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ORIGINAL ARTICLE

Pharmacological Investigation of Antidiabetic and Antihyper-lipidemic activity of Ethanolic fruit extract of *Calotropis procera*

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ABSTRACT
Medicinal plants play a vital role in the development of new drugs as lead compounds. In the past decade research has been focused on traditional plants for the treatment of various diseases. Medicinal plants are effective against diabetes with lesser side effects. The plant Calotropis procera (Family- Asclepiadaceae) has been reported as a traditional folk medicine for a variety of ailments.The present study was aimed to investigate the anti diabetic and anti hyperlipidemic activity of ethanolic extract of Calotropis procera fruit. Alloxan induced model was evaluated in albino rats of either sex weighing (160-200gm) were used for the study. The antidiabetic activity of ethanolic extract of calotropis procera (250mg/kg and 500mg/kg, p.o) was compared with standard drug Glibenclamide (500µg/kg). The blood glucose levels were estimated on 0 th, 7th and 15 th day of the treatment and serum lipid profile was also estimated. The present investigation justifies scientific support for the protective effect of Calotropis procera fruit as an anti diabetic agent.
***Key words**: Alloxan monohydrate (150 mg/kg, i.p) Fruit extract, Glibenclamide, Blood glucose levels.*

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INTRODUCTION

Diabetes mellitus (diabetes = overflow, mellitus = honeyed) is the most common pancreatic islet disorder caused by an inability to produce insulin or a defect in its utilization. The hallmark of diabetes mellitus is polyuria-excessive urine production, polydipsia-excessive thirst and polyphagia-excessive eating. It is also characterized by chronic hyperglycemia and glucosuria, caused by an absolute or relative deficiency of insulin. This derangement may results into the development of further metabolic and anatomic disturbances, among which the lipemia, hypercholesterolemia, loss of weight, ketosis, arteriosclerosis, gangrene, pathologic changes in the eye, neuropathy, renal disease and coma are more common [1].

To achieve glycemic control, therapeutic agents like insulin, sulfonylureas, biguanides and thiazolidinedione derivatives etc are used. However, on chronic usage most of these agents produced several side effects, including hypoglycemic coma, insulin resistance, hyper-sensitivity, jaundice, abdominal pain, anorexia and metallic taste [2]. In addition, increased cost of treatment and high rates of failure made it difficult to afford and use these agents for a prolonged period. Until the time insulin was invented, this disorder was managed principally by using medicinal plants due to their low cost, easy accessibility and less side effects. *Calotropis procera* (Ait.) R.Br. (giant milkweed) belongs to the family Asclepiadaceae locally known as "Aak" is distributed in tropical and sub-tropical Africa and Asia. *Calotropis procera* have large bushy shrub, leaves decussate, inflorescence extra axillary umbellate panicale, corolla purple, lobes erect [3]. The fruits are inflated, grey-green in colour and release flat, brown seeds with a tuft of white hair at one end [4]. The entire plant has been reported to contain alkaloids, sterols, flavonoids, cardiac glycosides, saponins, triterpinoids and uscharin [5]. *Calotropis procera* possess a wide range of biological activities such as analgesic, anti-inflammatory, antidiabetic, antiarthritic, antioxidant, anthelminthic, anticandidal, wound healing, anticonvulsant, antitumour, antiasthmatic and hepatoprotective. Literature reviews indicated that different parts of the plant have been claimed to be useful in ailments like anti-

inflammatory, analgesic, antimicrobial etc., but no scientific study on anti diabetic and anti hyperlipidemic of fruit has been reported. Therefore the present study was under taken to investigate the antidiabetic activity of fruit extract.

MATERIALS AND METHODS

Collection of plant material

Fresh fruits were collected during the month of January-February 2013 from the *calotropis procera* plant growing in the surrounding areas of West Godavari district, A.P. The plant was identified and Authenticated by Dr.T. V.Raghavarao, Taxonomist, Maharani's college, peddapuram.

Preparation of extract

The freshly collected fruits of the plant were cleared from dirt and dried under shade and then coarsely powdered manually. The dried fruit powder was macerated in ethanol for a period of 7 days and later subjected to hot percolation for 8 hrs. Then the solution was filtered, concentrated and subjected to drying.

Chemicals

Alloxan monohydrate was procured from LOBA CHEMIE laboratory reagents and fine chemicals, Mumbai. Glibenclamide was gifted sample from TABLETS INDIA PVT.LTD; Mumbai. Enzymatic kits for the estimation of lipid profile were obtained from CHEMA DIAGNOSTICA (INDIA).

Experimental animals

Albino rats (160-200gm) of either sex were used for the study. Animals were obtained from the animal house and are housed in colony cages at ambient temperature of 25±2ºc, 12hr light/dark cycle and 50±5% relative humidity with free access to food and water ad libitum. Rats were acclimatized for a period of 7 days before the experiment and placed randomly in separate cages with paddy husk as bedding. The animals were shifted from animal house to the laboratory one hour prior to the experiment.

EXPERIMENTAL DESIGN

The animals were randomly divided into five groups with 3 rats in each group and treated as follows:

Group A:

Served as normal control and did not receive any treatment except vehicle (0.3 ml of 5% aqueous gumacacia).

Group B:

Served as diabetic control and received single dose of alloxan monohydrate (150mg/kg b.w intra peritoneal,i.p.)

Group C :

Alloxan monohydrate + Glibenclamide (500µg/kg b.w, p.o) and served as Standard

Group D:

Alloxan monohydrate + Ethanolic Fruit Extract (250mg/kg b.w, p.o)

Group E:

Alloxan monohydrate + Ethanolic Fruit Extract (500mg/kg b.w, p.o)

The present study was designed to investigate the antidiabetic activity of *calotropis procera* fruit extract by estimating the Blood glucose levels in both normal and diabetic rats. Both acute study and chronic study was evaluated during the experiment. The change in body weight and fasting Blood Glucose Levels of all the rats were recorded at regular intervals during the experiment. For Acute Study, the BGL's were monitored after 0 and at time intervals of 1, 2, 4 and 6 hrs of administration of a single dose of the extract and for Chronic Study, the BGL's were estimated on 0, 7 and 15 th day of the treatment.The BGL's were monitored in the blood of the diabetic rats by tail tipping method. The blood glucose was estimated by' Accu Check Activ Glucometer. The serum was collected on 15th day of the treatment and used for the estimation of biochemical parameters, lipid profile and was analyzed by auto-biochemistry analyzer.

Induction of Diabetes

The method was slightly modified and used in this study. Albino rats were used for the study. The animal were deprived of food but given free access to water for 18 h before drug treatment. Alloxan monohydrate 150 mg/kg (b.wt.) was administered intraperitoneally to the rats to induce hyperglycemia. The animals were allowed to resume feeding one hour after the drug administration[6]. The blood glucose levels were measured using Glucometer through tail tipping 72 h after alloxan administration [7]. The rats with blood glucose level above 200 mg/dl were considered to be hyperglycemic and were selected for extract treatment.

ACUTE STUDY

1. Blood Glucose Estimation on Normal Rats:

The normal animals were randomized into the 4 groups of 3 rats each: group I served as normal control, group II received Glibenclamide (500µg/kg bwt) , groups III and IV received graded doses of the extract at (250and 500 mg/kg bwt) respectively by orogastric tubes. Blood samples were collected by tail vein puncture just prior to drug administration i.e. at 0 hr and at 1, 2, 4, and 6 hrs. The blood glucose was estimated by Accu check glucometer.

2. Blood Glucose Estimation on Diabetic Rats:

Animals were randomized into 5 groups of 3 rats each; group I served as normal control, group II as diabetic control, Group III received glibenclamide (500µg/kg bwt). groups IV and V received graded doses of the extract at (250and 500 mg/kg bwt) respectively by orogastric tubes and Blood samples were collected by tail vein puncture just prior to drug administration i.e. at 0 hr and at 1, 2, 4, and 6 hrs. The blood glucose was estimated by Accucheck glucometer.

CHRONIC STUDY

Chronic study was performed for the treatment of 15 days and each group containing 3 rats were selected in randomized manner. The animals were fasted for 18 hrs and diabetes was induced by intraperitoneal injection of alloxan monohydrate (150mg/kg) in ice cold 0.9% saline solution. .After 72 h, Alloxan-treated animals were considered as diabetic when the fasting plasma levels were observed above 200 mg/dL. The experiments were conducted on animal groups to see the effect of Fruit extract on diabetic rats. Three rats were used in each of the five groups i.e,.Vehicle, fruit extract (250 and 500mg/kg b.w) and glibenclamide were administered once daily for 15 days from the day of induction. Blood was drawn from tip of the tail, and blood glucose level was estimated on 0, 7th, and 15th day of experiment with the help of glucometer (AccuChek Activ) using strip method. On 15th day, blood sample was taken for measuring serum cholesterol and TG level using an auto- biochemistry analyzer.

Body weight:

Body weight was measured before the initiation of experiment and for every 5 days during the experiment to detect the changes.

Biochemical analysis

Fasting serum glucose level and lipid profile were evaluated. Serum glucose level was estimated by GOD/POD method. Lipid profiles including total cholesterol (CHOD/PAP method), triglycerides (GPO/PAP method), HDL-C (PEG Precipitation method), LDL-C (Freidewald's method) and VLDL-C were determined [8].

Statistical analysis

Data obtained from pharmacological experiments are expressed as mean ± SEM. Differences between the control and the treatments in these experiments were tested for significance using student's *t*–test. P - value < 0.05 were considered as significant.

RESULTS

ACUTE STUDY ON NORMAL RATS:

The resuts shows that there is a significant reduction occurs in fasting blood glucose levels at different time intervals in normal rats of both extracts (250 and 500 mg/kg, b.wt). Table 1 and Figure 1 show the effect of blood glucose levels on normal rats.

ACUTE STUDY ON DIABETIC RATS:

The ethanolic extract of Fruit on Alloxan induced Diabetic Rats shows a significant reduction in blood glucose levels at different time intervals. In Diabetic control group there is an increase in blood glucose levels is seen and reduction is seen in extract supplementation group. Table 2 and Figure 2 show the effect of blood glucose levels on Diabetic Rats.

CHRONIC STUDY

Body weight analysis

Table 3 and **Figure 3** shows the average weekly body weights of both control and treated groups. Reduction in body weight was observed in all the diabetic animals. Moreover, animals treated with Glibenclamide and extract shows a gradual increase in body weights on 15th day of the treatment and registered a less significant (P < 0.05) compared to diabetic control group.

Effect on Fasting Blood Glucose level

Diabetic control rats showed consistent rise in the Fasting blood glucose level up to 15 days of the study. Fruit extract (250 and 500 mg/kg) treated diabetic rats showed significant reduction in fasting blood glucose level on 7th and 15th day of the study as compared to 0 day of the experiment. Table 4 and Figure 4 show the fall in blood glucose level on Diabetic Rats.

Biochemical analysis

Animals of the Alloxan-induced diabetic control group showed a significant rise in serum total cholesterol, triglycerides, LDL-C and VLDL-C levels, whereas significant reduction was seen in serum HDL-C and in comparison to normal rats. After 15 days treatment with ethanolic Fruit extract (250 and 500 mg/kg), diabetic animals showed significant reduction in serum total cholesterol, triglycerides, LDL-C and VLDL-C levels with significantly elevated serum HDL-C levels. Glibenclamide (500 µg/kg) treated group showed significant reduction in total serum cholesterol, triglycerides, LDL-C and VLDL-C levels. On contrary, serum HDL-C levels were increased compared to diabetic control group. Table 5 and Figure 5 showed the effect of EECP Fruit Lipid Profile on Diabetic Rats.

TABLE 1.Effect of EECP fruit on Blood glucose levels in Normal rats (Acute study)

Group Treatment	Mean Blood Glucose levels (mg/dl)				
	0 hr	1 hr	2 hr	4 hr	6hr
Normal control	92.6±1.42	91.5±1.20	90.2±1.2	88±1.15	85±1.09
Glibenclamide(500µg/kg)	94.7±1.3	84.3±2.1	76±6.6	63±5.06	55.4±4.5
EECP Fruit(250mg/kg)	98±1.15	88±4.04	69±3.03	66±2.0	68±0.57
EECP Fruit(500mg/kg)	83±1.15	77±0.57	67±1.15	52±0.57	55±1.15

N=3 in each Group; Values of BGL's are expressed as mean ± SEM. *P < 0.001 when compared with normal control group animals

Figure 1: Effect of EECP fruit on Blood glucose levels in Normal rats (Acute study)

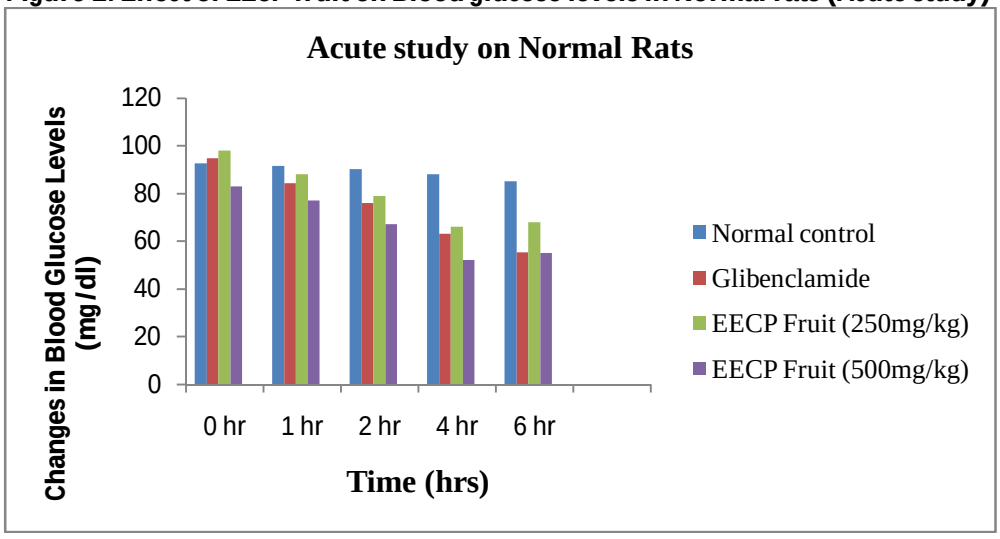


TABLE 2: Effect of EECP Fruit on Blood glucose levels in Diabetic Rats (Acute study)

Group Treatment	Mean Blood Glucose levels (mg/dl)				
	0 hr	1 hr	2 hr	4 hr	6 hr
Normal control	102.74±5.02	103.21±4.21	101.21±3.47	104.24±4.43	103.14±6.62
Diabetic control (150 mg/kg)	238.49±2.57	243.82±1.06	251.72±3.33	254.21±3.21	259.62±4.41
Glibenclamide (500µg/kg)	244.68±1.73	186.28±1.65	159.31±2.03	134.63±1.03	121.2±0.86
EECP Fruit(250mg/kg)	235.23±1.62	219.28±2.1	198.04±2.67	184.21±2.16	187.18±1.3
EECP Fruit(500 mg/kg)	239.45±1.6	214.63±2.07	194.42±3.3	179.48±2.78	181.31±2.5

N=3 in each group; Values of BGL are given in mean ± S.EM. *P < 0.001 when compared with control group animals

Figure 2: Effect of EECP Fruit on Blood Glucose Levels in Diabetic Rats (Acute Study)

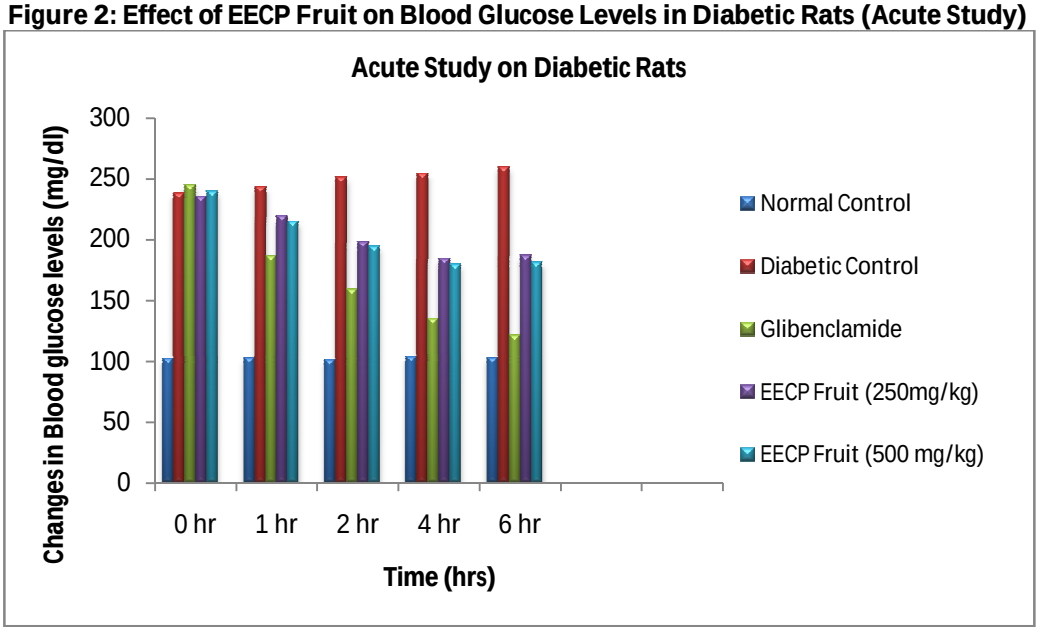


TABLE 3: Changes in Body weight of Alloxan induced Diabetic Rats after 15 Days Treatment

Group Treatment	Body weight changes (gm)		
	DAY 0	DAY 7	DAY 15
Normal control	177.33±3.30	176.00±3.56	177.33±4.99
Diabetic control(150mg/kg)	194.00±2.83	175.67±3.30	176.67±3.40
Glibenclamide(500µg/kg)	200.33±2.05	191.67±2.87	193.33±3.40
EECP Fruit (250mg/kg)	187.67±4.19	185.33±4.99	193.67±1.70
EECP Fruit (500mg/kg)	200.00±6.53	196.67±2.49	200.00±1.63

N=3 in each group; Values are given in mean ± S.EM.

Figure 3: Changes in Body weight of Alloxan Induced diabetic rats after 15 days Treatment

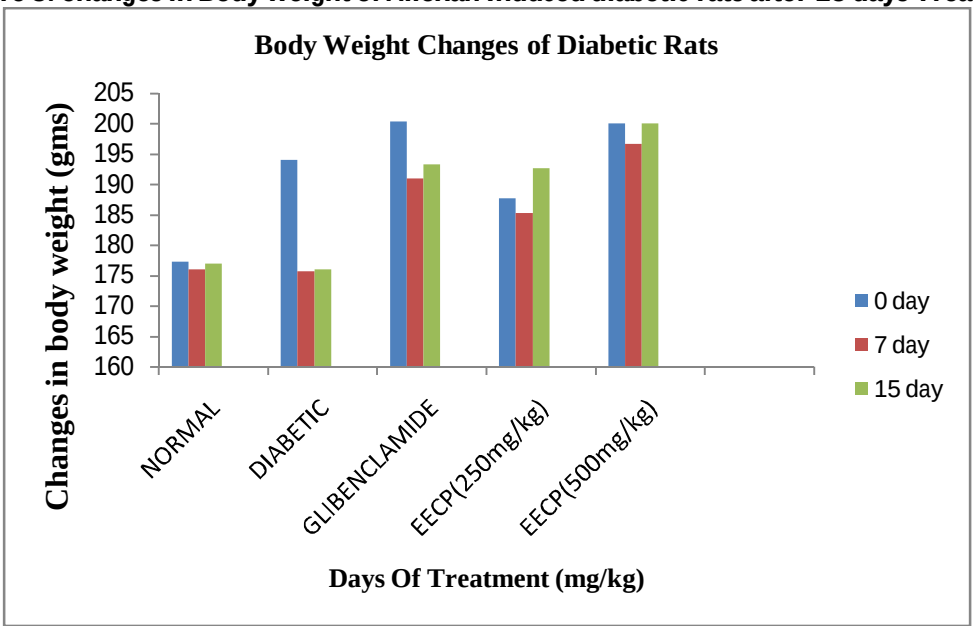


TABLE 4: Effect of EECP Fruit on BGL's in Diabetic Rats (Chronic Study)

Group Treatment	Mean Blood Glucose Levels (mg/dl)		
	DAY 0	DAY 7	DAY 15
Norrmal control	74.00±3.27	79.00±0.82	79.00±0.82
Diabetic control(150mg/kg)	318.67±2.62	338.00±5.72	353.00±3.27
Glibenclamide (500µg/kg)	311.33±7.36	215.67±3.30	146.67±4.99
EECP Fruit (250mg/kg)	311.33±3.40	280.33±6.34	213.67±4.64
EECP Fruit (500mg/kg)	316.67±4.11	254.33±6.34	190.33±4.19

N=3 rats in each Group; Values of BGL are given in mean ± SEM. * P < 0.001 when compared with control group animals

Figure 4: Effect of EECP fruit on BGL's in Diabetic Rats (Chronic Study).

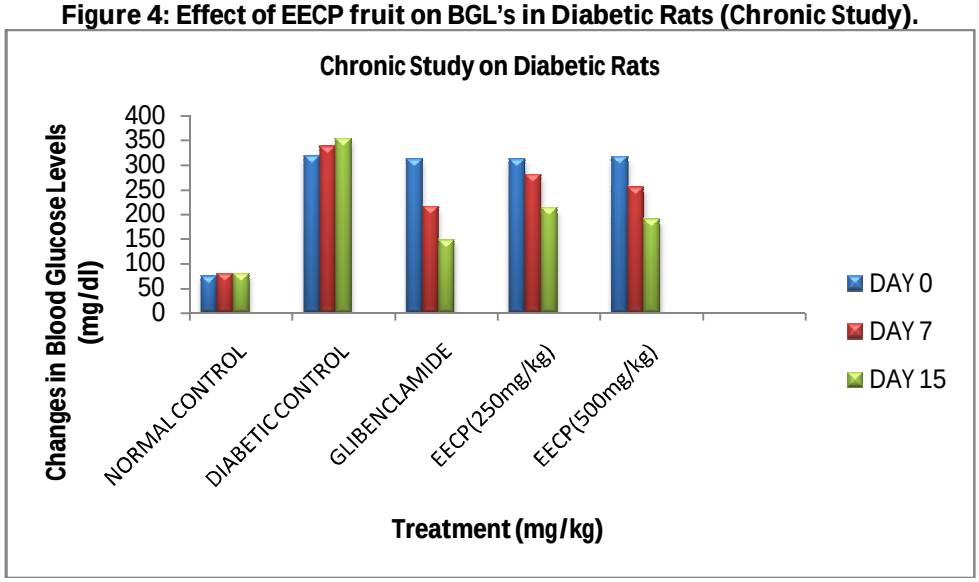
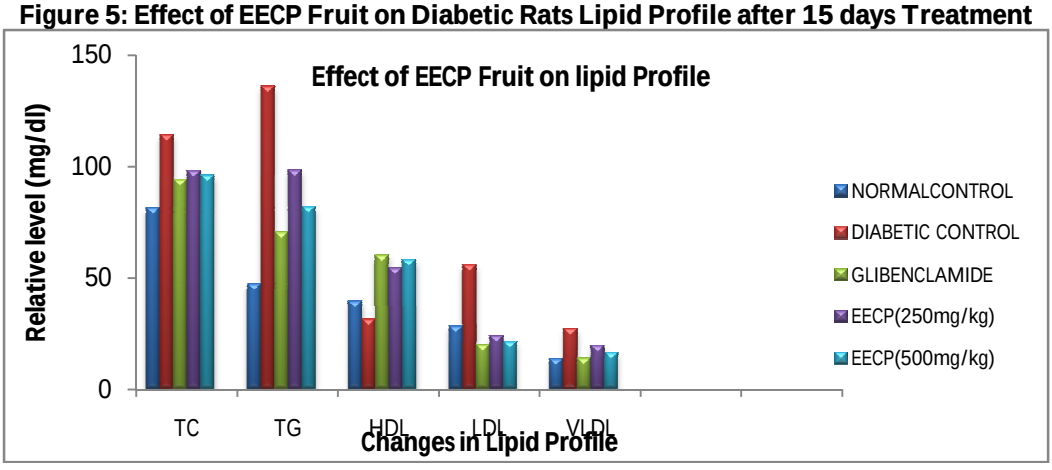


TABLE 5: Effect of EECP Fruit Lipid Profile on Diabetic Rats.

Group Treatment	Total cholesterol	Triglycerides	HDL	LDL	VLDL
Normal control	81.33±2.65	47.33±2.08	39.43±2.00	28.3±1.64	13.6±1.43
Diabetic control (150 mg/kg)	114.33±6.03	136.00±7.21	31.33±5.09	55.8±3.03	27.2±2.56
Glibenclamide (500µg/kg)	94.22±2.89	70.67±3.51	60.23±3.56	19.99±3.33	14.00±1.97
EECP Fruit (250mg/kg)	98.00±4.00	98.33±3.21	54.33±4.04	24.07±3.64	19.6± 2.20
EECP Fruit (500mg/kg)	96.12±2.52	82.00±1.73	58.24±4.23	21.48±3.53	16.4±2.89

N = 3 rats in each group; mean ± SEM. *P < 0.005 when compared with normal values.

Figure 5: Effect of EECP Fruit on Diabetic Rats Lipid Profile after 15 days Treatment



DISCUSSION

Diabetes mellitus is a heterogeneous group of metabolic disorders characterized by hyperglycemia and defective metabolism of glucose and lipids characterized by relative or absolute deficiency of insulin.[9] .Conventional stepwise approaches to clinical management generally focus on the consequences *i.e.* hyperglycemia, dyslipidemia and vascular complications rather than the causes of type 2 DM. [10]. Due to the uncertain allergic reactions and side effects of allopathic drugs, the attempts are being made to revert to herbal treatments all over the world. Hundreds of herbs, spices, fruits and vegetables have been shown to have remedial effects on certain diseases in humans. The experiments on laboratory animals, mostly rats, have led to considerable success in the treatment of diseases such as diabetes, hypertension and hyperlipidemia. With the same idea and previous evidence in other countries the extract supplementation to rats was attempted which showed significant reduction in serum glucose levels [11]. The various numbers of plants have been traditionally used to treat diabetes, and some have been proven to have hypoglycemic effects. These studies have identified that compounds such as polysaccharides, [12] flavonoids, [13] terpenoids and tannins, [14] and steroids, [15] are responsible for antidiabetic effect. *Calotropis procera* also contains flavonoids, saponins and carbohydrate, steroids, tannins, and phenolic compounds. The observed hypoglycemic effects of this plant could have resulted from the combined activity of these compounds present in the extract. Diabetes mellitus is one of the leading causes of death, illness and economic loss all over the world. Insulin-dependent (Type I, IDDM) diabetes is characterized by juvenile onset and by absolute insulin deficiency. Non-insulin-dependent (Type II, NIDDM) diabetes is characterized by mature onset, by varying basal insulin levels and a frequent association with obesity. We found an elevated blood glucose concentration accompanied by increase in total cholesterol, triglycerides, LDL,VLDL and decrease in HDL cholesterol in Alloxan- induced diabetic rats as compared to control animals. Oral administration of ethanolic extract of *Calotropis procera Fruit* decrease the levels of blood glucose and are shown in the table 1and 2. In recent years, considerable interest has been directed towards the investigation of plasma lipids and lipoproteins pattern in diabetes mellitus due to the fact that abnormal lipid level leads to the development of coronary artery disease in diabetic patients [16]. Reduced insulin secretion and defect in insulin function results in enhanced metabolism of lipids from adipose tissue to the plasma. Impairment in insulin sensitivity due to high concentration of lipids in the cells is responsible for the elevated cardiovascular risk in diabetes mellitus [17]. Thus, the altered lipid and lipoprotein pattern observed in diabetic rats could be due to defect in insulin secretion and/or action. Hypercholesterolemia and hypertriglyceridemia have been reported to occur in alloxan induced diabetic rats. Accumulation of cholesterol and phospholipids in liver due to elevated plasma free fatty acids has been reported in diabetic rats[18] . In the present study, ethanolic extract of *Calotropis procera* Fruit had significantly decreased Total Cholesterol, Triglycerides, VLDL, and LDL with increase in HDL which is having a protective function for the heart compared with diabetic control group. Oral administration of (250 and 500 mg/kg) of ethanolic extract as a test dose to the animals did not produce any death or symptoms of toxicity in the rats. This indicated that, the extract might be safe at the doses (250 and 500 mg/kg) selected for study. Administration of 150 mg/kg body weight of alloxan monohydrate induced hyperglycemia in the rats after 72 h. The chemical is reported to induce diabetes by forming highly reactive superoxide radicals which destroy the insulin producing cells in the pancreas [19]. Administration of ethanolic extract of *Calotropis procera* produced a significant reduction in blood glucose levels in the alloxan induced diabetic rats. No mortality was observed in both acute and chronic study and it is reported that the dose levels are to be safest for evaluating anti diabetic activity. According to earlier studies, plant extracts cause antihyperglycemic effect by promoting regeneration of β-cells or by protecting these cells from destruction, by restricting glucose load as well as by promoting unrestricted endogenous insulin action. Antihyperglycemic effect may also be caused by the effect of plant extract on β-cells to release insulin or activate the insulin receptors to absorb the blood sugar and stimulate the peripheral glucose consumption [20]. The present experimental studies reveal that the ethanolic extract of *Calotropis procera* fruits administered orally for 15 days produced a significant decrease in the blood glucose levels. The comparable effect of the plant extracts with glibenclamide may suggest similar mode of action. The present investigation hence proves the traditional claim regarding EECP Fruit for its anti-diabetic activity.

CONCLUSION

The present investigation justifies the scientific support that the ethanolic extract of *Calotropis procera* fruit has anti-diabetic activity and anti-hyperlipidemic activity. Further work can be carried out to isolate the compounds and to screen for their actual mechanism of action.

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