

# Evaluation of Treatment of Diabetic Complications like Neuropathy, Nephropathy & Cardiomyopathy in Rats by Combination Therapy of Ethanol Extract of Senna Auriculata Leaf, The Phyllanthus Emblica L. Fruits and Syzygium Cumini (L.) Skeels Seeds on Used to Treat Diabetes in Tribes of Telangana State

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## ABSTRACT

Purpose of research: India is “diabetes capital of the world”. Diabetes Atlas-2006: Rise to 69.9 million by 2025 unless urgent preventive steps are taken, considered major causes of morbidity and mortality.

### 1. Materials and methods:

Rats treated 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., for 42 days. Biochemical parameters of diabetic neuropathy, nephropathy and cardiomyopathy and histopathology of sciatic nerve, kidney and heart was done at end.

### 2. Results:

In Diabetic Group found Blood Glucose level(BGL)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; MGS49.06±0.962, 52.05±1.247, 54.06±1.268 & 56.79±1.125seconds; TPR6.54±0.841, 7.38±0.802, 6.45±1.062 & 6.14±0.837seconds; blood protein7.98±0.039, 8.02±0.053, 8.06±0.039 & 7.48±0.045mg/dl; urine protein1.22±0.058, 0.94±0.049, 0.96±0.056 & 0.82±0.062mg/dl; blood albumin1.64±0.033, 1.82±0.036, 1.87±0.044 & 1.96±0.039mg/dl; urine albumin0.122±0.008, 0.098±0.007, 0.132±0.009 & 0.108±0.011mg/dl; blood myoglobin0.045±0.00189, 0.036±0.00177, 0.041±0.00223 & 0.043±0.00175ng/dl; urine myoglobin0.0042±0.00129,

0.0052±0.00119, 0.0064±0.00126 &  
0.0036±0.00125mg/dl; BUN35.81±1.186,  
36.06±1.123, 34.53±1.177 & 29.03±1.229mg/dl;  
Serum Creatinine98.42±5.526, 99.73±6.064,  
101.97±6.052 & 94.83±6.678μMol/dl.

**3. Conclusions:** Ethanol extract of three plants(150mg/kg) and combination normalizes biochemical parameters & Morphological changes nerve, myocardium & kidney and improves behavioral parameters. Combination was found to be more effective in these diabetic complications.

### Introduction

Therapeutics agents like insulin, sulfonylureas, biguanides and thiazolidinedione derivatives and  $\alpha$  glucosidase inhibitors are preferred, to reduce the hyperglycemic condition. The drugs 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.  $\alpha$  – glucosidase inhibitors augment glucose utilisation and responsible for suppression of glucose production [1,2]. Apart from the therapeutic option for diabetes like oral hypoglycemic and insulin have some adverse effects [3]. Hence the current therapy is focused on herbal medicines [4]. And they are used for current therapy due to presumed effectiveness, relatively low cost, presumed fewer side effects and low toxicity [5]. 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 [6]. 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*, *Momordica 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  $\beta$  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 [7-9].

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[10-13]. 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. Study has been designed for effective treatment of diabetic complications like neuropathy, nephropathy & cardiomyopathy by combination therapy of ethanol extract of *Senna auriculata* leaf, *Syzygium cumini* (L.) Skeels seeds and *Syzygium cumini* (L.) Skeels seeds on used to treat diabetes in tribes of Telangana state.

### Materials and Methods

#### 1. 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. ManoharRao;

Professor & Head; Department of Horticulture, College of Agriculture, Professor Jayashankar Telangana Agriculture University, Rajendranagar, Hyderabad, Telagana-500030.; India. A voucher specimens was deposited in Ethnopharmacology unit, Department of Horticulture, College of Agriculture, Professor Jayashankar Telangana Agriculture University, Rajendranagar, Hyderabad, Telagana-500030.

Preparation of plant extract for antidiabetic studies [14,15]:

(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.

## 2. Animals

Normal healthy male Wistar albino rats (180-240g) were housed under standard environmental conditions at

temperature ( $25 \pm 2^\circ$  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 have been approved by Institutional Animal Ethical Commeeettee, Arya College of Pharmacy S-40, RIICO Industrial Area, Delhi Road Kukas, Jaipur, Rajasthan. India. CPCSEA No. 1013/PO/c/06/CPCSEA.

## 3. 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.

## 4. Induction of Experimental Diabetes [16]

Rats were induced diabetes by the administration of simple intraperitoneal 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.

### 4.1. 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:

### 5. 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.

**5.1: 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.

**5.2. 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.

### 6. 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 3000g for 10 minutes. Following parameters of diabetic complications (cardiomyopathy, neuropathy & nephropathy) analyzed in the normal, diabetic induced and drug treated rats.

**6.1. 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 0.33mol/liter). After mixtures had stood for 30 min at room temperature, the precipitates were centrifuged for 20min 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[17]:

**6.2. BromoCreso Green (BCG) Method** (using Span and Ranbaxy diagnostic kits by autoanalyser (Echo, Logotech Pvt. Ltd, India).

**6.3. 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

**6.4. 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°C) in a polypropylene container.

**6.5. Brij - 35..... 30g/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 30g solid Brij in a beaker in a small volume of distilled water to dissolve and make up to 100ml with distilled water.

**6.6. Bromo Cresol Green (BCG) dye solution:** Transfer 25ml of 1 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 3.5ml 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 in a polyethylene container. Stable for 6 months at room temperature (25-35°C).

**6.7. Standard:** Bovine Serum Albumin: 4g/dl.

## 7. 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 creatine phosphate, which is used by the skeletal muscle. The daily production depends on muscle 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.

**7.1. 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

**7.2. Serum and urine myoglobin estimation [18]:** 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.

## 8. 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 [18]  
(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 speed adjusted to 25rpm. The rod is 23cm 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 150-200gm under a pretested on the apparatus. Only those animal which have demonstrated their ability to remain on the revolving rod for, at least 1 minute 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 [19,20]. (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  $55\pm 1^{\circ}\text{C}$  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 [19-21].

### 9. 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  $-20^{\circ}\text{C}$  until processed for histopathology. Tissues were preserved in 1% w/v glutaraldehyde 4% w/v formaldehyde in phosphate buffer, pH-7.2 at  $4^{\circ}\text{C}$  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

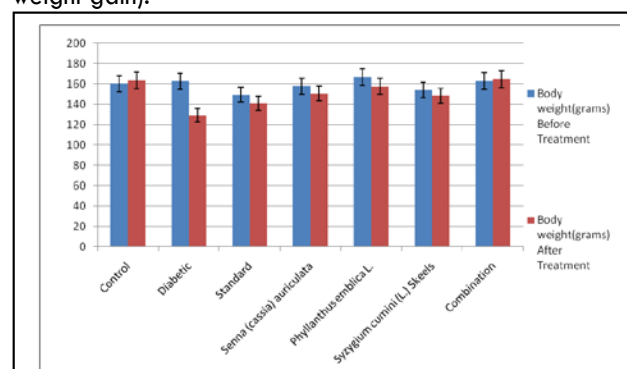
Quantitative data were expressed as Mean $\pm$ Standard Error Mean(SEM). Statistical significance was calculated using one-way analysis of variance. Dunnett's test and Student's t-test were employed as post hoc tests for comparison with the control group and for multiple comparisons between groups, respectively. A value of

$P < 0.05$  is considered to be statistically significant. Statistical Analysis was performed with Graph SYSTAT 12 software.

## Results

### 1. 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:  $167.41\pm 4.958$  to  $162.62\pm 5.78$ ,  $p < 0.01$ ). A progressive loss of body weight was noted after 14-days in negative control group (Pre:  $167.41\pm 4.958$  to  $156.89\pm 6.203$ ,  $P < 0.001$ ). The maximum decrease in body weight was observed after 6 weeks of Alloxan injection (Pre:  $167.41\pm 4.958$  to  $129.03\pm 5.932$ ,  $P < 0.001$ ). The weight of the animals of other groups was also decreased significantly till day 21 as compared to negative control group (Table 5.1). 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).

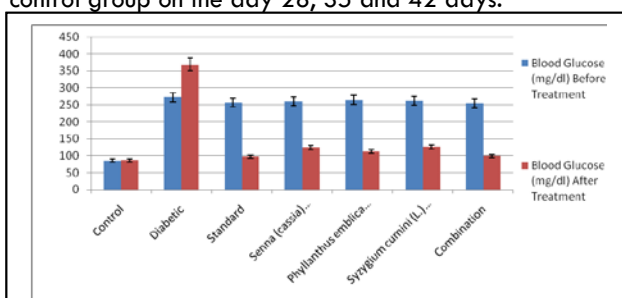


**Figure 1:** The Effect of Senna (cassia) auriculata; Phyllanthus emblica L.; Syzygium cumini (L.) Skeels and its combination on body weight in Alloxan induced diabetic in rats.

### 2. 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 increase 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  $369.36 \pm 7.784$  mg/dl on day 42 and found to be significant increased ( $P < 0.001$ ) compared to the value of day 0 was  $84.42 \pm 6.384$  mg/dl. In control animals remain normoglycaemic during the entire testing period of 42 days (Table 2). 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 it was observed that significant decreased in blood glucose level ( $P < 0.001$ ) compared to normal control group on the day 28, 35 and 42 days.



**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.

**Table 1:** Effect of Senna (cassia) auriculata; Phyllanthus emblica L.; Syzygium cumini (L.) Skeels and its combination on body weight in rats (in grams). Values are expressed MEAN  $\pm$  SEM, n=6, \* =  $P < 0.05$ , Standard = Glimipiride.

Body weight(grams)		
Groups	Before Treatment	After Treatment
Control	160.62 $\pm$ 6.412	163.64 $\pm$ 5.832
Diabetic	167.41 $\pm$ 4.958	129.03 $\pm$ 5.932*
Standard	153.83 $\pm$ 5.132	141.27 $\pm$ 6.057
Senna (cassia) auriculata	162.47 $\pm$ 5.334	150.67 $\pm$ 4.893
Phyllanthus emblica L.	171.93 $\pm$ 6.052	157.81 $\pm$ 5.846
Syzygium cumini (L.) Skeels	159.06 $\pm$ 6.429	148.62 $\pm$ 5.734
Combination	160.56 $\pm$ 6.274	164.38 $\pm$ 5.942

### 3. Effect of drugs in diabetic rats on muscle grip strength

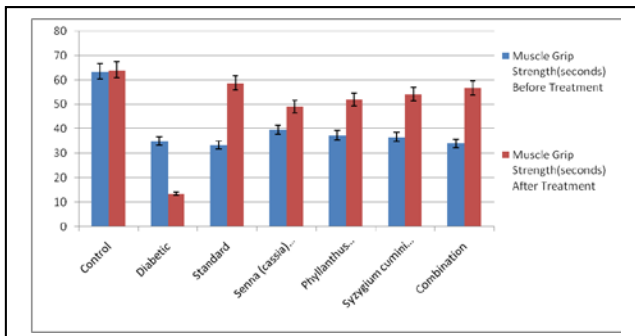
Measurement of muscle grip strength was used to diagnose the diabetic neuropathy after 14 days of Alloxan injection. The muscle grip strength reduced

significantly in all Alloxan treated groups that showed the induction of diabetic neuropathy. In the normal control group the muscle grip strength was normal ( $63.57 \pm 0.893$  to  $64.07 \pm 1.036$  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 ( $59.32 \pm 1.052$  to  $13.52 \pm 0.883$ ;  $P < 0.001$ ). 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 and Figure 3)

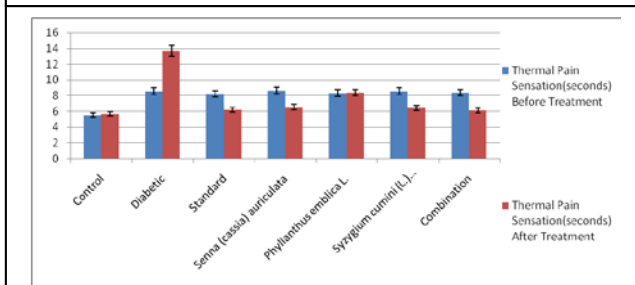
### 4. Effect of drugs in diabetic rats on pain sensation (Thermal Pain)

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 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 ( $5.33 \pm 0.618$  to  $5.68 \pm 0.647$ ). In diabetic induce rats (negative control) there was significant increase found in paw jumping response ( $5.55 \pm 0.621$  to  $13.67 \pm 1.164$ ). 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 28, 35 and 42 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

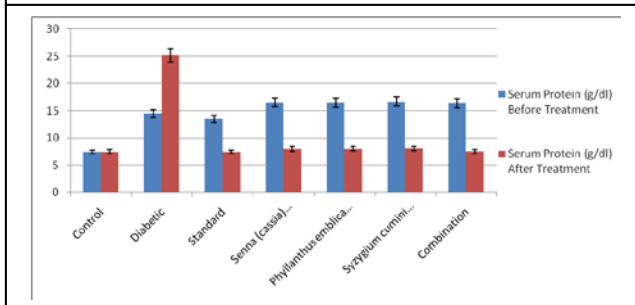
negative control (Diabetic)group. (Table 4 and Figure 4).



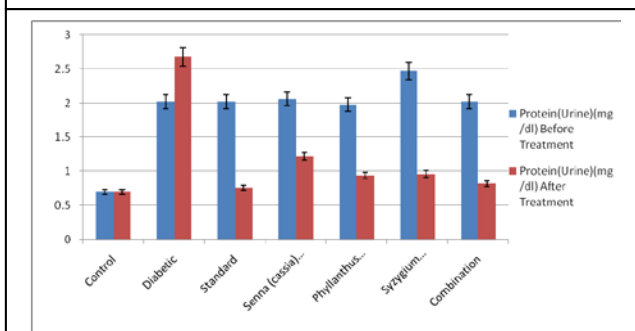
**Figure 3:**Effect of Senna (cassia) auriculata;Phyllanthus emblica L.; Syzygium cumini (L.) Skeels and its combination on muscle grip strength in rats (in seconds).



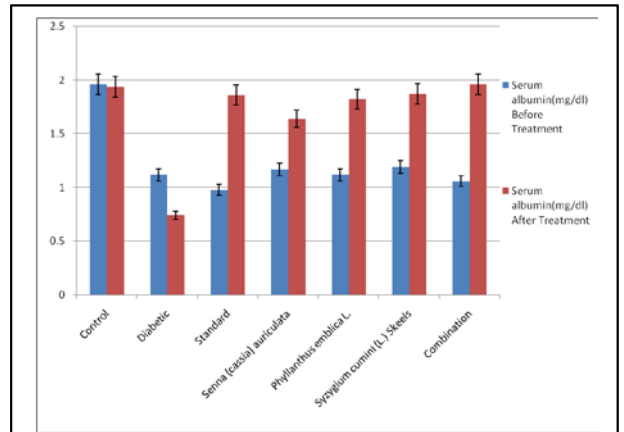
**Figure 4:** The Effect of Senna (cassia) auriculata;Phyllanthus emblica L.; Syzygium cumini (L.) Skeels and its combination on hot plate induced thermal pain in Alloxan induced Diabetic Neuropathy in rats.



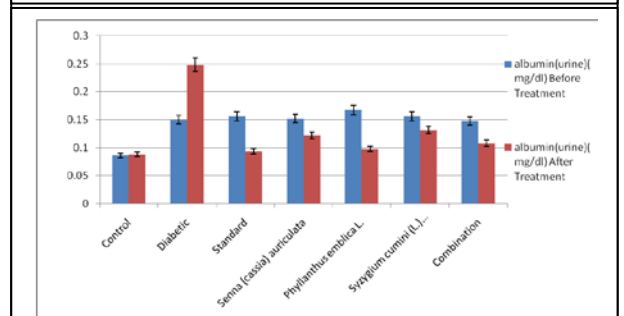
**Figure 5a:** The Effect of Senna (cassia) auriculata;Phyllanthus emblica L.; Syzygium cumini (L.) Skeels and its combination on Blood Protein Level in rats (in mg/dl) in Alloxan induced Diabetic rats.



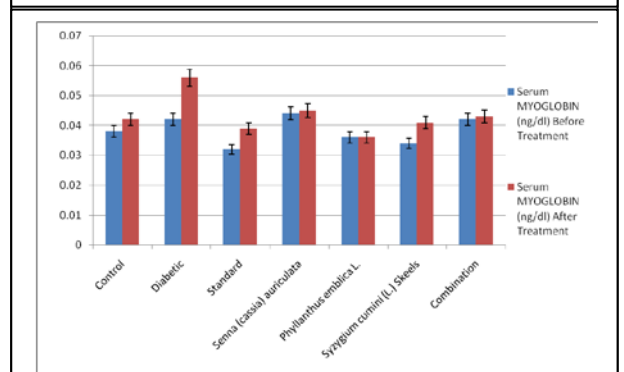
**Figure 5b:** The Effect of Senna (cassia) auriculata;Phyllanthus emblica L.; Syzygium cumini (L.) Skeels and its combination on Protein Level in Urine in Alloxan induced Diabetic rats.



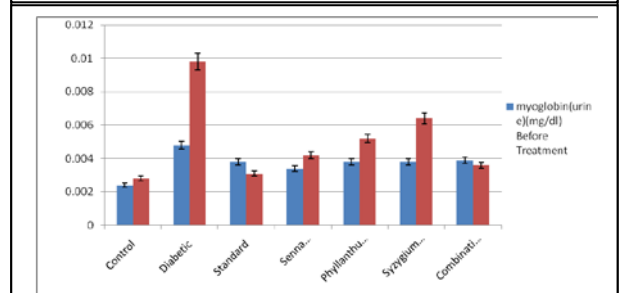
**Figure 6a:** The Effect of Senna (cassia) auriculata;Phyllanthus emblica L.; Syzygium cumini (L.) Skeels and its combination on Serum Albumin (mg/dl) in Alloxan induced Diabetic rats.



**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<0.05, Standard = Glimipiride.

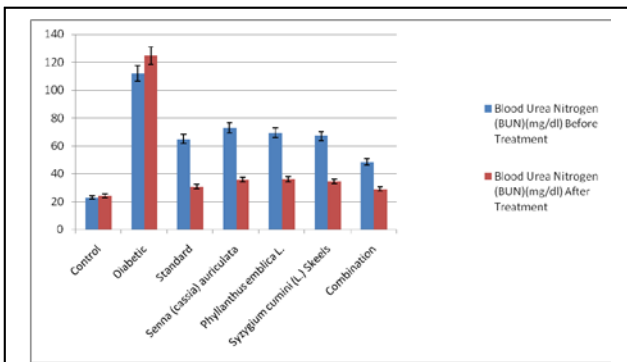


**Figure 7a:**Effect of Senna (cassia) auriculata;Phyllanthus emblica L.; Syzygium cumini (L.) Skeels and its combination on Myoglobin serum(ng/dl). Values are expressed MEAN±SEM, n=6, \* = P<0.05, Standard = Glimipiride.

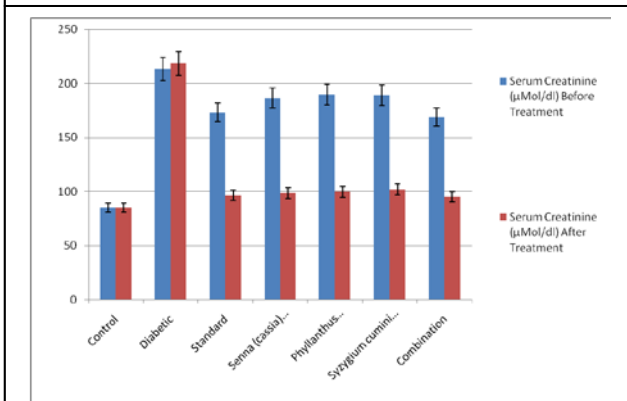


**Figure 7b:**Effect of Senna (cassia) auriculata;Phyllanthus emblica L.; Syzygium cumini (L.) Skeels and its combination on Myoglobin serum(ng/dl). Values are expressed MEAN±SEM, n=6, \* = P<0.05, Standard = Glimipiride.





**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<0.05, 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<0.05, Standard = Glimipiride.

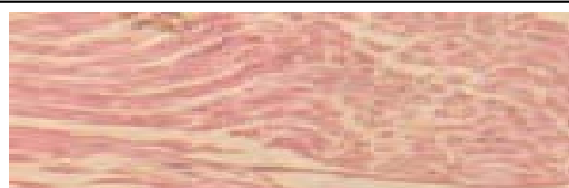
**HISTOPATHOLOGICAL STUDIES**



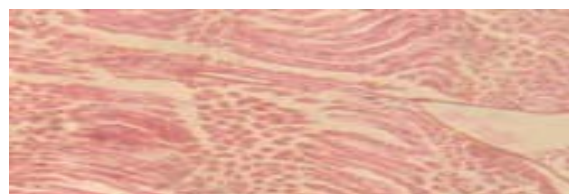
**Figure 10:** Normal, untreated rats. Cross sectional view of rat sciatic nerve showing normal structure.



**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.

**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). Values are expressed MEAN±SEM, n=6, \* = P<0.05, Standard = Glimipiride.

Groups	Blood Glucose (mg/dl)	
	Before Treatment	After Treatment
Control	85.82±7.042	85.87±7.146
Diabetic	84.42±6.384	369.36±7.784*
Standard	256.52±7.428	96.86±6.631*
Senna (cassia) auriculata	259.98±6.931	124.42±7.042*
Phyllanthus emblica L.	264.63±7.156	112.07±6.942*
Syzygium cumini (L.) Skeels	262.44±6.582	126.25±7.051*
Combination	253.94±6.745	98.83±6.932*

### 5. 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 Alloxan injection (Table 5a, 5b and Figure 5a, 5b). On the 21, 28 and 35 of diabetes induction, the negative control (Diabetic) group with statistical significant increase 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  $7.48 \pm 0.051$  to  $25.18 \pm 0.046$  mg/dl and urine protein level increased  $0.692 \pm 0.061$  to  $2.68 \pm 0.056$  and found to be statistical significance ( $P < 0.001$ ). In contrast, control group shows normal protein level in blood ( $7.42 \pm 0.044$  to  $7.46 \pm 0.043$ ) during the entire testing period of 42-days (Table 5a, 5b and Figure 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  $7.48 \pm 0.045$  which was significant compared with negative control (Diabetic) group.

### 6. 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 (Table 6a, 6b and Figure 6a, 6b). On the 21, 28 and 35 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  $1.12 \pm 0.033$  to  $0.74 \pm 0.043$  mg/dl and urine albumin level increased  $0.152 \pm 0.012$  to  $0.248 \pm 0.007$  mg/dl and found to be statistical significance ( $P < 0.001$ ). In contrast, control

group shows normal albumin level in blood ( $1.96 \pm 0.043$  to  $1.94 \pm 0.042$ ) during the entire testing period of 42-days (Table 6a, 6b and figure 6a, 6b). 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  $1.96 \pm 0.039$  which was significant compared with negative control (Diabetic) group.

### 7. 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 control group, was significantly increased and in urine, myoglobin excretion rate is increased after Alloxan injection (Table 7a,7b and Figure 7a,7b). On the 21, 28 and 35 of diabetes induction, the negative control (Diabetic) group with statistical significant increase 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  $0.042 \pm 0.00274$  to  $0.056 \pm 0.00207$  ng/dl and urine myoglobin level increased  $0.0048 \pm 0.00142$  to  $0.0098 \pm 0.00107$  mg/dl and found to be statistical significance ( $P < 0.001$ ). In contrast, control group shows normal myoglobin level in blood ( $0.038 \pm 0.00238$  to  $0.042 \pm 0.00276$ ) during the entire testing period of 42-days (Table 7a,7b and figure 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  $0.043 \pm 0.00175$  which was significant compared with negative control (Diabetic) group.

**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.). Values are expressed MEAN±SEM, n=6, \* = P<0.05, Standard = Glimipiride.

Muscle Grip Strength(seconds)		
Groups	Before Treatment	After Treatment
Control	63.57±0.893	64.07±1.036
Diabetic	59.32±1.052	13.52±0.883*
Standard	33.47±0.942	58.76±1.051*
Senna (cassia) auriculata	39.65±0.938	49.06±0.962*
Phyllanthus emblica L.	37.38±0.869	52.05±1.247*
Syzygium cumini (L.) Skeels	36.72±1.045	54.06±1.268*
Combination	34.08±1.206	56.79±1.125*

**Table 4:** Effect of Senna (cassia) auriculata;Phyllanthus emblica L.; Syzygium cumini (L.)Skeels and its combination on thermal pain in rats (in sec.). Values are expressed MEAN±SEM, n=6, \* = P<0.05, Standard = Glimipiride.

Thermal Pain Sensation(seconds)		
Groups	Before Treatment	After Treatment
Control	5.53±0.636	5.68±0.647
Diabetic	8.56±0.719	13.67±1.164*
Standard	8.23±7.784	6.23±0.736*
Senna (cassia) auriculata	8.66±0.783	6.54±0.841*
Phyllanthus emblica L.	8.33±0.847	7.38±0.802*
Syzygium cumini (L.) Skeels	8.56±1.058	6.45±1.062*
Combination	8.39±0.736	6.14±0.837*

**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).Values are expressed MEAN±SEM, n=6, \* = P<0.05, Standard = Glimipiride.

Serum Protein (g/dl)		
Groups	Before Treatment	After Treatment
Control	7.42±0.039	7.46±0.043
Diabetic	7.48±0.051	25.18±0.046*
Standard	13.53±0.058	7.42±0.039*
Senna (cassia) auriculata	16.52±0.052	7.98±0.039*
Phyllanthus emblica L.	16.48±0.048	8.02±0.053*
Syzygium cumini (L.) Skeels	16.68±0.043	8.06±0.039*
Combination	16.35±0.036	7.48±0.045*

**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). Values are expressed MEAN±SEM, n=6, \* = P<0.05, Standard = Glimipiride.

Protein(Urine)(mg/dl)		
Groups	Before Treatment	After Treatment
Control	0.696±0.047	0.698±0.055
Diabetic	0.692±0.061	2.68±0.056*
Standard	2.02±0.063	0.76±0.063*
Senna (cassia) auriculata	2.06±0.063	1.22±0.058*
Phyllanthus emblica L.	1.98±0.062	0.94±0.049*
Syzygium cumini (L.) Skeels	2.47±0.053	0.96±0.056*
Combination	2.02±0.054	0.82±0.062*

**Table 6a:** Effect of Senna (cassia) auriculata;Phyllanthus emblica L.; Syzygium cumini (L.)Skeels and its combination on Serum Albumin (g/dl). Values are expressed MEAN±SEM, n=6, \* = P<0.05,Standard = Glimipiride.

Serum albumin(mg/dl)		
Groups	Before Treatment	After Treatment
Control	1.96±0.043	1.94±0.042
Diabetic	0.082±0.009	0.74±0.043*
Standard	0.98±0.041	1.86±0.042*
Senna (cassia) auriculata	1.17±0.038	1.64±0.033*
Phyllanthus emblica L.	1.12±0.039	1.82±0.036*
Syzygium cumini (L.) Skeels	1.19±0.043	1.87±0.044*
Combination	1.06±0.038	1.96±0.039*

**Table 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, n=6, \* = P<0.05, Standard = Glimipiride.

Albumin(urine)(mg/dl)		
Groups	Before Treatment	After Treatment
Control	0.086±0.009	0.088±0.012
Diabetic	0.152±0.012	0.248±0.007*
Standard	0.156±0.008	0.094±0.012*
Senna (cassia) auriculata	0.152±0.007	0.122±0.008*
Phyllanthus emblica L.	0.167±0.014	0.098±0.007*
Syzygium cumini (L.) Skeels	0.156±0.009	0.132±0.009*
Combination	0.148±0.008	0.108±0.011*

**Table 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<0.05, Standard = Glimipiride.

Serum MYOGLOBIN (ng/dl)		
Groups	Before Treatment	After Treatment
Control	0.038±0.00219	0.042±0.00276
Diabetic	0.042±0.00274	0.056±0.00207*
Standard	0.032±0.00215	0.039±0.00219*
Senna (cassia) auriculata	0.044±0.00223	0.045±0.00189*
Phyllanthus emblica L.	0.036±0.00169	0.036±0.00177*
Syzygium cumini (L.) Skeels	0.034±0.00208	0.041±0.00223*
Combination	0.042±0.00241	0.043±0.00175*

**Table 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<0.05, Standard = Glimipiride.

Myoglobin(urine)(mg/dl)		
Groups	Before Treatment	After Treatment
Control	0.0024±0.00131	0.0028±0.00119
Diabetic	0.0048±0.00142	0.0098±0.00107*
Standard	0.0038±0.00139	0.0031±0.00134*
Senna (cassia) auriculata	0.0034±0.00125	0.0042±0.00129*
Phyllanthus emblica L.	0.0038±0.00134	0.0052±0.00119*
Syzygium cumini (L.) Skeels	0.0038±0.00129	0.0064±0.00126*
Combination	0.0039±0.00134	0.0036±0.00125*

**Table 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<0.05, Standard = Glimipiride.

Blood Urea Nitrogen (BUN)(mg/dl)		
Groups	Before Treatment	After Treatment
Control	23.04±1.093	24.04±1.246
Diabetic	23.04±1.093	124.81±1.238*
Standard	72.93±1.146	30.64±1.263*
Senna (cassia) auriculata	69.41±1.072	35.81±1.186*
Phyllanthus emblica L.	67.08±1.226	36.06±1.123*
Syzygium cumini (L.) Skeels	48.46±1.173	34.53±1.177*
Combination	64.73±1.238	29.03±1.229*

**Table 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<0.05, Standard = Glimipiride.

Serum Creatinine (µMol/dl)		
Groups	Before Treatment	After Treatment
Control	84.87±6.042	84.93±5.936
Diabetic	212.94±5.472	218.56±7.586
Standard	172.93±5.832	96.47±5.908*
Senna (cassia) auriculata	186.52±6.894	98.42±5.526*
Phyllanthus emblica L.	189.41±8.172	99.73±6.064*
Syzygium cumini (L.) Skeels	188.95±6.042	101.97±6.052*
Combination	168.97±6.493	94.83±6.678*

### 8. 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 21, 28 and 35 of diabetes induction, the negative control (Diabetic) group with statistical significant increase 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  $23.04 \pm 1.093$  to  $124.81 \pm 1.238$  mg/dl and found to be statistical significance ( $P < 0.001$ ). In contrast, control group shows normal Blood Urea Nitrogen (BUN) level in blood ( $23.04 \pm 1.093$  to  $24.04 \pm 1.246$ ) during the entire testing period of 42-days (Table 5.8 and Figure 5.8). 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  $29.03 \pm 1.229$  which was significant compared with negative control (Diabetic) group.

### 9. Effect of drugs in diabetic rats on serum creatinine ( $\mu\text{Mol/dl}$ )

Serum Creatinine ( $\mu\text{Mol/dl}$ ) level in blood is indicated in diabetic nephropathy. The Serum Creatinine ( $\mu\text{Mol/dl}$ ) level in blood in all experimental groups, except normal control group, was significantly increased after Alloxan injection (Table 9 and Figure 9). On the 21, 28 and 35 of diabetes induction, the negative control (Diabetic) group with statistical significant increase in Serum Creatinine ( $\mu\text{Mol/dl}$ ) ( $P < 0.001$ ). In diabetic group (negative control group) the Serum Creatinine ( $\mu\text{Mol/dl}$ ) increased to the maximum value of  $84.06 \pm 6.723$  to  $218.56 \pm 7.586$  ( $\mu\text{Mol/dl}$ ) and found to be statistical significance ( $P < 0.001$ ). In contrast, control group shows normal Serum Creatinine ( $\mu\text{Mol/dl}$ ) level in blood ( $84.87 \pm 6.042$  to  $84.93 \pm 5.936$ ) during the entire testing period of 42-days (Table 9 and Figure 9). The animal

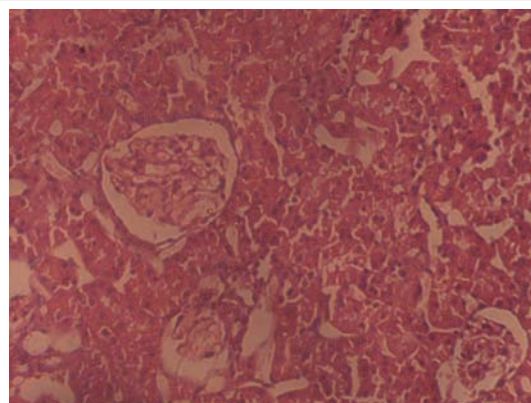
treated with *Senna (cassia) auriculata*; *Phyllanthus emblica* L.; *Syzygium cumini* (L.) Skeels and its combination were observed with significant normal Serum Creatinine ( $\mu\text{Mol/dl}$ ) level ( $P < 0.001$ ) compared to negative control group on day 21st, 28th, 35th and 42nd day. The Serum Creatinine ( $\mu\text{Mol/dl}$ ) level in Combination therapy on day 42nd was  $94.83 \pm 6.678$  which was significant compared with negative control (Diabetic) group.



**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.

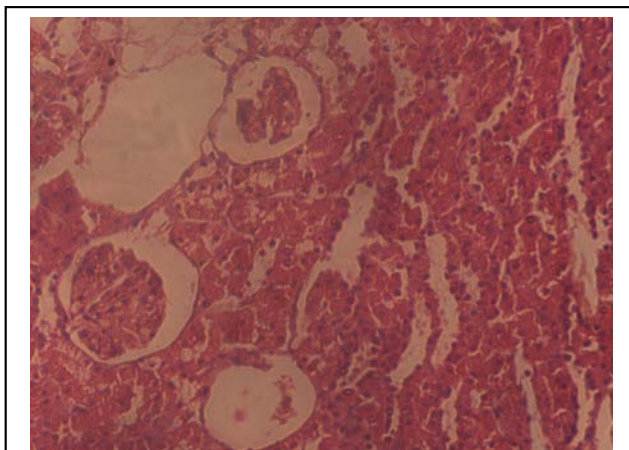


**Figure 16:** Diabetic rats given Combination of ethanol extract of *Senna auriculata* leaf (150 mg/kg of body weight), *Phyllanthus emblica* L.fruit. (150 mg/kg of body weight) and *Syzygium cumini* (L.) Skeels seeds (150 mg/kg of body weight). Cross sectional view rat sciatic nerve.

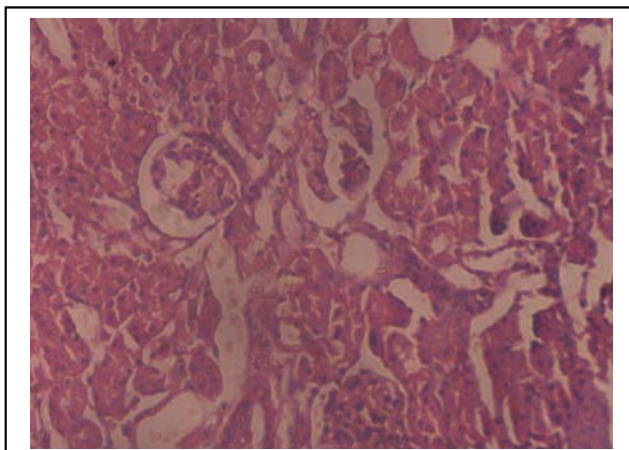


**Figure 17:** Photomicrograph of section of the normal control group(after 7 weeks treatment). There is no degree of deterioration byTubular Vacuolization(TV),Thickening of the glomerular basement membrane,Mesangial Matrix expansion(ME), Nodular Lesion(NL).

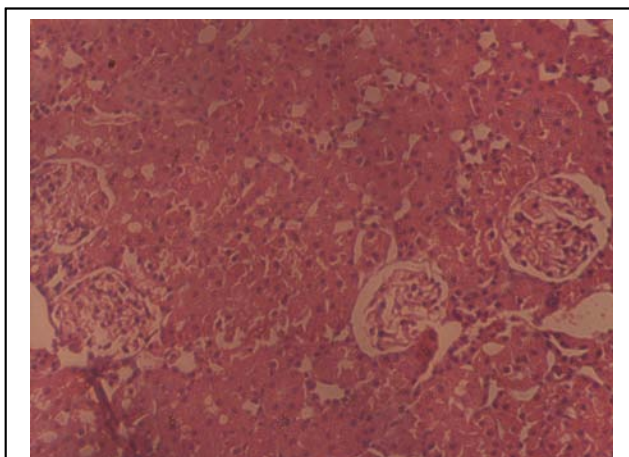




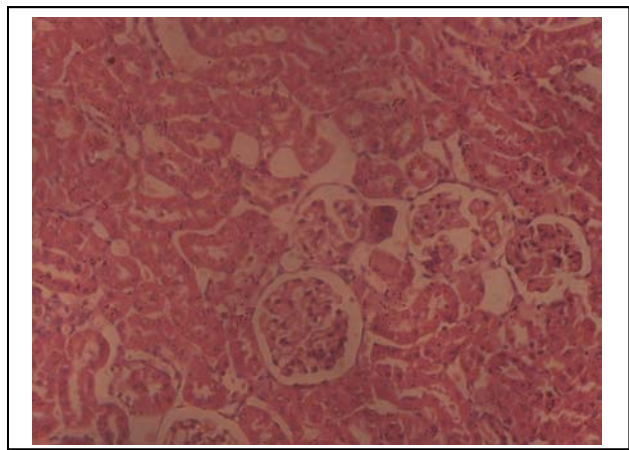
**Figure 18:** Photomicrograph of section of the Diabetic control group showing nephropathy (after 7 weeks treatment). There is high degree of deterioration by Tubular Vacuolization (TV), Thickening of the glomerular basement membrane, Mesangial matrix expansion (ME), Nodular Lesion (NL).



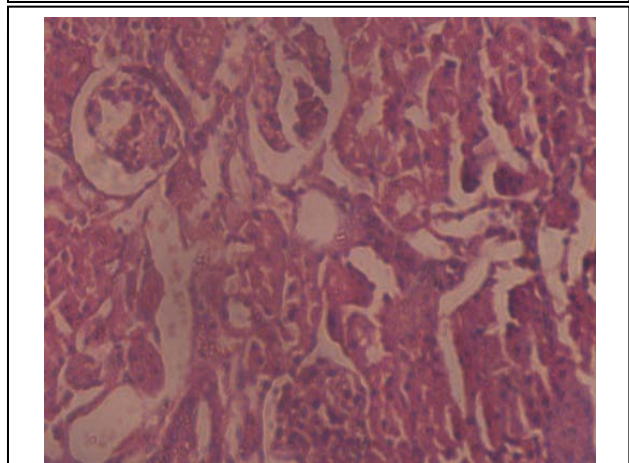
**Figure 19:** Photomicrograph of section of the Standard group treated with glibenclamide showing nephropathy (after 7 weeks treatment). There is less degree of deterioration by Tubular Vacuolization (TV), Thickening of the glomerular basement membrane, Mesangial matrix expansion (ME), Nodular Lesion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



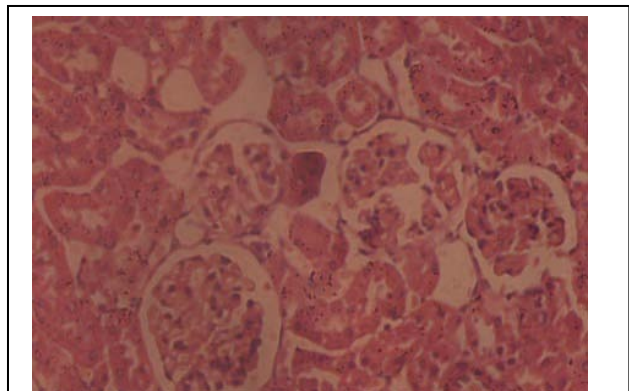
**Figure 20:** Photomicrograph of section of the diabetic treatment group, treated with ethanol extract of Senna auriculata leaf (150 mg/kg of body weight). showing nephropathy (after 7 weeks treatment). There is less degree of deterioration by Tubular Vacuolization (TV), Thickening of the glomerular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



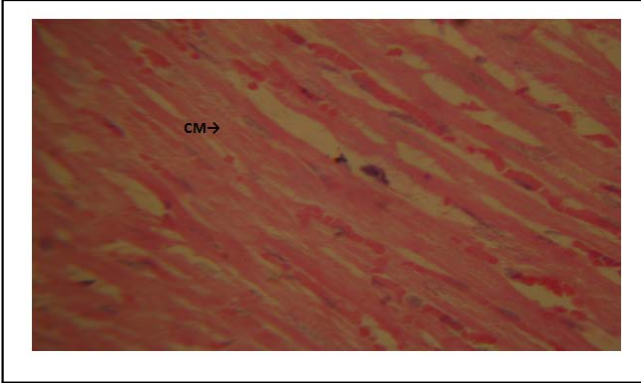
**Figure 21:** Photomicrograph of section of the diabetic treatment group, treated with ethanol extract of Phyllanthus emblica L. fruit. (150 mg/kg of body weight) showing nephropathy (after 7 weeks treatment). There is less degree of deterioration by Tubular Vacuolization (TV), Thickening of the glomerular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



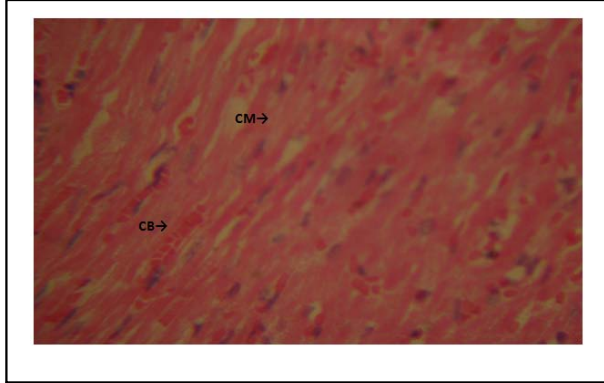
**Figure 22:** Photomicrograph of section of the diabetic treatment group, treated with ethanol extract of Syzygium cumini (L.) Skeels seeds (150 mg/kg of body weight) showing nephropathy (after 7 weeks treatment). There is less degree of deterioration by Tubular Vacuolization (TV), Thickening of the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



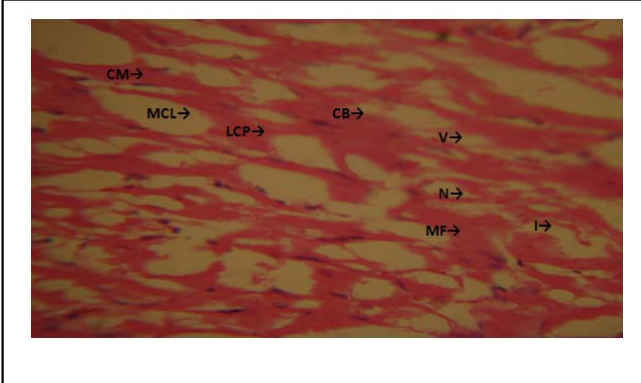
**Figure 23:** Photomicrograph of section of the diabetic treatment group, treated with combination of ethanol extract of Senna auriculata leaf, Phyllanthus emblica L. fruit and Syzygium cumini (L.) Skeels seeds (150 mg/kg of body weight) showing nephropathy (after 7 weeks treatment). There is less degree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



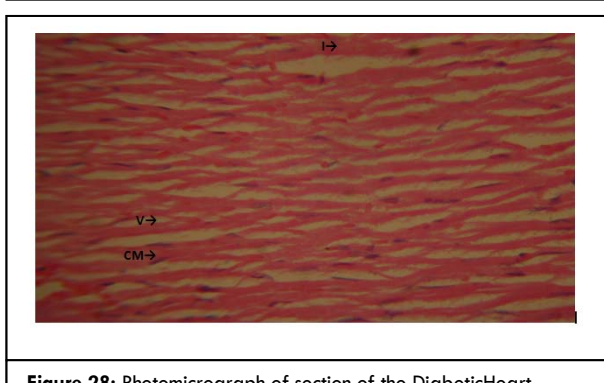
**Figure 24:** Photomicrograph of section of the Normal Heart from rats in control group showing cardiomyocytes (CM). 150x,(After 7 weeks treatment). There is no deterioration by Loss of Contractile Protein (LCP), Vacuolization(V), Myelin Formations(MF), Myocytolysis(MCL), necrosis(N) and Contracture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



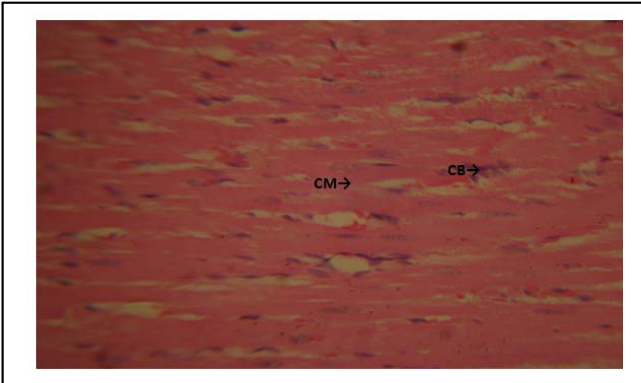
**Figure 27:** Photomicrograph of section of the Diabetic Heart treated with ethanol extract of Senna auriculata leaf (150 mg/kg of body weight). group showing cardiomyocytes (CM).150x, (After 7 weeks treatment) There is less degree of deterioration by Loss of Contractile Protein (LCP), Vacuolization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



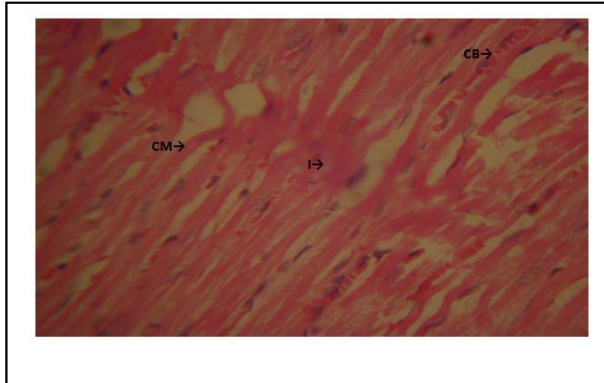
**Figure 25:** Photomicrograph of section of the Diabetic Heart group showing cardiomyocytes (CM).150x,(After 7 weeks treatment). There is high degree of deterioration by Loss of Contractile Protein(LCP), Vacuolization(V), Myelin Formations (MF), Myocytolysis(MCL), Necrosis(N) and Contracture Bands(CB)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

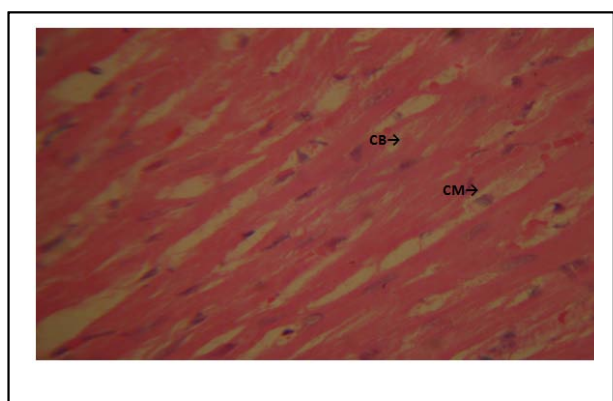


**Figure 26:** Photomicrograph of section of the Standard group treated with glimepiride Diabetic Heart group showing cardiomyocytes (CM).150x, (After 7 weeks treatment). There is no deterioration by Loss of Contractile Protein(LCP), Vacuolization(V), Myelin Formations(MF), Myocytolysis(MCL), Necrosis(N)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



**Figure 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 DiabeticHeart , 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 normal heart.

## Discussion

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  $\beta$  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  $\beta$  cells of islets of langerhans of pancreas. The STZ 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, MesangialMatrix Expantion(ME), Nodular Lesion(NL).

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 conforms 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 histopathological 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, single therapy of cilastazol and sitagliptin partially significant. 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 markedly changes in albuminuria, proteinuria 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, the 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 was observed, 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. 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

diabetic 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.

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