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Research Article

Evaluation of anti-Diabetic Activity for Ethanoic extract of Syzygium *cumini* leaf in Dexamethasone induced diabetic rats

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ABSTRACT

Syzygium cumini is commonly known as insulin plant in India. Consumption of leaves of this plant are believed to lower blood glucose level in healthy normal and diabetic individuals. The present study was planned to evaluate the effect of the leaves of syzygium cumini leaves on dexamethasone induced hyperglycemic rats. MaleWistar rats (n=6) were treated with 10mg/kg of dexamethasone subcutaneously for 20 days from 11th day to 20 days. Different groups received 100mg/kg plant extract in distill water and glibenclamide 500µg/kg per orally on plasma blood glucose level, serum total cholesterol, triglyceride level, HDL, LDL and Serum VLDL were observed. Dexamethasone caused an increase blood glucose level, serum total cholesterol, serum triglyceride level, Serum HDL ,Serum LDL and Serum VLD and compare with normal control[**P<0.01]. In the dexamethasone model 100mg/kg p.o. of Ethanolic extract of syzygium cumini leaf showed significant decrease in blood glucose level, serum total cholesterol , serum triglyceride level, Serum HDL ,Serum LDL and Serum VLDL when compared to dexamethasone control[I.**P<0.01]. The study results concluded *Syzygium cumini* proved to be effective in treatment of Type-II Diabetes mellitus owing to its ability to decrease insulin resistance.

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INTRODUCTION

The Diabetogenic effect of exogenous or endogenous Glucocorticoid excess results in part from the development of peripheral insulin resistance [1], a wherein insulin fails to normally stimulate glucose uptake into skeletal muscle, the main site of insulin-mediated glucose disposal [2]. Glucocorticoid induced insulin resistance is attributed mainly to a post receptor defect of insulin action [3,4]. About 90% of type 2 diabetes is attributable to excess weight. Further-more, approximately 197 million people worldwide have impaired glucose tolerance, most commonly because of obesity and the associated metabolic syndrome [4]. It is projected that one in three American adults will have diabetes in 2050 if this trend continues [5]. This form of diabetes is characterized by insulin resistance and at least initially, a relative lack of insulin secretion. Most individuals with type 2 diabetes exhibit abdominal obesity which itself causes insulin resistance. In addition, hypertension, dyslipidemia (high triglyceride levels and low HDL-cholesterol levels), and elevated inhibitor plasminogen activator-1 (PAI-1) levels are often present in these individuals. This clustering of abnormalities is referred to as the "insulin resistance syndrome" or the "metabolic syndrome." Because of these abnormalities, patients with type 2 diabetes are at increased risk of developing macro vascular complications [6].

Syzygium cumini (L.) Skeels. a polyembryonic species(family-Myrtaceae), is a tropical fruit tree of great economic importance [7]. It is a large evergreen tree up to30 meters height and girth of 3.6 meters with a bole up to 15meters. *S. cumini* has been widely used forth treatment of various diseases in traditional and folk medicine.Unani system of medicine describes the use of the plant in liver tonic, enrich blood, strengthen teeth and gums and form good lotion for removing ringworm infection of the head [8]. The leaves are antibacterial and used tostrengthen the teeth and gums. The leaves have also beenextensively used to treat diabetes, constipation, leucorrhoea, stomachalgia, fever, gastropathy, stranger, dermopathy and toinhibit blood discharge in the feces[9].It has been also showedbefore that the leaf, bark, stem and pulp of *S. cumini* plants possess potent antidiabetic activity [10]. The major phytoconstituents are reported to contain vitamin C, gallic acid, tannins, anthocyanins, includes cyanidin,petunidin, malvidinglucoside and other components [11]. Preliminaryphytochemical analysis also showed the presence of phenols, terpenoids, tannins, saponins, phytosterols, carbohydrates, flavonoids, amino acids in stem bark of *S. cumini* [12].Previous investigations also revealed the bark of *S. cumini*contains butulinic acid, β-sitosterol, friedelin, epi-friedelanol[13]. It also contains new esters of epifriedelanol(eugenin), D-glucoside, kaempterol-3-O-glucoside,quercetin, myricetin, astragal in and Gallic acid[14]. The presentstudy was designed to investigate the phytochemical

bioactivecompounds of the Ethanolic extract of *S. cumini* leaves toestablish its antidiabetic activity.

Materials and methods

Drugs and chemicals

Dexamethasone and all other reagents used were of analytical grade. Diagnostic kits used in this study were procured from Span Diagnostics Ltd., India, NR Chemicals, Mumbai and Excel diagnostics Ltd., India.

Plant material

The leaves of *Syzygium cumini* were collected fromGuntur and were shade dried, Powdered and extracted in Maceration process successivelywith Ethanolrespectively due to their nature of polarity. After extraction, the ethanol extracts were filtered through Whatman No.1 filter paper and stored for further use.

Preparation of Extraction

The powdered plant material (400 g) was first defatted with Ethanolic solvent and then macerated at room temperature (24-26ºC) with Ethanolic (850 mL) for 4 days with occasional shaking, followed by re-maceration with the same solvent for 3 more days. The macerates were combined, filtered and distilled off in reduced pressure. The resulting concentrate was vacuum dried at 40°C.The dry extract was kept in a vacuum desiccators until use. Preliminary phytochemical studies on Ethanolic extract of syzygium cumini leaf revealed the presence of alkaloids, triterpenoids, steroids and tannins. The percentage yield of the extract was calculated by using the formula below:

% yield= (weight of extract/weight of plant material) ×100%

Phytochemical screenings

The leaf extracts of *Syzygium cumini* were analyzed for the presence of flavonoids, alkaloids,Glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides and tannins according to standardmethods [15].

Experimental design

The antidiabetic activity of Ethanolic extract of s. cumini leaf was assessed in normal, glucose loaded and Dexamethasone induced diabetic rats. In all studies, the animals were fasted overnight for 16 h with free access to water throughout the duration of the experiment.

Animals used

Experiments were performed with male wistar rats procured from Albino Research & Training institute (Hyderabad, Andhra Pradesh, India), weighing about 200-230 g. The animals were housed in individual polypropylene cages under standard laboratory conditions of light, temperature (23 ± 1°C) and relative humidity for at least one week before the beginning of experiment, to adjust to the new environment and to overcome stress possibly incurred during transit. Animals were given standard rat pellets and drinking water ad libitum. The animals were fasted 12 hours before the conduct of experiment and during the experiment they were withdrawn from food and water . The experiments were planned after the approval of Institutional Animal Ethical Committee (Approval number is 1722/RO/ERe/S/13/CPCSEA).

Acute toxicity studies

Acute oral toxicity study was performed as per OECD-421 guidelines (acute toxic class method). Albino rats (n=6) of either sex selected by random sampling techniques were employed in this study. The animal were kept fasting for overnight providing only water. Then the extracts (Leaf) were administered orally at the dose of 800 mg/kg by intragastric tube and observed for 2 days for the gross behavioral changes and mortality [12].

Methodology

Methods

1. Dexamethasone – induced diabetes mellitus [16]

Thirty Male Wistar rats weighing 200-250gm were randomly divided into 4 groups of 5 each and kept in their cages for 10 days prior dosing to allow for acclimatization to the laboratory conditions.

Group 1 served as normal control; group 2, 3, 4, received Dexamethasone 10mg/kg/day subcutaneously for 10 days; on day 11, after overnight fasting, retro-orbital puncture was performed to obtain blood sample for estimation of lasting and postprandial blood sugar. Only those rats whose fasting and postprandial blood glucose levels were higher than those of the normal controls were utilized for further study. From day 11 to day 20, group 2, 3, 4, continued to receive dexamethasone 10mg/kg/day subcutaneously. Group 3 received 100mg/kg/day of *Syzigium cumini* pant leaf powder in 1ml of distilled water per oral, in addition to dexamethasone in addition to dexamethasone. Group 4 received Glibenclamide 500µg/kg per oral, in addition to dexamethasone.

On the 20th day, after overnight fasting retro orbital puncture was done on the left eye to obtain blood for estimation of fasting blood glucose using autoanalyser. Immediately after this, a glucose load for estimation of postprandial blood glucose levels

Group1: Administered vehicle serves as Normal control.
Group2: Administered Dexamethasone (10mg/kg s.c.) Serves as diabetic control
Group3: Diabetic rats treated with Dexamethasone (10mg/kg, s.c. once daily)
[Dexamethasone (10mg/kg, s.c.) + *Syzigium cumini* 100mg/kg p.o.]
Group4: Administered Reference standard, Glibenclamide (500µg/kg, p.o. once daily) [Dexamethasone (10mg/kg, s.c.) + Glibenclamide (500µg/kg, p.o)]
On day 21, rats were sacrificed and serum was analyzed for serum triglycerides, serum cholesterol, serum HDL-cholesterol, serum LDL-cholesterol, serum VLDL-cholesterol, and serum glucose.

Statistical analysis

The results were represented as Mean ± SD. The statistical significance was computed using One Way ANOVA followed by Tukeys multiple comparison test and compared with diabetic control group with Standard drug, fig ;1.2 where the n=6 animals in each group were used.** P <0.01was considered statistically significant.

Result:

Table 1: Effect of administration of *Syzygium cumini* leaf extract (100 mg/kg, p.o) + Glibenclamide (500µg/kg, p.o) for 21 days on Blood Glucose levels in diabetic rats

GROUP	TREATMENT	GLUCOSE LEVEL AT 11th DAY	GLUCOSE LEVEL AT 16th DAY	GLUCOSE LEVEL AT 21st DAY
Group – 1	Control (Normal control)	106 ±1.61	99.86 ± 1.56	95.58 ± 1.32
Group – 2	Diabetic control	287.53 ±5.51*	274.93± 9.31**	270.6 ± 9.16**
Group – 3	*Syzigium cumini* leaf extract (100 mg/kg , p.o)	294.13 ±15.41*	199.73±36.50**	150.06±4.48**
Group - 4	Glibenclamide (500 µg/kg , p.o) (reference standard)	285.43 ±5.48*	189.65±34.65**	145.58±5.49**

Value expressed in MEAN ±SEM ,n=6 Experimental groups statically compared with control groups where significant *p<0.05, moderately significant **p<0.01 All the values are compared with the Dexamethasone control group.

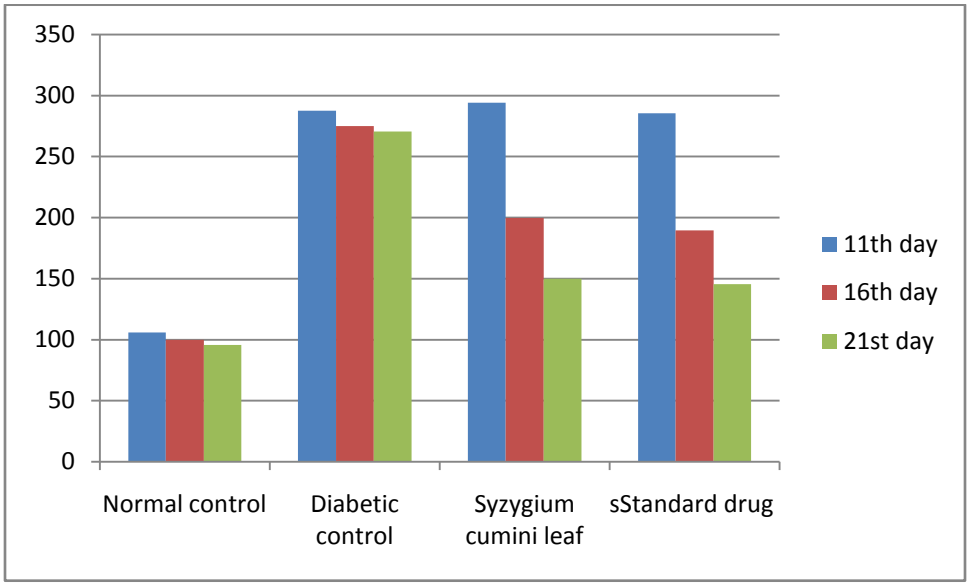

Fig -1: : Effect of administration of *Syzigium cumini* leaf extract (100 mg/kg, p.o) Glibenclamide (500 µg/kg, p.o), for 21 days on serum blood glucose levels in diabetic rats

Table 2: Effect of administration of *Syzigium cumini* leaf extract (100 mg/kg, p.o) p.o) + Glibenclamide (500 µg/kg, p.o), for 21 days on serum Total cholesterol, Triglycerides, HDL, LDL VLDL levels in diabetic rats

Groups	SERUM CHOLESTEROL(mg/dl)	SERUM TRIGLYCERIDES(mg/dl)	SERUM HDL(mg/dl)	SERUM LDL(mg/dl)	SERUM VLDL(mg/dl)
Normal control	90.76±2.45	60.78±0.009	49.90±0.021	20.89±0.025	17.35±0.042
Diabetic control	146.1±6.41**	156.45±0.171**	29.32±0.020**	84.23±0.026**	37.64±0.017**
Syzigium cumini leaf(100mg/kg)	100.05±3.57**	67.81±0.030**	54.90±0.013**	34.64±0.036**	22.58±0.014**
Glibenclamide (500µg/kg)	94.9±3.88**	62.91±0.018**	51.28±0.014**	30.66±0.035**	19.11±0.019**

Value expressed in MEAN ±SEM ,n=6 Experimental groups statically compared with control Groups Where significant *p<0.05, moderately significant **p<0.01 All the values are compared with the Dexamethasone control group.

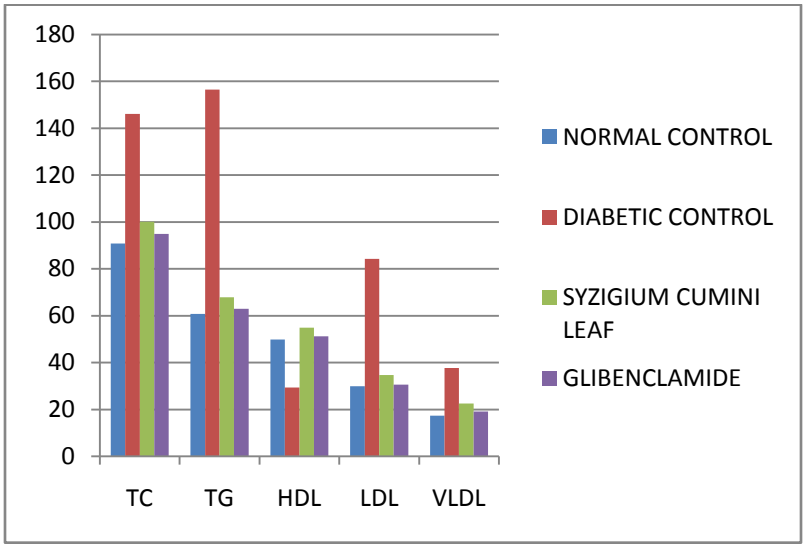

Fig -2: Effect of administration of *Syzigium cumini* leaf extract (100 mg/kg, p.o) Glibenclamide (5 µg/kg, p.o), for 21 days on serum Total cholesterol, Triglycerides, HDL, LDL VLDL levels in diabetic rats

DISCUSSION

In Dexamethasone control group there was significant increase in blood glucose levels (p<0.05) when compared to the vehicle control. Ethanol extract of syzygium cumini leaf (100mg/kg p.o) increased blood glucose level as compared with normal control. Glibenclamide (500µg/lgp.o) increased blood glucose level. On 16th day collecting with blood sample retro orbital puncture in estimation of blood glucose and compare with normal control diabetic control increase in blood glucose level .plant extract of syzygium cumini leaf significantly reducing blood glucose level (**p<0.01).glibenclamide (5µg/kg p.o)show as significant action in reducing the blood glucose level (**P<0.01.)21st day collecting blood sample in retro orbital puncture and then centrifugation and collecting the plasma to estimation of blood glucose level. Plant extract of syzygium cumini leaf(100mg/kg p.o_ show as significant reducing the blood glucose level and compare in dexamethasone control **P<0.01)Glibenclamide (5µg/kg p.o) show as significant reducing blood glucose and compare to dexamethasone control group**P<0.01).The values of blood glucose levels are shown in the Table No.1

Serum total cholesterol(mg/dl)level increase (146.1±6.41mg/dl)) in the untreated dexamethasone induced rats compares to the normal control rats(90.76±2.45 mg/dl) after treatment with the 100mg/kg p.o dose of syzygium cumini leaf Shown a significant increase in the total cholesterol level compared with the normal control animals .Glibenclamide(5µg/kg)a significant activity in increase the total cholesterol level. The values of serum total cholesterol levels are shown in the Table No 2

Serum Triglycerides (mg/dl) level increase (156.45±0.017mg/dl)) in the untreated dexamethasone induced rats compares to the normal control rats(60.78±0.009 mg/dl) after treatment with the (100mg/kg p.o) dose of syzygium cumini leaf Shown a significant increase in the Triglycerides'level compared with the normal control animals .Glibenclamide(5µg/kg p.o)a significant activity in increase the Triglycerides level. Serum HDL level decrease (29.32±0.02 mg/dl) diabetic control and compare to normal control rats (49.90±0.02 mg/dl) after treatment with the (100mg/kg p.o) dose of syzygium cumini leaf Shown a significant increase in the serum HDL level compared with the normal control animals .Glibenclamide(5µg/kg p.o)a significant activity in increase the Serum HDL level. The values of serum HDL levels are shown in the Table No 2.
Serum LDL (mg/dl)level increase 84.23±0.026mg/dl)) in the untreated dexamethasone induced rats compares to the normal control rats(29.89±0.025 mg/dl) after treatment with the(100mg/kg p.o) dose of syzygium cumini leaf shown a significant increase in the LDL level compared with the normal control animals .Glibenclamide(5µg/kg p.o)a significant activity in increase the LDL level. Serum VLDL (mg/dl)level increase (37.64±0.017mg/dl)) in the untreated dexamethasone induced rats compares to the normal control rats(17.35±0.042 mg/dl) after treatment with the(100mg/kg p.o) dose of syzygium cumin leaf shown a significant increase in the Serum VLDL level compared with the normal control animals .Glibenclamide(5µg/kg p.o)a significant activity in increase the VLDL level. Insulin resistance in humans has been shown to be present in conditions like NIDDM, obesity and dyslipidemia. Thus interventions to decrease insulin resistance may postpone the development of NIDDM and its complications. Treatment with natural herbs is likely to be with lesser side effects compared to the presently used synthetic oral antidiabetic agents [22-24].

Dexamethasone increases triglyceride levels, causing an imbalance in lipid metabolism leading to hyperlipidemia [25] and an increase in glucose levels leading to hyperglycemia [26]. The pharmacological doses of Glucocorticoid induce gene expression in rats adipocyte tissue with in 24h. This is followed by complex metabolic changes resulting in decrease in food consumption reduction in body weight, profound obesity often accompanied by diabetes and development of insulin resistance with enhanced blood glucose and triglyceride levels. Dexamethasone induced insulin resistance model study for 21 days was carried out in Wistar rats. Ethanolic extract of syzygium cumini leaf (100 mg/kg, p.o.) showed maximum decrease in blood glucose levels, triglyceride levels and totalcholesterol, Serum HDL, LDL, and VLDL at 21ST day, when compared with the dexamethasone control.

Conclusion

These observations concluded that the grains extract of the *plant Syzygium cumini leaf* possesses insulin resistance activity. The *Syzygium cumini*showed significant and dose dependent decrease in blood glucose levels, triglyceride levels and Total cholesterol, HDL, LDL and VLDL.

References

1. Amatruda JM, Livingston JN, Lockwood DH. Cellular mechanisms in selected states of insulin resistance: Human obesity, glucocorticoid excess, and chronic renal failure Diabetes Metab Rev 1985; 1:293-317.

2. DeFronzo RA, Tripathy D. Skeletal Muscle Insulin Resistance Is the Primary Defect in Type 2 Diabetes. Diabetes Care. 2009;32(2):S157-S163.

3. Lenzen S, Bailey CJ. Thyroid hormones, gonadal and adrenocortical steroids and the function of the islets of Langerhans. Endocr Rev. 1984 Summer;5(3):411–434.

4. Block NE, Buse MG. Effects of hypercortisolemia and diabetes on skeletal muscle insulin receptor function in vitro and in vivo. Am J Physiol. 1989;256: E39-E48.

5. Hosein D, Ahmad A, Khan MU, Dhingra S. Canagliflozin: A First-in-Class Medication for the Treatment of Type 2 Diabetes Mellitus. International Journal of Toxicological and Pharmacological Research 2015; 7(2); 105-107.

6. Van Tilburg J, van Haeften TW, Pearson P, Wijimenga C. Defining the genetic contribution of type 2 diabetes mellitus. J Med Genet 2001; 38:569-578.

7. Aratanechemuge Y., Hibasami H., Sanpin K., Katsuzaki H.,Imail K., Komiya T. Induction of apoptosis by lupeol isolated frommokumen (Gossampinus malabarica L. Merr) in human promyelotic leukemia HL-60 cells. J. Oncol, Rep. 2004; 11: 289-292.

8. Ayyanar M., Subash-Babu P. Syzygium cumini (L.) Skeels: Areview of its phytochemical constituents and traditional uses. Asian Pacific Journal of Tropical Biomedicine. 2012;(2) : 240-246.

9. Gowri SS. Vasantha K. Phytochemical Screening and Antibacterial Activity of *Syzygium cumini* (L.) (Myrtaceae) Leaves Extracts. International Journal of PharmTech Research. 2010; 2:1569-1573.

10. Chaudhary B., Mukhopadhyay K. Syzygium cumini (L.) skeels: a potential source of nutraceuticals. Intrnational J. of Pharm. and Bio. Sci. 2012; 2(1): 46-53

11. Martinez SB. Del Valle MJ. Storage stability and sensory quality of duhat*Syzygium cumini* Linn. Anthocyanins as food colorant. UP Home Economic Journal. 1981; 9(1).

12. Kuncha J., Ayyachamy M., Mariappan M. In-vitro evaluation of nitric oxide scavenging activity of methanol and aqueous extract of syzygium cumini Linn. Bark (Myrtaceae). Int. J Pharm Sci Res. 2012; 3(2): 615-619.

13. Wealth of India. Ed. 10, CSIR, New Delhi. 1982; 100-104. Wealth of India. Raw materials, New Delhi: CSIR. 1976; 10:100–104.

14. Williamsen EM. Major Herbs in Ayurveda. Churchill Livingstone. 2002; 279-282.

15. Harborne JB. Phytochemical methods, A Guide to Modern Techniques of plant analysis, Chapman and Hall, London, Ltd, 1973,49-188.

16. Shetty AJ, Choudhury D, Rejeesh, Nair V, Kuruvilla M, Kotian S. Effect of the insulin plant (Costusigneus) leaves on dexamethasone-induced hyperglycemia. Int J Ayurveda Res. 2010;1(2):100-102.

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