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COMPARATIVE EVALUATION OF ANTI DIABETIC AND ANTI LIPIDEMIC ACTIVITY OF *Dypsis lutescens* (H.Wendl.) AND *Caryota urens* (L.) of F: ARECACEAE IN STREPTOZOTOCIN INDUCED DIABETES IN RATS

MANASA CHIDURUPPA* AND P PANDIAN

1: Associate Professor, Department of Pharmacognosy, Nizam Institute of Pharmacy, Near Ramoji Film City, Deshmukhi, Yadadri Bhongir-508264,Telangana,India

2: Associate Professor, Department of Pharmacy, Annamalai University, chidambaram -608002, Tamil Nadu, India

***Corresponding Author: Manasa Chiduruppa: E Mail: manasa0405@gmail.com**

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ABSTRACT

The present study was undertaken to assess the effects of leaves extracts of *Dypsis lutescens* (H.Wendl.) and *Caryota urens* (L.) both belonging to the family Arecaceae on the blood sugar and lipid levels in diabetic rats. The Leaves of *Dypsis lutescens* and *Caryota urens* were used to prepare ethanolic and aqueous extract. Diabetes was induced by streptozotocin injection Intra peritoneally (i.p) in all experimental animals groups except normal control group. Group I (normal control) rats received distilled water and Group II to Group VII animals treated with streptozotocin to induce diabetes and titled diabetic rats. Group III rats were treated with glibenclamide and served as standard group, whereas Group IV to VII rats were treated with leaves extract of *Dypsis lutescens* and *Caryota urens* respectively. Physical parameters (Feed Intake, Body Weight), Biochemical parameters (Blood Glucose, Liver Glycogen, Serum a-amylase, Serum Insulin, Total cholesterol, TG, HDL and LDL level) were measured timely. The study results indicate that oral administration of leaves extracts of *Dypsis lutescens* has proven to be more effective and safe anti-diabetic agent in comparison to leaves extracts of *Caryota urens*.

Keywords: Diabetes, *Dypsis lutescens, Caryota Urens*, Streptozotocin

1. INTRODUCTION

Diabetes Mellitus (DM) is a major metabolic disorder characterized by increased blood glucose and disturbance in carbohydrate, lipid and protein metabolism and insulin secretion **[1]**. The major cause of Diabetes Mellitus is the shortage of insulin secretion and decline in cell response towards insulin. As per recent WHO Report almost 580 million adults living with diabetes around the globe. India also has more than 80 million diabetic individuals who are currently diagnosed with the disease **[2]**.

The spread of DM is more in recent years due to modern lifestyle linked with increase in overweight and sedentary population. Diabetic Patients with diabetes have dyslipidemia and an increased risk of stroke, coronary heart disease, myocardial infarction and peripheral vascular disease **[3]**. Many researches indicate that multiple abnormalities of lipoprotein metabolism also observed in diabetic patients. Hyperlipidemia represents a major risk factor for the premature development of atherosclerosis and its cardiovascular complications **[4]**. The American Heart Association (AHA) has identified the primary risk factor associated with progression of atherosclerotic lesions as elevated levels of total cholesterol (TC) and triglycerides (TG) in serum **[5]**. Untreated diabetes leads to many acute and chronic complications in diabetic population. Acute complications involve diabetic ketoacidosis, non ketotic hyperosmolar coma whereas chronic complications consist of cardiovascular disease, stroke, nephropathy, foot ulcers and retinopathy.

Many research investigations suggested that the medicinal plants are the best alternative to treat diabetic conditions like lowering lipid and glucose levels and management of diabetic complications.

The current study was planned to investigate the comparative evaluation of Anti diabetic and Anti lipidemic activity of *Dypsis lutescens* and *Caryota urens* in streptozotocin (STZ) induced diabetes in rats.

2. MATERIAL AND METHODS

Collection and authentication of plant material

The leaves of *Dypsis lutescens* (H.Wendl)) and *Caryota urens* (L) required for the study were collected from in and around Hyderabad (Dist), Telangana; in the month of December 2019. The plant parts were authenticated by P.V.Prasanna, Scientist 'G' and HoO, Botanical Survey of India, Hyderabad and Dr. A Vijaya Bhaskar Reddy, Head of the Dept. of Botany, Osmania University, Hyderabad and the voucher

specimens were kept for further reference in the department.

Extraction:

The leaves of *Dypsis lutescens* and *Caryota urens* were cleaned and shade dried for about 7 days. The shade dried leaves were then ground to coarse powder using a mechanical grinder. The coarse powder of leaves of *Dypsis lutescens* and *Caryota urens* were stored properly for further extraction procedures.The stored leaves were subjected to soxhlet extraction using Petroleum ether, Ethanol and water as per increasing the polarity order of solvents. The marc obtained was air dried. The filtrate obtained was subjected to steam distillation, to concentrate the extract and the solvent was recollected and was used for further extraction process. A dark green residue was obtained on further concentrating and evaporating the extract on a water bath. The dried extracts thus obtained were kept in the desiccator and was used for further phytochemical and pharmacological investigations **[6]**.

Acute Toxicity Testing

Ethanolic and Aqueous extracts of *Dypsis lutescens* and *Caryota urens* were administered at dose rate of 50, 500, 1000, 2000, 4000 and 5000 mg/kg to the test groups. Changes in behaviors of rats were observed for 14 days. The mortality rate was used to calculate mean lethal dose (LD_{50}) value **[7]**.

Experimental Animals setup

We housed Forty two (42) healthy albino wistar rats of either sex weighing between 140- 160 g in an animal facility. The experiment was conducted according to approved methods of Institutional Animal Ethics Committee. The animals were kept for two weeks as acclimatization period prior to the start of experiment and received normal diet and water *ad libitum.* After adaptation period, rats were randomly divided into following groups **[8]**.

Group I: Non-diabetic (ND) rats received Distilled water and served as normal control

Group II: STZ diabetic rats received Distilled water and served as diabetic control

Group III: STZ diabetic rats received Glibenclamide at 0.25 mg / kg/oral and served as standard

Group IV: STZ diabetic rats treated with Ethanolic extract of *Dypsis lutescens* (400 mg/kg/day, p.o)

Group V: STZ diabetic rats treated with Aqueous extract of *Dypsis lutescens* (400 mg/kg/day, p.o)

Group VI: STZ diabetic rats treated with Ethanolic extract of *Caryota urens* (400 mg/kg/day, p.o)

Group VII: STZ diabetic rats treated with Aqueous extract of *Caryota urens* (400 mg/kg/day, p.o)

Induction of Diabetes

The study was performed for 14 days by repeated oral administration. Diabetes was induced in overnight fasted rats of Group II to VII by injecting intraperitoneally with a single dose of STZ (50 mg/kg b w) once dissolved freshly in cold 20 mM citrate buffer adjusted to pH 4.5 **[9]**. After injection, they were provided with 2% sucrose solution for 48 h to alleviate the discomfort after initiating the hypoglycemic phase. Three days after injection, the rats were examined for fasting plasma glucose to confirm their diabetic stage. The rats with fasting plasma glucose higher than 126 mg/dL were used in the experiments **[10]**.

Monitoring of body weight

Body weights and Food intake of rats were measured on digital weighing machine and recorded weekly up to 14 days of experiment.

Monitoring of Fasting Blood Glucose

Fasting Blood Glucose was measured by commercially available glucometer. Blood samples of rats were collected by cutting tip of tails and Blood sugar level checked by using blood glucose test strips with Accu Check glucometer.

Monitoring of Liver Glycogen

A total of 50 mg of liver tissue was weighed and transferred to 200 µl 30% Potassium Hydroxide (KOH) and heated in boiling water bath for 10 minute with regular mixing. The sample was cooled and ethanol was added at a final concentration of 55%, the mixture was vortexed and centrifuged for 10 minute at 1700rpm. The supernatant was decanted off and the pellet re-suspended in 2 ml of distilled water and 10 µl was analyzed for total glycogen in triplicate **[11]**.

Monitoring of Serum α-amylase

Amylase analysis of the pancreas of the animals used was carried out with Van Loon's amyloclastic method **[12]**.

Monitoring of Serum Insulin

Insulin concentration in the serum samples were examined by enzyme-linked immunosorbent assay (ELISA) through commercially available ELISA® kit

Monitoring of Lipid level

After completion of anti-diabetic activity on the 14th day, all animals were anesthetized by pentobarbital sodium (35 mg/kg) and euthanized by cervical dislocation and blood samples were collected through cardiac puncture for biochemical parameters studies. Collected blood was centrifuged to separate the supernatant plasma serum and used to determine the various biochemical

parameters like Total cholesterol (TC), low density lipoproteins (LDL), high density lipoprotein (HDL) triglycerides (TG).

Histopathalogical Examination

Histopathology was performed on collected tissue samples of pancreas by placing tissue samples in 10% neutral buffered formalin (NBF) and embedded in paraffin wax to form the homogenous mass. After mounting the tissues were stained with eosin and hematoxylin **[13]**.

Statistical Analysis

Results were expressed as (mean ±SE). Data was analyzed statistically by analysis of variance (ANOVA). Duncan multiple range (DMR) test was applied in case of significant difference among the experimental groups at 5% level of significance **[14]**.

3. RESULTS

Acute Toxicity Study

Acute toxicity studies of *Dypsis lutescens* and *Caryota urens* leaves extracts were done as per OECD guidelines. The results revealed the extracts did not show any significant fluctuations in behavioral or neurological responses up to 4000mg/kg body weight. There was a small changes observed in behavioral responses but no mortality or toxicity reaction observed up to 5000mg/kg body weight of ethanolic and aqueous extract of *Dypsis lutescens* and *Caryota urens* in all groups after 14 days, suggesting that LD_{50} of the extracts 4000 mg/kg b.w. So LD_{50} of herbal formulation was considered 1/10 of 4000 mg/kg **[15]**.

Effect of various treatments on physical parameters

Feed Intake (mg/day) (Table 1; Graph 1)

Weight (grams) (Table 2)

Blood Glucose (mg/dl) level (Table 3)

Liver Glycogen, Serum a-amylase and Serum Insulin (U/L) (Table 4)

Lipid Profile (Table 5)

Histopathology

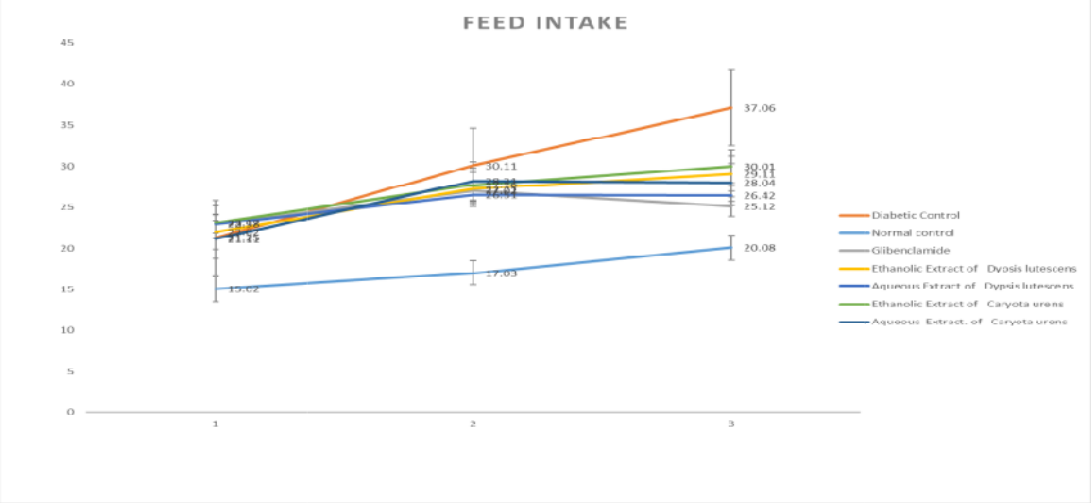
Histopathology of Pancreas

Tissue samples of pancreas of rats of all experimental groups were collected and processed for histopathological examinations. Photomicrographs obtained after histopathological evaluation of pancreas are presented in **(Figure 1 A-G)**.

Table 1: Effect of various treatments on Mean±SE Feed intake (mg/day) in rats

Group	Treatments	Days		
		Day 0	Day 7	Day 14
I	Normal control	15.02 ± 0.57	17.03 ± .84	20.08 ± .88
II	Diabetic Control	21.25 ± 0.82	30.11 ± 0.81	37.06 ± 0.74
III	Glibenclamide	23.00 ± 0.67	27.02 ± 0.61	25.12 ± 0.88
IV	Ethanolic Extract of *Dypsis lutescens*	22.02 ± 0.63	27.21 ± 0.82	29.11 ± 0.71
V	Aqueous Extract of *Dypsis lutescens*	22.98 ± 0.83	26.51 ± 0.99	26.42 ± 0.87
VI	Ethanolic Extract of *Caryota urens*	23.17 ± 0.87	27.76 ± 0.92	30.01 ± 1.01
VII	Aqueous Extract of *Caryota urens*	21.11 ± 0.93	28.21 ± 0.94	28.04 ± 0.81

Mean values with in a row or column, not bearing similar letters differ significantly (P≤0.01)



Graph 1

Table 2: Effect of various treatments on Mean±SE Body weight (gm) in rats

Group	Treatments	Days		
		Day 0	Day 7	Day 14
I	Normal control	153.12 ± 3.51	165.11 ± 4.90	178.60 ± 6.01
II	Diabetic Control	152.54 ± 4.12	145.71 ± 5.01	133.16 ± 4.24
III	Glibenclamide	153.23 ± 4.15	170.32 ± 3.91	188.74 ±4.01
IV	Ethanolic Extract of *Dypsis lutescens*	154.54 ± 4.23	158.42 ± 4.82	165.32 ± 4.21
V	Aqueous Extract of *Dypsis lutescens*	151.27 ± 5.21	166.95 ± 4.01	179.98 ± 4.25
VI	Ethanolic Extract of *Caryota urens*	152.32 ± 4.99	155.26 ± 3.61	157.71 ± 5.41
VII	Aqueous Extract. of *Caryota urens*	153.65 ± 3.59	156.54 ± 3.62	168.61 ± 3.09

Mean values within a row or column not having similar letter differ significantly (P≤0.01)

Table 3: Effect of various treatments on Mean ±SE Blood Glucose (mg/dl) level in rats

Group	Treatments	Days		
		Day 0	Day 7	Day 14
I	Normal control	92.3 ± 3.11	95.5 ± 3.14	100 ± 3.98
II	Diabetic Control	271.3 ± 7.12	300.8 ± 6.47	342.7 ± 7.12
III	Glibenclamide	265.8 ± 5.31	194.5 ± 4.01	106.2 ± 3.40
IV	Ethanolic Extract of *Dypsis lutescens*	277.7 ± 5.18	245.7 ± 5.20	144.3 ± 3.02
V	Aqueous Extract of *Dypsis lutescens*	273 ± 4.52	142.1 ± 3.16	110.8 ± 2.93
VI	Ethanolic Extract of *Caryota urens*	276.7 ± 4.53	210.8 ± 3.10	180.6 ± 3.04
VII	Aqueous Extract of *Caryota urens*	271.4 ± 3.98	195.7± 2.80	164.3 ± 2.12

Mean values within a row or column not having similar letter differ significantly (P≤0.01)

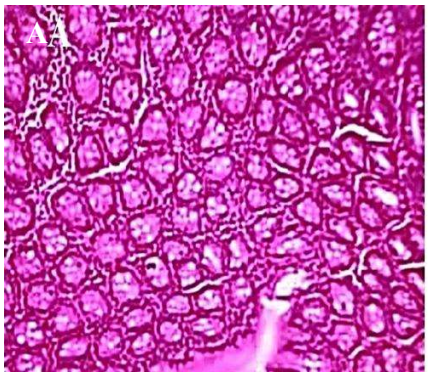
Table 4: Effect of various treatments on Mean±SE on Liver Glycogen, Serum a-amylase and Serum Insulin (U/L) level in rats

Group	Treatment	Liver Glycogen (mg/g tissue)	Serum a-amylase (u/1)	Insulin (IU/ml)
I	Normal control	75.62 ± 2.77	518.86 ± 11.14	20.85 ± 3.56
II	Diabetic Control	52.31 ± 1.17	950.31 ± 19.26	10.21 ± 2.04
III	Glibenclamide	87.72 ± 2.99	489.13 ± 16.50	39.1 ±2.3
IV	Ethanolic Extract of *Dypsis lutescens*	64.11 ± 2.78	701.11 ± 19.30	15.56 ± 3.51
V	Aqueous Extract of *Dypsis lutescens*	78.34 ± 3.15	590.21 ± 19.12	18.61 ± 2.59
VI	Ethanolic Extract of *Caryota urens*	60.53 ± 2.44	750.22 ± 21.71	13.15 ± 3.05
VII	Aqueous Extract. of *Caryota urens*	67.42 ± 2.98	665.20 ± 21.02	15.99 ± 3.24

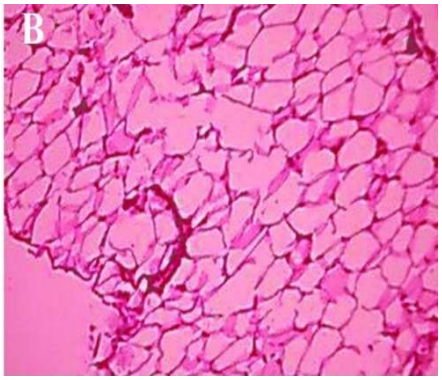
Mean values with in a row or column, not bearing similar letters differ significantly (P≤0.01)

Table 5: Effect of various treatments on Mean±SE on Total cholesterol, TG, HDL and LDL level in rats

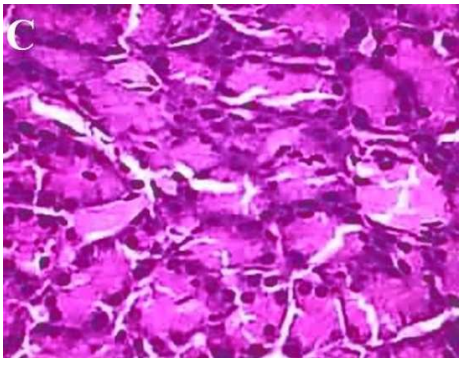
Group	Treatment	Total cholesterol (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
I	Normal control	120 ± 9.12	125 ± 8.80	34±2.2	81± 2.5
II	Diabetic Control	185.3 ± 4.4	169.0 ± 7.9	30.2 ±2.4	177.4±8.9
III	Glibenclamide	137.7± 5.3	128.3 ± 6.5	39.1 ±2.3	90.3± 3.5
IV	Ethanolic Extract of *Dypsis lutescens*	140.5±7.5	136.8±4.5	35.6±5.5	93.5±3.6
V	Aqueous Extract of *Dypsis lutescens*	134.4±2.7	136.2±8.1	38.2±1.5	81.3±4.0
VI	Ethanolic Extract of *Caryota urens*	144.2± 4.3	138.5± 4.7	34.5 ±3.1	95.6± 3.2
VII	Aqueous Extract. of *Caryota urens*	136.9± 8.5	145.4± 2.2	37.2 ±3	148.8±5.5



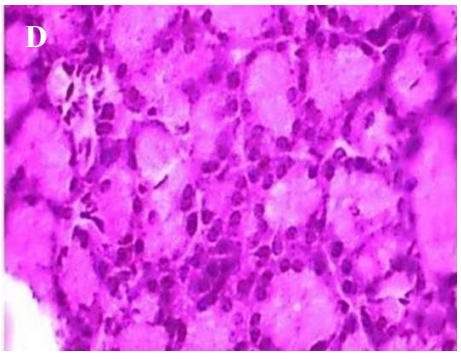
Normal control



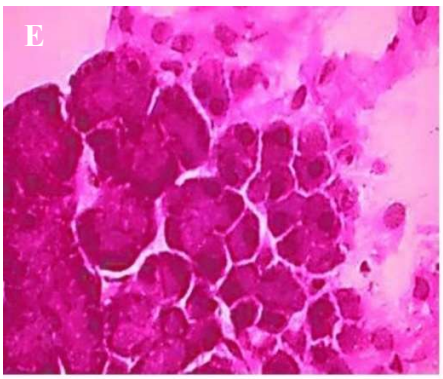
Diabetic Control



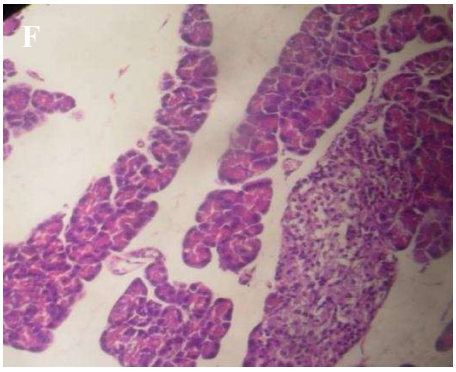
Glibenclamide



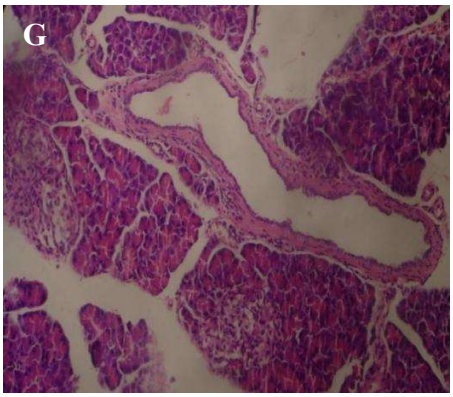
Eth. Extract of *Dypsis lutescens*



Aq. Extract of *Dypsis lutescens*



Eth. Extract of *Caryota urens*



Aq. Extract of *Caryota urens*

Figure 1 (A-G): Photomicrographs of pancreas sections of Normal control, Diabetic Control, Glibenclamide, Eth. Extract of Dypsis lutescens, Aq. Extract of Dypsis lutescens, Eth. Extract of Caryota urens, Aq. Extract of Caryota urens treated groups of rats (H & E staining 40X)

(A) Pancreas of control group showing normal pancreatic cells and fully active islets of Langerhans in pancreatic parenchyma.

(B) Pancreas of Diabetic Control showed Islets cells of pancreas were seen Shrinkage, No change of exocrine cells was observed and Islets cells of pancreas were decreased in size.

(C) Pancreas of the Glibenclamide treated group indicated Restoration of normal cellular size of islet and normal nuclei of cells with hyperplasia.

(D) Pancreas of Eth. Extract of *Dypsis lutescens* treated group showed lymphocytes infiltration was seen, Cytoplasmic Vaculization was seen, Duct of pancreas was normal .

(E) Pancreas of Aq. Extract of *Dypsis lutescens* treated group showed Size of islets cells was showed normal, No fibrosis was seen, Pancreatic duct was not dilated, Pancreatic duct was not obstructed.

(F) Pancreas of Eth. Extract of *Caryota urens* treated group showed Size of islets cells of pancreas was decreased, Structure of islets cells of pancreas were not well defined.

(G) Pancreas of Aq. Extract of *Caryota urens* treated group showed No Vaculization, less number of islets cells of pancreas with No congestion in blood vessels.

4. DISCUSSION

Diabetic mellitus is a leading metabolic disorders in the world which causes both microvascular and macrovascular complications. Presently, more then 170 million people suffering from diabetes mellitus globally and number increasing exponentially over the last few years and is

on the rise. It is projected to that upto approximately 380 million people will be affected by diabetic mellitus by 2030.

Two medicinal plants were selected for the present activity which are easily available and very economic. Medicinal plants have been used in the treatment of various diseases and ailments from centuries becoming more common throughout the world for therapeutic purposes.

Acute oral toxicity of leaves extracts of *Dypsis lutescens, Caryota urens* were performed as per OECD guidelines. On the basis of acute oral toxicity doses have been selected to assess the anti-diabetic activity of leaves extract of these plants in STZ induced diabetic rats.

During the study, physical parameters including body weight and food intake were observed. The results shown in **Table 1** read that animals treated with ethanolic and aqueous extracts of *Dypsis lutescens* minimized significantly diabetic effects produced by Streptozotocin. It was observed that diabetic rats have significant effect on feed intake and increased feed consumption in comparison to Glibenclamide treated rats during the study. Extracts of *Dypsis lutescens* produced significant effects on feed intake and reduce feed intake in comparing to leaves extracts of *Caryota urens*.

The effect of various treatments on changes in body weight of experimental rats shown in **Table 2**. Streptozotocin induced diabetic rats produced significant loss in body weight in compared to normal rats during the study. In diabetic control rats, continued weight loss was observed till the end of the study. Whereas Streptozotocin mediated body weight reduction was reversed by leaves extracts of Dypsis *lutescens* more effectively when compared to leaves extracts of *Caryota urens*. The improvement in body weight was compared with glibenclamide. Reduction in the body weight could be due to increased gluconeogenesis and lipolysis of triglycerides under the influence of experimental diabetes **[16]**.

Effect of various treatments on blood glucose presented in **Table 3**. Oral administration of the leaves extract of *Dypsis lutescens* caused a significant reduction in blood glucose level compared to diabetic control. Ethanolic and aqueous extracts of *Dypsis lutescens leaves* decreased blood glucose level from 277.7 ± 5.18 to 144.3 ± 3.02 mg/dl and from 273 ± 4.52 to 110.8 ± 2.93 mg/dl respectively. The effect was evident from the 7^{th} day and onwards.

Insulin releasing effect by the leaves extracts of *Dypsis lutescens* were substantiated by significantly increased

levels of plasma insulin in **Table 4**. Aqueous extract of *Dypsis lutescens* produced a significant effect on Insulin level by synthesis/release of insulin from pancreatic beta cells in Diabetic rats. Possible mechanism of action of anti-hyperglycemic activity of the extract may be due to increased insulin discharge from existing β cells as well as increased transfer of glucose into peripheral tissues **[17]**.

α-Amylase play a key role in Controlling the catalytic activity in reduction of glucose production in the postprandial stage, which could be a therapeutic benefit for people with diabetes. Inhibition of the α-amylase inhibits the breakdown of carbohydrates present in food, a major dietary source of carbohydrates is present in food. Leaves extracts of *Dypsis lutescens* produced significant decrease in the serum α-Amylase level 590.21 ± 19.12 in comparison to 950.31 ± 19.26 in Diabetic Control **[18]**.

Hyperlipidaemia is a common complication of diabetes mellitus. The present results showed significant (P≤0.01) rise in serum cholesterol, triglycerides, LDL and VLDL levels in the diabetic rats while HDL level was decreased. Treatment of diabetic rats with leaves extracts of *Dypsis lutescens* results gradual decrease in Serum cholesterol, Triglycerides, VLDL and LDL levels with increase in HDL level at 14^{th} days of treatment shown in **Table 5**. The lipid-lowering trend of the leaves extract may be due to the presence of flavonoids which are reported to lower the levels of cholesterol and triglycerides and also due to the action of HMG-CoA reductase enzyme that is cholesterol biosynthesis rate-limiting enzyme **[19]**. Flavanones (flavonoids) also reported to lower the activity of another key cholesterol-regulating enzyme (ACAT) that plays important role in the absorption and esterification of cholesterol **[20]**. Flavonoids have also been reported to play a major role in the anti-diabetic activity according to the researches done on various flavonoidal compounds and presence of these secondary metabolites in the plant extracts of dypsis lutescens and caryota urens may be a possible outcome for the reported positive anti-diabetic activity **[21, 22]**.

5. CONCLUSION

Results indicate that leaves extracts of *Dypsis lutescens* proven as a more effective, safe anti-diabetic agent in comparison to leaves extracts of *Caryota urens*. There is need to obtain more information about the nature of the cellular stress pathways. Role of hyperglycemia and hyperlipidemia in the development of diabetes and oxidative stress should be analyzed in more detail.

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The authors declare that they have no funding support for this study.

8. Conflict of Interest

The authors declare that they have no conflict of Interest for this study

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