0.00169	0.056±0.00207
Standard	0.038±0.00186	0.032±0.00215	0.038±0.00236	0.038±0.00183	0.037±0.00206	0.037±0.00294	0.039±0.00219
Senna (cassia) auriculata	0.042±0.00193	0.044±0.00223	0.044±0.00193	0.044±0.00266	0.042±0.00184	0.042±0.00213	0.045±0.00189
Phyllanthus emblica L.	0.036±0.00273	0.036±0.00169	0.034±0.00235	0.032±0.00186	0.036±0.00212	0.036±0.00255	0.036±0.00177
Syzygium cumini (L.) Skeels	0.042±0.00403	0.034±0.00208	0.032±0.00241	0.038±0.00193	0.037±0.00213	0.038±0.00158	0.041±0.00223
Combination	0.043±0.00257	0.042±0.00241	0.045±0.00183	0.043±0.00199	0.045±0.00225	0.043±0.00214	0.043±0.00175

Note: Values are expressed MEAN±SEM, n=6.

Table 7b: Effect of Senna (cassia) auriculata; Phyllanthus emblica L.; Syzygium cumini (L.) Skeels and its combination on Myoglobin serum (ng/dl).

Myoglobin(urine)(mg/dl)							
Groups	**0 Days**	**7 Days**	**14 Days**	**21 Days**	**28 Days**	**35 Days**	**42 Days**
Control	0.0023±0.00142	0.0024±0.00131	0.0022±0.00129	0.0024±0.00106	0.0026±0.00118	0.0029±0.00108	0.0028±0.00119
Diabetic	0.0031±0.00148	0.0048±0.00142	0.0068±0.00127	0.0071±0.00124	0.0831±0.00106	0.0082±0.00120	0.0098±0.00107
Standard	0.0025±0.00152	0.0038±0.00139	0.0036±0.00122	0.0036±0.00136	0.0036±0.00104	0.0038±0.00142	0.0031±0.00134
Senna (cassia) auriculata	0.0028±0.00128	0.0034±0.00125	0.0058±0.00137	0.0042±0.00128	0.0032±0.00126	0.0059±0.00141	0.0042±0.00129
Phyllanthus emblica L.	0.0026±0.00126	0.0038±0.00134	0.0064±0.00153	0.0058±0.00142	0.0056±0.00148	0.0058±0.00108	0.0052±0.00119
Syzygium cumini (L.) Skeels	0.0024±0.00147	0.0038±0.00129	0.0064±0.00125	0.0032±0.00139	0.0042±0.00134	0.0058±0.00130	0.0064±0.00126
Combination	0.0023±0.00136	0.0039±0.00134	0.0061±0.00152	0.0035±0.00124	0.0031±0.00128	0.0041±0.00132	0.0036±0.00125

Note: Values are expressed MEAN±SEM, n=6.

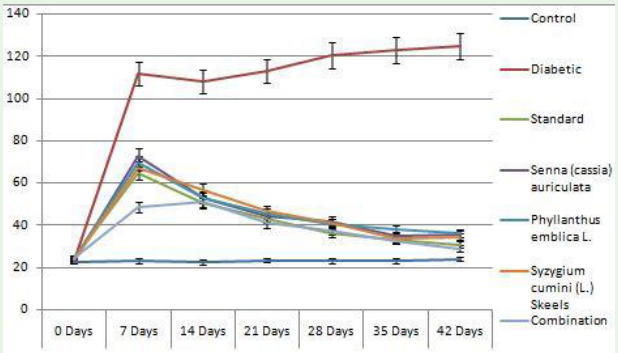

Figure 8: Effect of *Senna (cassia) auriculata*; *Phyllanthus emblica L.*; Syzygium cumini (L.) Skeels and its combination on Blood Urea Nitrogen (BUN) (mg/dl). Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, b = ns when compared to normal control group, a*** = P<0.001 when compared to negative control group, c = ns when compared to standard group. Standard = Glimipiride.

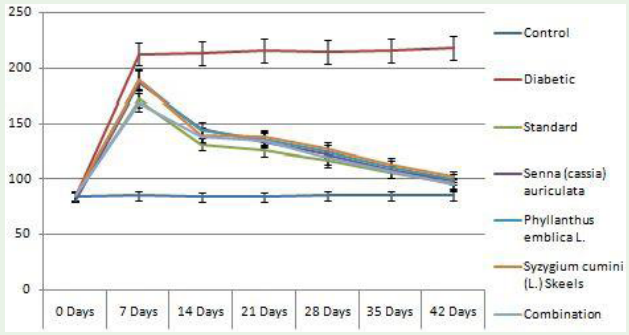

Figure 9: Effect of *Senna (cassia) auriculata*; *Phyllanthus emblica L.*; Syzygium cumini (L.) Skeels and its combination on Serum Creatinine (µMol/dl). Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, b = ns when compared to normal control group, a*** = P<0.001 when compared to negative control group, c = ns when compared to standard group. Standard = Glimipiride.

Serum Creatinine (µMol/dl) level in blood is indicated in diabetic nephropathy. The Serum Creatinine (µMol/dl) level in blood in all experimental groups, except normal control group, was significantly increased after Alloxan injection (Table 8 and Figure 8). On the 21, 28 and 35 of diabetes induction, the negative control (Diabetic) group with statistical significant increased in Serum Creatinine (µMol/dl) (P<0.001). In diabetic group (negative control group) the Serum Creatinine (µMol/dl) increased to the maximum value of 84.06±6.723 to 218.56±7.586 (µMol/dl) and found to be statistical significance (P<0.001). In contrast, control group shows normal Serum Creatinine (µMol/dl) level in blood (83.92±5.926 to 84.93±5.936) during the entire testing period of 42-days (Table 9 and Figure 9). The animal treated with *Senna (cassia) auriculata*; *Phyllanthus emblica L.*; *Syzygium cumini (L.)* Skeels and its combination were observed with significant normal Serum Creatinine (µMol/dl) level (P<0.001)

Table 8: Effect of Senna (cassia) auriculata; Phyllanthus emblica L.; Syzygium cumini (L.) Skeels and its combination on Blood Urea Nitrogen (BUN)(mg/dl).

Blood Urea Nitrogen (BUN)(mg/dl)							
Groups	**0 Days**	**7 Days**	**14 Days**	**21 Days**	**28 Days**	**35 Days**	**42 Days**
Control	22.76±1.352	23.02±1.043	22.57±0.937	23.41±1.042	22.94±1.093	23.02±1.129	24.04±1.246
Diabetic	23.04±1.093	111.98±0.951	108.09±1.303	112.75±1.205	120.47±1.047	123.06±0.859	124.81±1.238
Standard	24.31±0.936	64.73±1.238	50.36±1.152	43.06±1.243	36.09±0.981	33.06±1.204	30.64±1.263
Senna (cassia) auriculata	23.08±1.149	72.93±1.146	53.03±1.142	44.07±1.041	42.07±1.192	34.82±1.206	35.81±1.186
Phyllanthus emblica L.	24.63±1.206	69.41±1.072	52.94±1.327	45.72±1.24	40.88±1.093	38.02±0.941	36.06±1.123
Syzygium cumini (L.) Skeels	23.84±1.125	67.08±1.226	56.85±1.082	46.91±1.118	41.04±0.894	33.63±1.283	34.53±1.177
Combination	24.45±0.892	48.46±1.173	50.92±1.307	40.82±1.262	37.09±1.139	32.62±1.284	29.03±1.229

Note: Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, b = ns when compared to normal control group, a*** = P<0.001 when compared to negative control group, c = ns when compared to standard group. Standard = Glimipiride.

Table 9: Effect of Senna (cassia) auriculata; Phyllanthus emblica L.; Syzygium cumini (L.) Skeels and its combination on Serum Creatinine (µMol/dl).

Serum Creatinine (µMol/dl)							
Groups	**0 Days**	**7 Days**	**14 Days**	**21 Days**	**28 Days**	**35 Days**	**42 Days**
Control	83.92±5.926	84.87±6.042	84.06±7.231	83.85±8.216	84.72±6.732	84.62±8.564	84.93±5.936
Diabetic	84.06±6.723	212.94±5.472	213.45±6.261	215.78±7.319	214.94±8.031	216.02±5.932	218.56±7.586
Standard	84.06±6.674	172.93±5.832	132.03±7.341	126.92±8.639	116.58±6.455	105.83±5.942	96.47±5.908
Senna (cassia) auriculata	80.87±7.041	186.52±6.894	146.08±5.834	133.43±8.172	122.62±6.846	108.94±7.493	98.42±5.526
Phyllanthus emblica L.	83.62±6.942	189.41±8.172	144.09±8.058	135.81±6.905	124.76±7.453	110.63±6.639	99.73±6.064
Syzygium cumini (L.) Skeels	84.75±5.723	188.95±6.042	139.74±5.745	137.64±6.437	126.81±7.043	112.77±7.586	101.97±6.052
Combination	84.74±5.832	168.97±6.493	138.38±7.630	134.79±5.842	118.36±7.936	106.42±5.946	94.83±6.678

Note: Values are expressed MEAN±SEM, n=6, ** = P<0.01, *** = P<0.001 when compared to normal control group, b = ns when compared to normal control group, a*** = P<0.001 when compared to negative control group, c = ns when compared to standard group. Standard = Glimipiride.

Citation: Hussain SA and Sharma AK. Evaluation of Antidiabetic Plants used by Tribes of Telangana State on Diabetic Complications like Neuropathy, Nephropathy and Cardiomyopathy in Rats. J Nephrol Kidney Dis. 2017; 1(2): 1005.
https://dx.doi.org/10.36876/smjnkd.1005

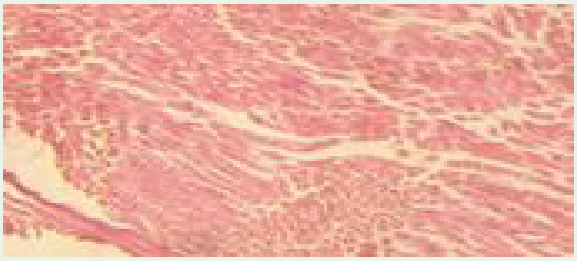

Figure 10: Normal, untreated rats. Cross sectional view of rat sciatic nerve showing normal structure.

compared to negative control group on day 21st, 28th, 35th and 42nd day. The Serum Creatinine (µMol/dl) level in Combination therapy on day 42nd was 94.83±6.678 which was significant compared with negative control (Diabetic) group.

Histopathological study

In histopathological study of Diabetic Neuropathy, Nephropathy & Cardiomyopathy which is a long-term complication of diabetes observed in 60-70% of all diabetic patients that develops early in the course of the disease. in Diabetic neuropathy there was nerve degeneration characterized by nerve fiber demylination, axonal degeneration, and a reduction in the number of medium to large diameter nerve fiber, particularly in peripheral nerve. Diabetic cardiomyopathy is demonstrated as there is high degree of deterioration by Loss of Contractile Protein (LCP), Vacuolization (V), Myelin Formations (MP), Myocytolysis (MCL), Necrosis (N) and Contracture Bands (CB) or Inflammation (I) in cardiomyocytes. Diabetic Nephropathy is characterized by deterioration of kidney by Tubular Vacuolization (TV), Thickening of the glomerular basement

Figure 11: Diabetic control rats. Cross sectional view rat sciatic nerve treated with Alloxan (150 mg/kg).* showing significant degeneration of nerve cells. * = single dose.

Figure 12: Diabetic rats given standard drug glimepiride (100mg/kg of body weight). Cross sectional view rat sciatic nerve.

Figure 13: Diabetic rats given ethanol extract of *Senna auriculata* leaf (150 mg/kg of body weight). Cross sectional view rat sciatic nerve.

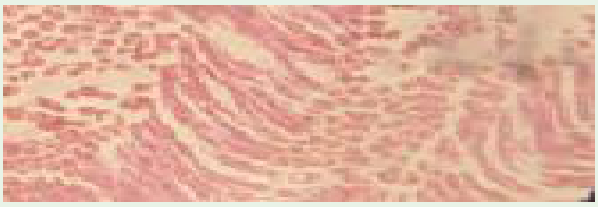

Figure 14: Diabetic rats given ethanol extract of *Phyllanthus emblica* L. fruit. (150 mg/kg of body weight). Cross sectional view rat sciatic nerve.

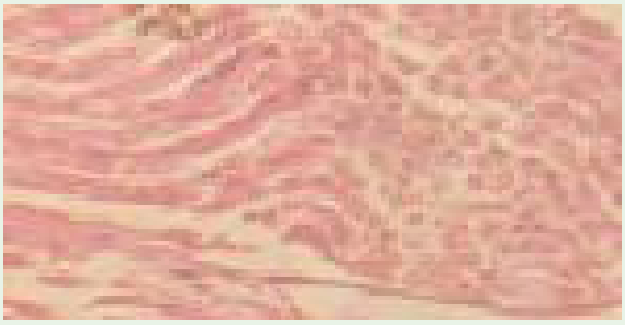

Figure 15: Diabetic rats given ethanol extract of *Syzygium cumini* (L.) Skeels seeds (150 mg/kg of body weight). Cross sectional view rat sciatic nerve.

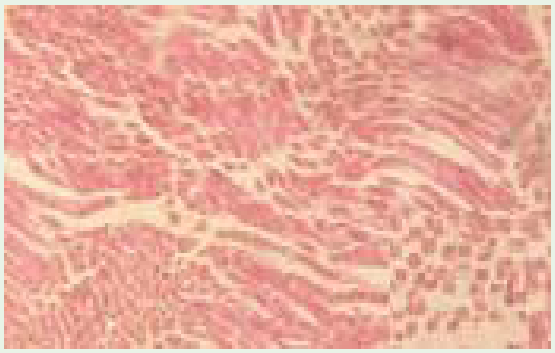

Figure 16: Diabetic rats given Combination of ethanol extract of *Senna auriculata* leaf (150 mg/kg of body weight), *Phyllanthus emblica* L. fruit. (150 mg/kg of body weight) and *Syzygium cumini* (L.) Skeels seeds (150 mg/kg of body weight). Cross sectional view rat sciatic nerve.

membrane, Mesangial Matrix Expantion (ME), Nodular Lesion (NL) Ethanol extract of *Senna auriculata leaf, Phyllanthus emblica L.* fruit and *Syzygium cumini (L.)* Skeels seeds (150mg/kg) and its combination normalizes morphological changes in sciatic nerve, myocardium & kidney. Combination was found to be more effective in normalization of histopathological alterations these diabetic complications (Figures 10 to 30).

Citation: Hussain SA and Sharma AK. Evaluation of Antidiabetic Plants used by Tribes of Telangana State on Diabetic Complications like Neuropathy, Nephropathy and Cardiomyopathy in Rats. J Nephrol Kidney Dis. 2017; 1(2): 1005.
https://dx.doi.org/10.36876/smjnkd.1005

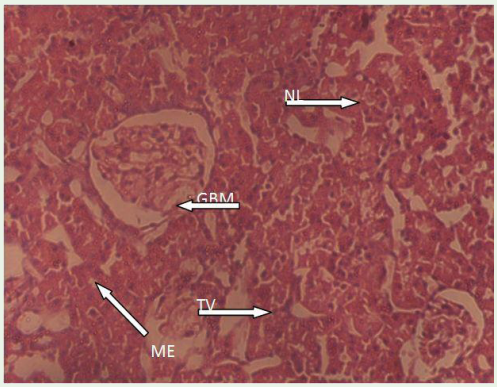

Figure 17: Photomicrograph of section of the normal control group (after 7 weeks treatment). There is no degree of deterioration by Tubular Vacuolization (TV),Thickening of the glomerular basement membrane, Mesangial Matrix Expantion (ME), Nodular Lesion (NL).

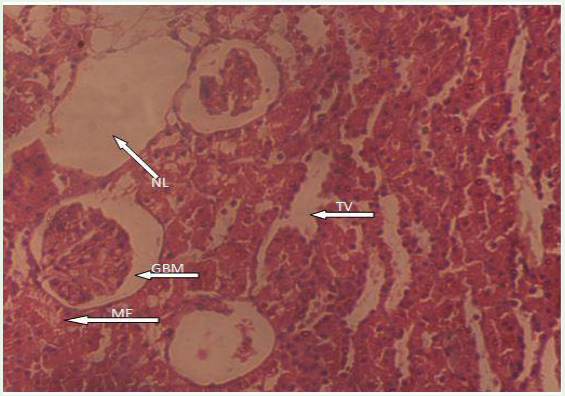

Figure 18: Photomicrograph of section of the Diabetic control group showing nephropathy. (after 7 weeks treatment). There is high degree of deterioration by Tubular Vacuolization (TV), Thickening of the glomerular basement membrane, Mesangial Matrix Expantion (ME), Nodular Lesion (NL).

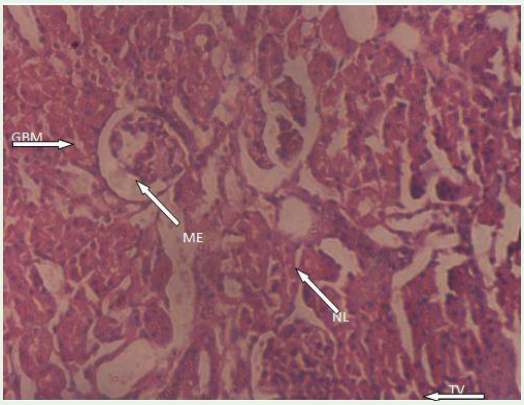

Figure 19: Photomicrograph of section of the Standard group treated with glimepiride showing nephropathy (after 7 weeks treatment). There is less degree of deterioration by Tubular Vacuolization(TV), Thickening of the glomerular basement membrane, Mesangial Matrix Expantion (ME), Nodular Lesion (NL), but not significant as like dual therapy.

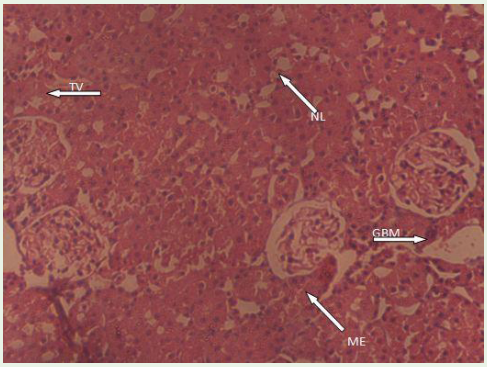

Figure 20: Photomicrograph of section of the diabetic treatment group, treated with ethanol extract of *Senna auriculata leaf* (150 mg/kg of body weight). showing nephropathy (after 7 weeks treatment). There is less degree of deterioration by Tubular Vacuolization (TV), Thickening of the glomerular basement membrane, Mesangial Matrix Expantion (ME), Nodular Lesion (NL).

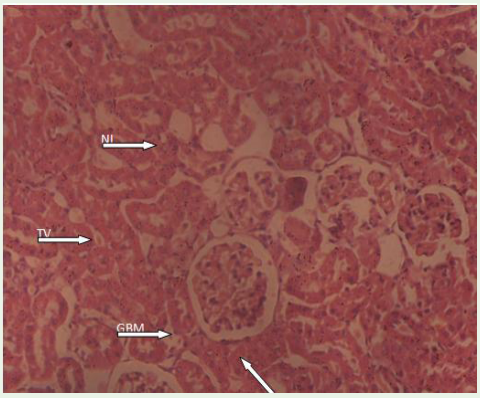

Figure 21: Photomicrograph of section of the diabetic treatment group, treated with ethanol extract of *Phyllanthus emblica* L. fruit. (150 mg/kg of body weight) showing nephropathy (after 7 weeks treatment). There is less degree of deterioration by Tubular Vacuolization (TV),Thickening of the glomerular basement membrane, Mesangial Matrix Expantion (ME), Nodular lesion(NL),but not significant as compared to dual therapy.

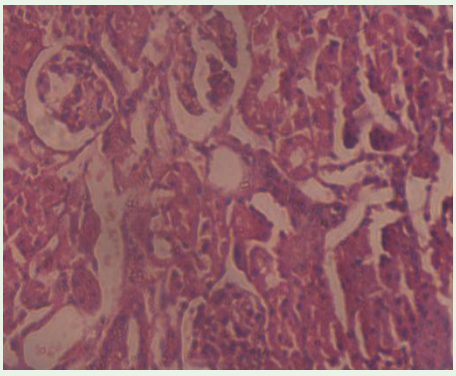

Figure 22: Photomicrograph of section of the diabetic treatment group, treated with ethanol extract of Syzygium cumini (L.) Skeels seeds (150 mg/kg of body weight) showing nephropathy (after 7 weeks treatment). There is less degree of deterioration by Tubular vacuolization (TV),Thickening of the glomerular basement membrane, Mesangial Matrix Expantion (ME), Nodular Lesion (NL),but not significant as compared to dual therapy.

Citation: Hussain SA and Sharma AK. Evaluation of Antidiabetic Plants used by Tribes of Telangana State on Diabetic Complications like Neuropathy, Nephropathy and Cardiomyopathy in Rats. J Nephrol Kidney Dis. 2017; 1(2): 1005.
https://dx.doi.org/10.36876/smjnkd.1005

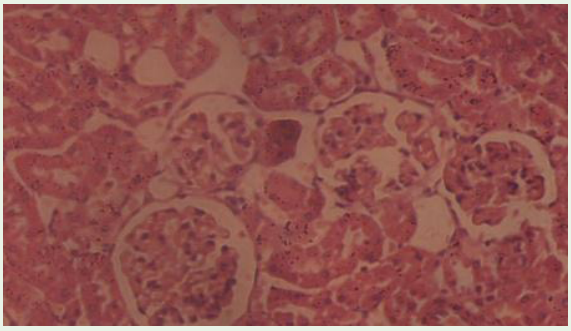

Figure 23: Photomicrograph of section of the diabetic treatment group, treated with combination of ethanol extract of *Senna auriculata* leaf, *Phyllanthus emblica* L. fruit and Syzygium cumini (L.) Skeels seeds (150 mg/kg of body weight) showing nephropathy (after 7 weeks treatment). There is less degree of deterioration by Tubular Vacuolization (TV),Thickening of the glomerular basement membrane, Mesangial Matrix Expantion (ME), Nodular Lesion (NL),but not significant as compared to dual therapy.

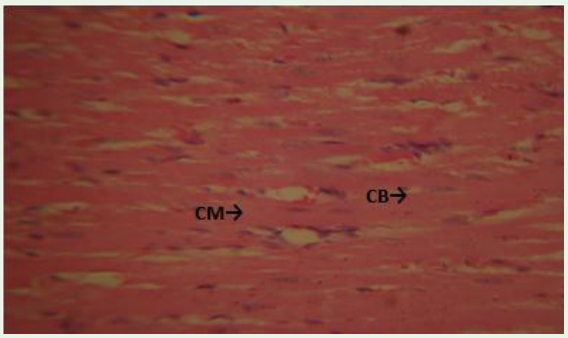

Figure 26: Photomicrograph of section of the Standard group treated with glimepiride Diabetic Heart group showing cardiomyocytes (CM). 150x, (After 7 weeks treatment). There is no deterioration by Loss of Contractile Protein (LCP), vacuolization (V), Myelin Formations (MF), Myocytolysis (MCL), Necrosis (N) and Contracture Bands (CB) or inflammation. This group shows near normal heart.

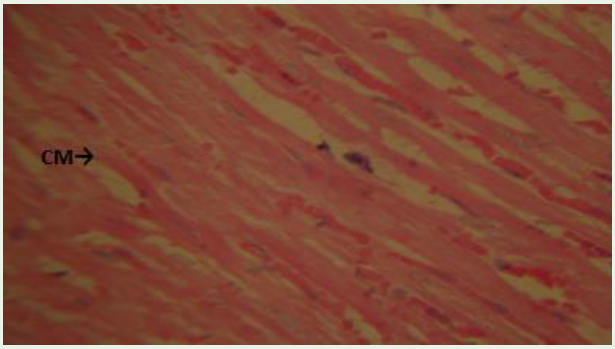

Figure 24: Photomicrograph of section of the Normal Heart from rats in control group showing Cardiomyocytes (CM). 150x, (After 7 weeks treatment). There is no deterioration by Loss of Contractile Protein (LCP), Vacuolization (V), Myelin Formations (MF), Myocytolysis (MCL), Necrosis (N) and Contracture Bands (CB) or Inflammation.

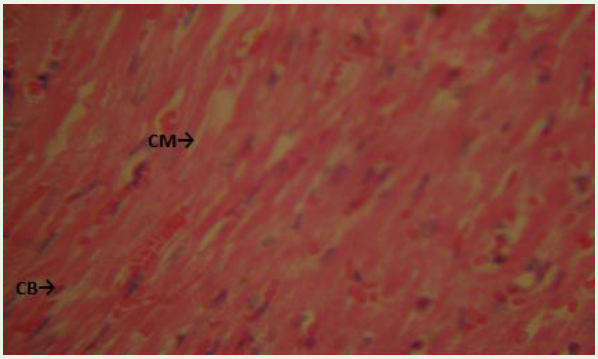

Figure 27: Photomicrograph of section of the Diabetic Heart treated with ethanol extract of *Senna auriculata* leaf (150 mg/kg of body weight). group showing Cardiomyocytes (CM). 150x, (After 7 weeks treatment) There is less degree of deterioration by Loss of Contractile Protein (LCP), Vacuolization (V), Myelin Formations (MF), Myocytolysis (MCL), Necrosis (N) and Contracture Bands (CB) or Inflammation. This group shows near normal heart.

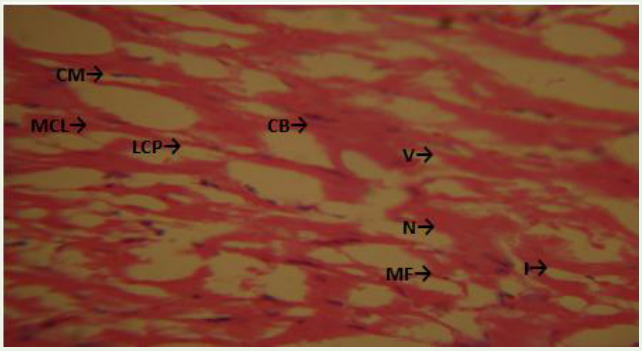

Figure 25: Photomicrograph of section of the Diabetic Heart group showing Cardiomyocytes (CM). 150x, (After 7 weeks treatment). There is high degree of deterioration by Loss of Contractile Protein (LCP), Vacuolization (V), Myelin Formations (MP), Myocytolysis (MCL), Necrosis (N) and Contracture Bands (CB) or Inflammation (I).

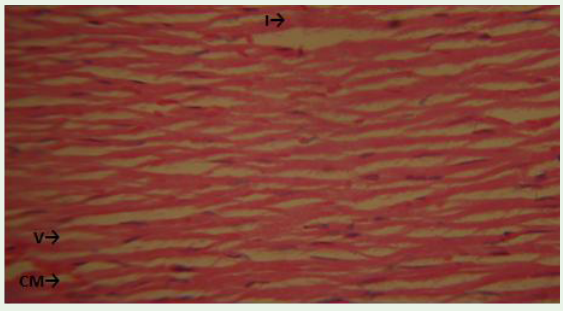

Figure 28: Photomicrograph of section of the Diabetic Heart treated with ethanol extract of *Phyllanthus emblica* L. fruit. (150 mg/kg of body weight) showing Cardiomyocytes (CM). 150x, (After 7 weeks treatment) There is less degree of deterioration by Loss of Contractile Protein (LCP), Vacuolization (V), Myelin Formations (MF), Myocytolysis (MCL), Necrosis (N) and Contracture Bands (CB) or Inflammation (I). This group shows near normal heart.

Citation: Hussain SA and Sharma AK. Evaluation of Antidiabetic Plants used by Tribes of Telangana State on Diabetic Complications like Neuropathy, Nephropathy and Cardiomyopathy in Rats. J Nephrol Kidney Dis. 2017; 1(2): 1005.
https://dx.doi.org/10.36876/smjnkd.1005

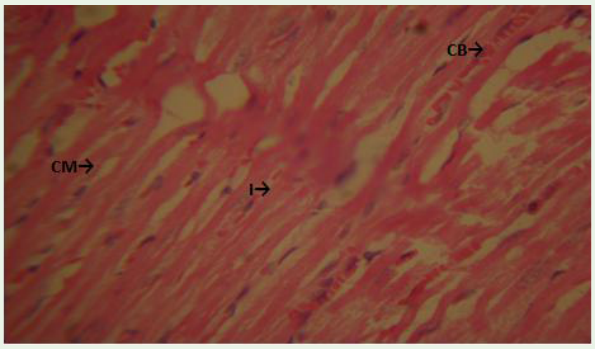

Figure 29: Photomicrograph of section of the Diabetic Heart treated with ethanol extract of *Syzygium cumini (*L.*)* Skeels seeds (150 mg/kg of body weight) showing Cardiomyocytes (CM). 150x, (After 7 weeks treatment) There is less degree of deterioration by Loss of Contractile Protein (LCP), Vacuolization (V), Myelin Formations (MF), Myocytolysis (MCL), Necrosis (N) and Contracture Bands (CB) or inflammation (I). This group shows near normal heart.

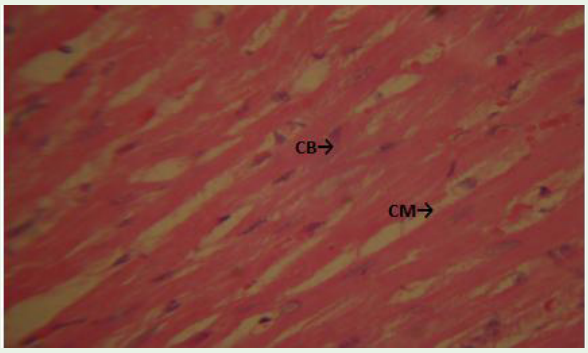

Figure 30: Photomicrograph of section of Diabetic Heart , treated with combination of ethanol extract of *Senna auriculata* leaf, *Phyllanthus emblica* L. fruit and *Syzygium cumini* (L.) Skeels seeds (150 mg/kg of body weight) showing cardiomyocytes (CM). 150x, (After 7 weeks treatment) There is less degree of deterioration by Loss of Contractile Protein (LCP), vacuolization (V), Myelin Formations (MF), Myocytolysis (MCL), Necrosis (N) and Contracture Bands (CB) or Inflammation (I). This group shows near n

Discussion

India is "diabetes capital of the world". Diabetes Atlas 2006 published by International Diabetes Federation, India currently around 40.9 million is expected to rise to 69.9 million by 2025 unless urgent preventive steps are taken. Over the past 30 yr, the status of diabetes has changed from being considered as a mild disorder to major causes of morbidity and mortality.

Therapeutics agents like insulin, sulfonylureas, biguanides and thiazolidinedione derivatives and α glucosidase inhibitors are preferred. To reduce the hyperglycemic condition, the drugs that are preferred for treatment such as sulfonylureas which stimulates pancreatic islets to secrete insulin. Biguanides are responsible for the reduction of hepatic glucose output. Thiazolidinedione derivatives exert their peripheral action by lowering insulin resistance in peripheral tissue. α - glucosidase inhibitors augment glucose utilisation and responsible for suppression of glucose production. Apart from the therapeutic option for diabetes like oral hypoglycemic and insulin have some adverse effects. Hence the current therapy is focused on herbal medicines. And they are used for current therapy due to presumed effectiveness, relatively low cost, presumed fewer side effects and low toxicity. The medicinal plants might provide a useful source of new oral hypoglycemic compounds, and this may lead to the development of pharmaceutical entities, and this may act as a dietary ~ 32 ~. The Pharma Innovation Journal adjunct to existing therapies. Worldwide there are more than 1200 plant species, some of the medicinal plants that are used to control blood glucose levels such as *Azadirachta indica*, *Catharanthus roseus*, *Allium sativum*, *Memordica judaica*, *Aloe vera*, Trigonellafoenum graecum. Due to the presence of active principles in medicinal plants they have been reported to possess some characteristic properties like pancreatic β cell regenerating, insulin-releasing and fighting the problem of insulin resistance. India is well known for its great heritage of herbal medicinal knowledge. Large number of tribals and ethnic people living in the remote forest areas depend on plants to a great extent for foods, medicine, pharmaceuticals and agrochemicals. From the decades studies on ethnobotany have gained importance. Diabetes is an important chronic disorder afflicting many from various walks of life around the world. Though they are various allopathic drugs used to treat the worse effects of diabetes, herbal formulations are preferred to minimize the risk of side effects and due to low cost [11-19].

According to WHO's estimation 80% of the world's population use herbal medicine. Now a days traditional medicine with good clinical practice is showing a lively future in treating diabetes and its complications. From the decades vigorous research on ethnobotany shows that plant and its derivatives are useful in the treatment of diabetes mellitus. Though there are numerous approaches to treat diabetes but traditional medicine is preferred due to its lesser side effects and low cost. In Indian systems of herbal medicine most traditional practitioners formulate and give out their own recipes. India is the largest producer of medicinal plants and approx. 2,500 species of plants are used for medicinal purposes [20-22]. The current study was undertaken in the tribal region of Telangana state in order to list out the plant species having antidiabetic activity used by the traditional practitioners.

The present work has detected the effect of ethanol extract of *Senna auriculata leaf*, *Phyllanthus emblica L.* fruit and *Syzygium cumini (L.)* Skeels seeds (150 mg/kg of body weight) and its combination in Alloxan induced diabetic complications like neuropathy, nephropathy and cardiomyopathy in rats. Alloxan injection caused diabetic neuropathy, nephropathy and cardiomyopathy probably due to destruction of the β cells of islets of langerhans of the pancreas, over the production of high blood glucose level and decreased utilization by tissues from the fundamental bases of hyperglycemia in diabetes mellitus. Alloxan prevent the DNA synthesis, and also prevent cellular reproduction with a much smaller dose that that dose needed for inhibiting the substance concentration of DNA or inhibiting many of enzymes involved in DNA synthesis. Hyperglycemia accompanied by weight loss were seen in adult rats treated with Alloxan which were stable for weeks, which indicates the irreversible destruction of β cells of islets of lengerhans of pancreas. The Alloxan is most commonly used to induce diabetes in experimental animals, because it is simple, inexpensive and available method.

Diabetic Neuropathy, Nephropathy & Cardiomyopathy is a long-term complication of diabetes observed in 60-70% of all diabetic patients that develops early in the course of the disease. In Diabetic neuropathy there is nerve degeneration disease characterized by nerve fiber demylination, axonal degeneration, and a reduction in the number of medium to large diameter nerve fiber, particularly in peripheral nerve. Diabetic cardiomyopathy is demonstrated as there is high degree of deterioration by Loss of Contractile Protein (LCP), Vacuolization (V), Myelin Formations (MP), Myocytolysis (MCL), Necrosis (N) and Contracture Bands (CB) or Inflammation (I) in cardiomyocytes. Diabetic Nephropathy is characterized by deterioration of kidney by Tubular vacuolization (TV), Thickening of the glomerular basement membrane, Mesangial matrix expantion (ME), Nodular lesion (NL) (Figure 10 to 30).

Diabetic Neuropathy, Nephropathy & Cardiomyopathy is triggered by hyperglycemia, which leads to a persistent accelerated flux of glucose through the polyol pathway. The rate limiting enzyme in this pathway is aldose reductase. The increased flux through the polyol pathway is followed by abnormal PKC metabolism, oxidative stress, accelerated glycation, and decreased endoneural capillary perfusion, leading eventually to nerve, cardiac and nephron degeneration.

The hypoglycemic effect was observed with the treatment of ethanol extract of *Senna auriculata leaf*, *Phyllanthus emblica L.* fruit and *Syzygium cumini (L.)* Skeels seeds (150 mg/kg of body weight) and its combination in Alloxan induced hyperglycemic rats, with the maximum effect seen in combination group, which may be due to its antidiabetic effect because all the three drugs are use in type-2 DM.

Induction of DN with Alloxan is also associated with characteristic loss of body weight, which is due to increased muscle wasting, and also of proteins. Diabetic rats treated with ethanol extract of *Senna auriculata leaf*, *Phyllanthus emblica L.* fruit and *Syzygium cumini (L.)* Skeels seeds (150 mg/kg of body weight) and its combination showed a neutral effect in body weight (no weight gain) as compared to diabetic control, which may be due to its effect in controlling muscle wasting.

Presence and severity of DN has been shown to be associated with decrease muscle strength in DM. In the present study, significant improvement in motor behavior, in particular grip strength after treatment of diabetic animals with ethanol extract of Senna auriculata leaf, *Phyllanthus emblica L.* fruit and *Syzygium cumini (L.)* Skeels seeds (150 mg/kg of body weight) and its combination has been observed. Per as treatment of ethanol extract of Senna auriculata leaf, *Phyllanthus emblica L.* fruit and *Syzygium cumini (L.)* Skeels seeds (150 mg/kg of body weight) and its combination showed significant increase in grip strength when compared with diabetic control group, where significant decrease in grip strength was observed in negative control group (diabetic group).

Hyperalgesia is a constant feature of sensory dysfunction in spontaneously and experimental model of DN, Observation indicted that improvement in hot plate response that is pain threshold of diabetic animal with ethanol extract of Senna auriculata leaf, *Phyllanthus emblica L.* fruit and *Syzygium cumini (L.)* Skeels seeds (150 mg/kg of body weight) and its combination. The response with that group was found to be better than other groups of treatments. The analgesic was near normal with standard drug. Significant increase in pain threshold was observed in ethanol extract of *Senna auriculata leaf*, *Phyllanthus emblica L.* fruit and *Syzygium cumini (L.)* Skeels seeds (150 mg/kg of body weight) and its combination. This confirms the usefulness of ethanol extract of Senna auriculata leaf, *Phyllanthus emblica L.* fruit and *Syzygium cumini (L.)* Skeels seeds (150 mg/kg of body weight) and its combination in symptomatic treatment of painful diabetic neuropathy.

Large numbers of studies have reported that in DN, blood protein level decrease and excretion of protein from the urine increase in diabetic neuropathy. In Alloxan induced DN in rats showed increased in blood protein level and increased in urine protein level in diabetic control group. After the treatment with standard group, blood protein level was near of normal level. The negative control group showed the loss of blood protein level may be due to its metabolic and excretion rat from the urine. Treatment with ethanol extract of *Senna auriculata leaf*, *Phyllanthus emblica L.* fruit and *Syzygium cumini (L.)* Skeels seeds (150 mg/kg of body weight) and its combination significantly reduces the protein excretion from the urine comparative with negative control group.

It has been reported that peripheral nerve becomes ischemic and hypoxic due to osmotic shrinkage or retardation of normal axonal maturation in Alloxan treated rats. An alternation of nerve function or structure was present in 42 days' of diabetic rats, which was evident from the hystopathological slides. T.S. of sciatic nerve of diabetic group showed significant degeneration of nerve fibers, which was in consistent with previous reported findings. Ethanol extract of Senna auriculata leaf, *Phyllanthus emblica L.* fruit and *Syzygium cumini (L.)* Skeels seeds (150 mg/kg of body weight) and its combination treatment showed the normal growth in sciatic nerve compared with normal control group.

Blood Glucose are increased in wide variety of diabetes, if elevation of blood glucose remains for long time, cardiovascular diseases such as diabetic cardiomyopathy develop in diabetic patients. Ethanol extract of Senna auriculata leaf, *Phyllanthus emblica L.* fruit and *Syzygium cumini (L.)* Skeels seeds (150 mg/kg of body weight) and its combination significantly reduced the blood glucose. Serum Protein are reduced in wide variety of type 2 diabetes, contributes considerably to increased risk of cardiovascular events. Serum Protein was found to be decrease significantly with combination of, partially significant with Ethanol extract of Senna auriculata leaf, *Phyllanthus emblica L.* fruit and *Syzygium cumini (L.)* Skeels seeds (150 mg/kg of body weight) in single therapy.

Serum Albumin are also reduced in diabetic cardiomyopathy group Serum Myoglobin is a biomarker for cardiovascular risk, is also reduced in diabetic cardiomyopathy groups. Combination is significantly effective to normalize serum myoglobin. Protein, Albumin & Myoglobin in urine are also elevated in diabetic cardiomyopathy groups, Ethanol extract of *Senna auriculata leaf*, *Phyllanthus emblica L.* fruit and *Syzygium cumini (L.)* Skeels seeds (150 mg/kg of body weight) and its combination showed significant effective to reduce these parameters in urine.

Diabetic rats treated with Ethanol extract of Senna auriculata leaf, *Phyllanthus emblica L.* fruit and *Syzygium cumini (L.)* Skeels seeds (150 mg/kg of body weight) and its combination showed a reduction in albumin excretion rate,serum creatinine rate, blood urea

nitrogen, fasting blood glucose and renal structural changes. There were also reported marked changes in albuminuria, protenuria which is a marker and potential contributor to renal injury, accompanies diabetic nephropathy. Interventions that have ameliorated the progression of DN have been associated with a reduction in urinary protein excretion. Finally the significant effect of combined therapy could be a result of synergistic/potentiative action in diabetic nephropathy and able to target multiple mechanism involved in the pathophysiology of diabetic nephropathy.

Conclusion

In conclusion, there is significant effect ethanol extract of *Senna auriculata leaf*, *Phyllanthus emblica L.* fruit and *Syzygium cumini (L.)* Skeels seeds (150 mg/kg of body weight) and its combination in diabetic complications like neuropathy, nephropathy and cardiomyopathy in rats. Significant effect could be result of synergistic/potentiative action of its combinations, since they contain a diverse array of active principles which are able to target multiple mechanisms involved in the pathophysiology of diabetic complications like neuropathy, nephropathy and cardiomyopathy. Ethanol extract of *Senna auriculata leaf*, *Phyllanthus emblica L.* fruit and *Syzygium cumini (L.)* Skeels seeds (150 mg/kg of body weight) and its combination showed no weight gain increased in grip strength and pain sensitivity. This indicates its protective role against neurons. Ethanol extract of Senna auriculata leaf, *Phyllanthus emblica L.* fruit and *Syzygium cumini (L.)* Skeels seeds (150mg/kg) and its combination normalizes biochemical parameters and morphological changes in sciatic nerve, myocardium & kidney. There was also improvement of the general behavioral parameters. Combination was found to be more effective in these diabetic complications.

In summary, ethanol extract of *Senna auriculata leaf*, *Phyllanthus emblica L.* fruit and *Syzygium cumini (L.)* Skeels seeds (150 mg/kg of body weight) and its combination treatment reversed the alteration in biochemical parameters. Morphological changes in sciatic nerve, myocardium & kidney and improvement of the general behavioral parameters occurs in ethanolic extract of *Senna auriculata leaf*, *Phyllanthus emblica L.* fruit and *Syzygium cumini (L.)* Skeels seeds (150 mg/kg of body weight) and its combination treated Alloxan induced diabteic rats. So, the combination of all the three plant parts can be formulated & can be effective in diabetic patients suffering from diabetic complications like neuropathy, nephropathy and cardiomyopathy with natural way of treatment with minimal side effects.

References

1. McCarron P, Davey Smith G. "Commentary: incubation of coronary heart disease--recent developments". Int J Epidemiol. 2005; 34: 248-250.
2. Huizinga MM, Rothman RL. Addressing the diabetes pandemic: A comprehensive approach. Indian J Med Res. 2006; 124: 481-484.
3. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care. 2004; 27: 1047-1053.
4. Sicree R, Shaw J, Zimmet P. Diabetes and impaired glucose tolerance. In: Gan D, editor. Diabetes Atlas. International Diabetes Federation. 3rd ed. Belgium: International Diabetes Federation. 2006; 15-103.
5. Karvounis HI, Papadopoulos CE, Zaglavara TA, Nouskas IG, Gemitzis KD, Parharidis GE, et al. "Evidence of left ventricular dysfunction in asymptomatic elderly patients with non-insulin-dependent diabetes mellitus". Angiology. 2004; 55: 549 - 555.
6. Vergely P. De l'angine de poitrine dans ses rapports avec le diabete. Gaz Hebd Med Chir. 1883; 20: 364-368.
7. Kannel WB, Hjortland M, Castelli WP. Role of diabetes in congestive heart failure: the Framingham study. Am J Cardiol .1974; 34: 29-34.
8. Abbott RD, Donahue RP, Kannel WB, Wilson PW. The impact of diabetes on survival following myocardial infarction in men vs. women. The Framingham Study. JAMA. 1988; 260: 3456-3460.
9. Apfel SC. "Introduction of Diabetic Neuropathy"; Am J Med. 1999; 107: 1S.
10. Gross JL, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T: Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes Care. 2005; 28: 164-176.
11. Sharma US, Kumar A. Anti-diabetic effect of Rubus ellipticus fruits extracts in alloxan-induced diabetic rats. J Diabetol. 2011; 2: 1-6.
12. Bastaki A. Diabetes mellitus and its treatment. International journal of Diabetes and Metabolism. 2005; 13: 111- 134.
13. Mohammed A, Adelaiye A, Abubakar M, Abdurahman E. Effects of aqueous extract of Ganoderma lucidum on blood glucose levels of normoglycemic and alloxan induced diabetic wistar rats. Journal of Medicinal Plants Research. 2007; 1: 34-37.
14. Are PC, Adidala RRR, Puchchakayala G. Hypoglycemic and antidiabetic activity of Glochidion velutinum on streptozotocin-nicotinamide induced type 2 diabetic rats. Eur J Biol Sci. 2011; 3: 126-130.
15. Chauhan P, Pandey I, Dhatwalia VK. Evaluation of the anti-diabetic effect of ethanolic and methanolic extracts of Centella asiatica leaves extract on alloxan induced diabetic rats. Adv Biol Res. 2010; 4: 27-30.
16. Poongothai K, Ponmurugan P, Ahmed KSZ, Kumar BS, Sheriff S. Antihyperglycemic and antioxidant effects of Solanum xanthocarpum leaves (field grown & in vitro raised) extracts on alloxan induced diabetic rats. Asian Pacific Journal of Tropical Medicine. 2011; 4: 778-785.
17. Niranjan SP, Dharmendra S, Kiran P. Antidiabetic activity of ethanolic extract of Dalbergis sissoo L. Leaves in Alloxan-Induced diabetic rats. International Journal of current pharmaceutical research. 2010; 2: 29-38.
18. Ekeanyanwu R, Udeme A, Onuigbo A, Etienajirhevwe O. Anti-diabetic effect of ethanol leaf extract of Cissampelos owariensis (lungwort) on alloxan induced diabetic rats. African Journal of Biotechnology. 2012; 11: 6758-6762.
19. Natarajan KSZA, Sundaresan S, Sivaraj A, Devi K, Senthil Kumar K. Effect of Aqueous flower extract of Catharanthus rosues on alloxan induced diabetes in male albino rats. International Journl of Pharmaceutical Sciences and Drug research. 2012; 4: 50-53.
20. Sreeramoju P, Mamidala E. An Ethnobotanical Survey of Antidiabetic plants used by tribes of Warangal District, Telangana State. The American Journal of Science & Medical Research. 2016; 2: 246-249.
21. Shanker K, Mohan GK, Hussain MA, Jayarambabu N, Pravallika PL. Green Biosynthesis, Characterization, In vitro Antidiabetic Activity, and Investigational Acute Toxicity Studies of Some Herbal-mediated Silver Nanoparticles on Animal Models. Pharmacogn Mag. 2017; 13: 188-192.
22. Karunasri Alli, Thirupathi AT, KNV Rao, Begum A, Suhas Agey, Rajeswar Dutt. Antidiabetic activity of the methanolic extract of the leaves of Basella rubra L. against alloxan induced diabetes in albino rats. World Journal of Pharmacy and Pharmaceutical Sciences. 2017; 6: 1295-1308.
23. Soren G, Sarita M , Prathyusha T. Antidiabetic activity of Actinidia deliciosa fruit in alloxan induced diabetic rats. The Pharma Innovation Journal 2016; 5: 31-34.
24. Brinda P, Sasikala P, Purushothaman KK: Pharmacognostic studies on Merugan kizhangu. Bulletin in Medical Ethnobotanical Research. 1981; 3: 84-96.
25. Lala PK. Lab manuals of Pharmacognosy CSI Publishers and Distributers, Kolkata. 1993.

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https://dx.doi.org/10.36876/smjnkd.1005

26. Nagappa AN, Thakurdesai PA, Venkat Rao N, Sing J. Antidiabetic activity of Terminalia catappa Linn Fruits. Journal of Ethnopharmacology. 2003; 88: 45-50.

27. Spencer K, Price CP. Ann Clin Biochem. 1977; 14: 105-115.

28. Robinson HW, Price JW, Hogden CG. J Biol Chem. 1937; 120: 481.

29. Dussoix P, Vaxillaire M, Iynejian PB, Tiercy JM, Ruiz J, Spinas GA. "Diagnostic heterogeneity of diabetes in lean young adults, Classification based on immunological and genetic parameters". Diabetes. 1997; 46: 622-631.

30. Fajans SS, Bell GI, Polonsky KS. "Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young". N Engl J Med. 2001; 345: 971.

31. Boron WF, Boulpaep EL. "Medical Physiology"; Philadelphia, Saunders. 2003.

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