

## Research in Pharmacy and Health Sciences

Research Article

### Evaluation of anti Diabetic Activity for ethanolic extract *Syzygium cumini* leaf in Alloxan induced diabetic rats

Maheswararao Chattu\*, Guptha Attyam

Department of pharmacology, Yalamarty Pharmacy College, Yalamarty Nagar, Tarluvada, Visakhapatnam-530052, India

#### ABSTRACT

The available drugs for treatment of diabetes, such as Insulin or Oral hypoglycemic agents have one or more side effects. So the search for newer anti-diabetic drugs with minimum or no side effects from herbal plants is a challenge as per world health organization (WHO) recommendations. Diabetes mellitus is a metabolic disorder of carbohydrate, protein and fat which are characterized by hyperglycemia, polyuria, polydipsia, and polyphagia which is deficient insulin production or ineffectiveness in insulin actions. The present study was aimed to evaluate the anti diabetic potency of syzygium cumini leaf on the blood glucose level in alloxan induced diabetic rats. Diabetic Wistar strain rats were treated with standard drug Glibenclamide and test drug syzygium cumini at 100mg, 200mg. The hypoglycemic effect was determined in the rats and the efficacy of the test drug was compared to the standard drug Glibenclamide. Syzygium cumini leaf was orally administered for 14 days in alloxan induced diabetic rats. At the end of the study duration blood glucose level and Biochemical parameters were statistically analyzed. Based on these results of the study this syzygium cumini produced a significant reduction in blood glucose levels when compared with diabetic control rats and hence the present research work proved that the syzygium cumini possess hypoglycemic effect.

**Received:** 18-08- 2015

**Revised:** 01-10-2015

**Accepted:** 29-10-2015

**\*Correspondence to:**

Mr. Maheshwararao Chattu,  
Email:

maheshbabupharmacy@gmail.com

**Funding:** The authors have no support or funding to report.

**Competing Interests:** The authors have declared that no competing interests exist.

**Keywords:** diabetes mellitus, alloxan, glibenclamide, syzygium cumini.

#### INTRODUCTION

The medicinal properties of several herbal plants have been documented in ancient Indian literature and the preparations have been found to be effective in the treatment of diseases. Therefore to meet the increasing demand of manufacturing modern medicines and export, the need of the medicinal plants have enormously increased. This demand is generally met with by cultivating uprooted medicinal plants [1].

During the last ten years pace of development of new antidiabetic drug has slowed down while the prevalence of resistance has increased astronomically [2]. Therefore, actions must be taken to reduce this problem, such as controlling the use of antibiotics and carrying out research for the better understanding of the genetic mechanism of resistance. This prompted us to evaluate plants as the source of potential chemotherapeutic agent, antimicrobial agent and their ethno medicinal use [3].

*Syzygium cumini* is belonging to the family Myrtaceae. Large trees cultivated throughout India for the edible fruits (Black Plum) and are reported to contain vitamin C, gallic acid, tannins, anthocyanins, includes cyanidin, petunidin, malvidinglucoside and other components [4]. Gowri *et al.*, conducted a study and showed that the juice of unripe fruits is considered to be a stomachic, carminative and diuretic. The ripe fruits are used for making preserves, squashes and

jellies [4]. The fruits showed astringent property and also used to prepare wine [4]. Leaves have been used in traditional medicine as a remedy for diabetes mellitus in many countries. The leaves are also used to strengthen the teeth and gums, to treat leucorrhoea, stomachalgia, fever, gastropathy, strangury, and dermopathy, constipation and to inhibit blood discharges in the faeces [5].

Diabetic mellitus (DM) is the condition arising due to abnormal metabolism of carbohydrate, proteins and fats. It is caused by insulin deficiency, often combined with insulin resistance. This disorder occurs worldwide and its occurrence is increasing quickly in most of the countries. Various complications develop as a consequence of the metabolic derangement in diabetes. The treatment of DM is based on parenteral insulin and oral anti-diabetic drug [6]. Oral hypoglycemic agents, currently used have serious side effect hence there is a need to search a newer anti-diabetic agents that having high therapeutic efficacy with minimum side effect [7]. This may be fulfilled by treating DM with traditional medicine using as anti-diabetic agents from medicinal plants. Diabetes mellitus (DM) consists of a group of syndromes characterized by hyperglycemia; altered metabolism of lipids, carbohydrates, and proteins; and an increased risk of complications from vascular disease. The worldwide prevalence of DM has raised dramatically over the past two decades, from an estimated

30 million cases in 1985 to 177 million in 2000. Based on the current trends > 360 million individuals will have diabetes by the year 2030 [8].

## MATERIAL AND METHODS

### Drugs and chemicals

Alloxan was purchased from Sigma Aldrich chemicals Pvt. Ltd., USA. Glibenclamide was obtained as a gift sample from Sanofi Aventis India Ltd. All other chemicals and reagents used were of analytical grade.

### Collection of plant material

Fresh plant leaves of *Syzygium cumini* was collected from botanical department Sri Venkateswara University, Tirupat, Andhra Pradesh.

### Preparation of extract

The collected leaves of *syzygium cumini* were washed generally and then kept for drying in number of days. The plant materials were then oven dried for 24 hours at low temperature. Powdered material of *syzygium cumini* leaves was macerated with ethanolic in round bottom flask. The containers were sealed with cotton plug and aluminum foil at room temperature for 12 days with occasional shaking. The mixture was filtered through cotton and then evaporated to dryness (450C) under reduced pressure by rotary evaporator [9]. The percentage yield of the extract was calculated by using the formula below:

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

### Phytochemical Tests

The leaf extracts of *syzygium cumini* were analysed for the presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins, terpenoid, cardiac glycosides and tannins according to standard methods [10].

### Experimental design

The antidiabetic activity of ethanolic extract of *syzygium cumini* leaf was assessed in normal, glucose loaded and Alloxan 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 gm. 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. The animals were

fasted 12 hours before the conduct of experiment and during the experiment they were withdrawn from food and water [11]. 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].

## METHODLOGY

### Methods

#### 1. Alloxan induced diabetes mellitus

Total 30 Male wistar rats weighing 200-250gm were used for the study. Male wistar rats (n=6) selected by random sampling were used for acute toxicity. All the animals were purchased from Hyderabad. After procuring, the animals were acclimatized for 10 days at normal laboratory condition. All animals were allowed free access to tap water and pellet diet and maintained at 18°C temperature in polyethylene cages.

Diabetes was induced in 48h fasted Male wistar rats (200-250gm) by intraperitoneal injection of 150 mg/kg body weight of Alloxan [13].

Alloxan was first weighed individually for each animal according to the body weight and then solubilized with 0.2ml saline (154mM NaCl) just prior to injection. Two days after Alloxan injection, rats with plasma glucose level greater than 140mg/dl were included in the study. Treatment with plant extract was started 48h after Alloxan injection.

The rats were divided into five groups consisting six rats each

Group 1: Administered 1%normal saline serves as Normal control.

Group 2: Administered Alloxan monohydrate (150mg/kg i.p.) serves as diabetic control

Group 3: Diabetic rats treated with *Syzygium cumini* (100mg/kg, per oral once daily)

Group 4: Diabetic rats treated with *Syzygium cumini* (200mg/kg, per oral once daily)

Group 5: Diabetic rats treated with Glibenclamide (5mg/kg, per oral once daily)

### Induction of Diabetes in Experimental Animals

Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg) [14]. Alloxan was first weighed individually for each animal according to the body weight and then solubilized with 0.2 ml saline (154 mm NaCl) just prior to injection. Two days after alloxan injection, rats with plasma glucose levels of less greter than 140 mg/dl were included in the study. Treatment with plant extracts was started 48 h after alloxan injection.

### Collection of Blood Sample and Blood Glucose Determination

The blood was drawn from the retro orbital plexus of the rats (fasted for 14 h) under light ether anaesthesia on different occasions i.e., 1, 7, 14 day respectively. The blood samples were allowed to clot for 30 min at room temperature and then they were centrifuged at 5000 rpm for 20 min. The resulting upper serum layer was collected in properly labelled, clean and dry micro-centrifuge tubes. The blood samples were stored at 2-8 °C and analyzed

within one week. This serum specimen was used for the estimation of different biochemical parameters. Fasting blood glucose estimation and body weight measurement were done on day 1, 7, and 14 of the study. Blood glucose estimation can be done by semi bio chemistry analyzer model CL-380

On day 14, blood was collected from retro-orbital plexus from overnight fasted rats and fasting blood sugar was estimated. Serum separated from ultra centrifuge or cold centrifuge 8000Rpm for 15 min and separated for plasma testing for lipids profile parameter estimated .serum Glucose, serum HDL, serum LDL, serum VLDL, serum triglycerides, serum cholesterol

### STATISTICAL ANALYSIS

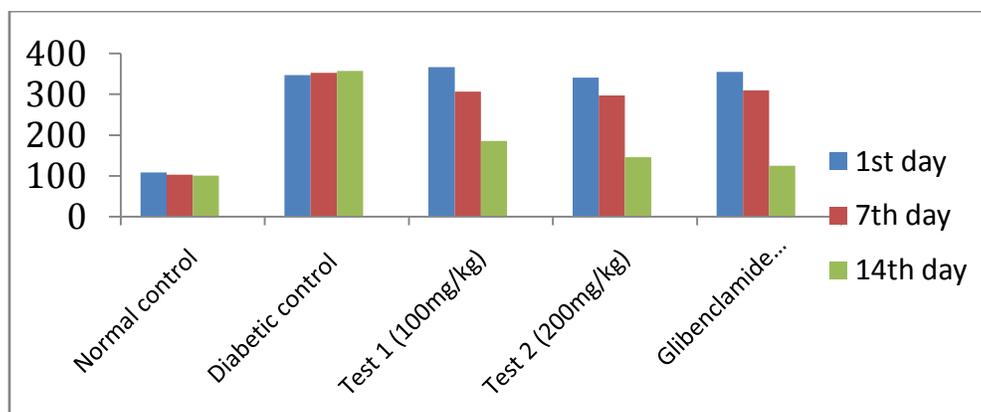
The results were represented as Mean ± SD. The statistical significance was computed using One Way ANOVA followed by tukeys multiple comparison test and compared with diabetic control group with Standard drug, FD39 where the n=6 animals in each group were used. P <0.001 was considered statistically significant.

## RESULTS AND DISCUSSION

**Table 1: Effect of administration of *Syzygium cumini* leaf extract) for 14 days on serum GLUCOSE levels in diabetic rats**

Group	Treatment	Glucose level at 1 <sup>st</sup> Day	Glucose level at 7 <sup>th</sup> Day	Glucose level at 14 <sup>th</sup> day
Group 1	Control (normal control)	108.33± 1.08	103.65 ± 0.14	100.56 ±2.34
Group 2	Diabetic control	347.1±10.59	352.6±0.14**	350.2±0.13**
Group 3	<i>Syzygium cumini</i> leaf extract (100 mg/kg, p.o)	366.25 ±3.81	206.86± 0.14**	185.65±12.7**
Group 4	<i>Syzygium cumini</i> leaf extract (200 mg/kg, per oral)	341.05 ± 7.23	199.8 ± 0.10**	145.86±6.34**
Group 5	Glibenclamide (5 mg/kg, per oral)	354.43 ± 5.18	189.7±0.15**	124.7 ± 2.46**

Values are mean±SD, n=6 in each group. \*\*\*P <0.01 (respectively as compared to normal control) ; a3P<0.01( as compared to diabetic control). One way ANNOVA followed by Tukeys multiple comparison test.

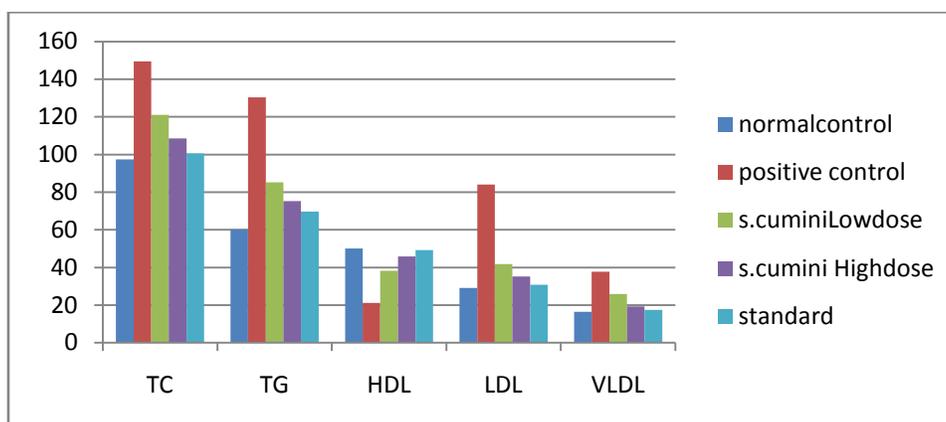


**Figure 1: Effect of pretreatment with *syzygium cumini* 100 mg/200mg per oral once daily for 14 days on serum glucose in insulin resistance rats:**

**Table 2: Effect of administration of *Syzygium cumini* leaf extract (100 mg/kg, p.o) + *Syzygium cumini* leaf extract (200 mg/kg, p.o) + Glibenclamide (5 mg/kg, p.o), for 14 days on serum Total cholesterol, Triglycerides, HDL,LDL VLDL levels in diabetic rats**

Group	Treatment	S. cholesterol (mg/dl)	S.Triglyceride (mg/dl)	S. HDL (mg/dl)	S. LDL (mg/dl)	S. VLDL (mg/dl)
Group 1	Control (normal control)	97.35± 0.54	60.35± 0.19	50.06±0.16	29.08±0.14	16.48±0.13
Group 2	Diabetic control	149.5 ±0.31**	130.33 ± 0.60**	21.1 ±0.15**	84.13±0.13**	37.76±0.12**
Group 3	<i>Syzygium cumini</i> leaf extract (100 mg/kg, p.o)	121.08 ±0.25**	85.26 ± 0.18**	38.3±0.17**	41.71±0.14**	25.9±0.11**
Group 4	<i>Syzygium cumini</i> leaf extract (200 mg/kg, per oral)	108.5 ±0.38**	75.26 ± 0.16**	45.83±0.15**	35.2±0.18**	19.3±0.17**
Group 5	Glibenclamide (5 mg/kg, per oral)	100.6 ± 0.46**	69.71 ± 0.13**	49.3 ±0.20*	30.76±0.18**	17.35±0.14**

Values are mean±SD, n=6 in each group. \*\*\*P <0.01 (respectively as compared to normal control) ; a3P<0.01( as compared to diabetic control). One way ANNOVA followed by Tukeys multiple comparison test.



**Figure 2: Effect of administration of *Syzygium cumini* leaf extract (100 mg/kg, p.o) + *Syzygium cumini* leaf extract (200 mg/kg, p.o) + Glibenclamide (5 mg/kg, p.o), for 14 days on serum Total cholesterol, Triglycerides, HDL, LDL VLDL levels in diabetic rats**

The commonly used chemical agent in laboratories for inducing diabetes in animal is alloxan which is an oxidized product of uric acid that causes destruction of beta cells of the pancreas by oxidation mechanism and produce Type 1 diabetes. The present study screened the anti diabetic activity of the *syzygium cumini* against alloxan induced diabetic rats. The continuous treatment of the *syzygium cumini* was done for a period of 14 days at 100mg/kg, 200mg of body weight. glibenclamide was the standard drug used to stimulate insulin from beta cells of islets of langerhans many years in research. So, glibenclamide (5mg/Kg) was selected as standard drug in the study. The results of the blood glucose level of the normal control group, diabetic control group, standard group (Glibenclamide 5mg/kg) and trial *syzygium cumini* were summarized in Table No 1 and Table No 2 respectively. Data are statistically obtained by using one way ANNOVA followed by Turkeys' multiple comparison test. In Table No.1, the blood glucose level of the normal control is near about same after 14 days. However in diabetic control

group was increased from 347.1± 10.59to 350.2±0.13 after 14 days. The blood glucose of standard control group is near about same after 14 days of treatment. The initial blood glucose of diabetic *syzygium cumini* test group is 185.65±12.7 and high dose 145.86±6.34 after 14 days of treatment the blood glucose was about near to 124.7±2.46, there was decreased in blood glucose found when compared with diabetic control group. The dose of the test *syzygium cumini* on biochemical parameters total cholesterol, triglycerides, HDL, LDL, VLDL level was studied in the animals. The test group showed a significant decrease in lipids profile parameters level on alloxan induced diabetic rats when compared to diabetic control group. After the 14 days period *syzygium cumini* produced significant reduction in the Biochemical parameters levels total cholesterol decreased in compared with positive control decreased in standard, triglycerides –decreased in plant extracts and compared with a disease control, HDL-treatment groups decreased in disease control and compared with a treatment groups LDL-decreased in

treatment groups and compared with a positive group VLDL –Decreased in treatment groups and compared with disease control. Which showed that the standard drug had produced maximum anti-diabetic effect? The diabetic control group showed rise in blood glucose level throughout the study period. The results of blood glucose level in rats were summarized in Table No.1. And on the basis of the results and biochemical parameters levels throughout the study period tab-2 it was observed that there was an significant reduction in blood glucose level and biochemical parameters by syzygium cumini leaf in Alloxan induced diabetic rats. The anti-diabetic activity of this syzygium cumini could be due to the increased release of insulin from beta cells of the pancreas or may be due to potentiating effect of insulin. Treatment of ethanolic leaf extract of syzygium cumini in diabetic rat also showed the significant blood glucose level and biochemical parameters property which proved its efficacy of this syzygium cumini in treating diabetic patients successfully.

## CONCLUSION

Ethanolic extract of syzygium cumini leaf extract and it is found to be more effective in the treatment of diabetes mellitus as determined by its statistically significant p-value < 0.01 in Alloxan induced diabetic rats. The mechanism of anti-diabetic activity of this ethanolic leaf extract of syzygium cumini leaf may be due to enhancing the effect of insulin and by stimulating the insulin secretion from beta cells of pancreas. Hence this study suggests that this Syzygium cumini leaf has a potent anti diabetic effect which could be used for the management of diabetes effectively.

## REFERENCES

- Aratanechemuge Y, Hibasami H, Sanpin K, Katsuzaki H, Imai K, Komiya T. Induction of apoptosis by lupeol isolated from mokumen (*Gossampinus malabarica* L. Merr) in human promyelotic leukemia HL-60 cells. *J Oncol Rep.* 2004;11:289-292.
- Ayyanar M, Babu SP. *Syzygium cumini* (L.) Skeels: A review of its phytochemical constituents and traditional uses. *Asia Paci J Tropi Biomed.* 2012;(2):240-246
- Batta AK, Xuab G, Honda A, Miyazaki T, Salen G. Stigmasterol reduces plasma cholesterol levels and inhibits hepatic synthesis and intestinal absorption in the rat. *J Pharm Sci.* 2006; 55(3): 292-299.
- Gowri SS, Vasantha K, Phytochemical Screening and anti bacterial activity of *Syzygium cumini* (L.) (Myrtaceae) Leaves extracts. *Int J PharmTech Res.* 2010;2(2):1569.
- Bhuyan MA, Mia MY, Rashid MA. Antibacterial principles of the seed of *Eugenia jambolana*. *Bangladesh J Botany.* 1996; 25: 239–241.
- Bopp A, Bona KS, Bellé LP, Moresco RN, Moretto MB. *Syzygium cumini* inhibits adenosine deaminase activity and reduces glucose levels in hyperglycemic patients. *Fundamental Clin Pharmacol.* 2009; 23: 501–507.
- Van de Laar FA, Lucassen PL, Akkermans RP, Van de Lisdonk EH, Rutten GE, Van Weel C. Alpha-glucosidase inhibitors for type 2 diabetes mellitus, *Cochrane Database Syst Rev.* 2005;18;(2):CD003639.
- Rhee EJ. Diabetes in Asians. *Endocrinol Metab (Seoul).* 2015;30(3):263-9.
- Chaudhwei AKN, Pal S, Gomes A, Bhattacharya S. Antiinflammatory and related actions of *Syzygium cumini* seed extract. *Phytotherapy Research.* 1990;4:5-10.
- Chiu HL, Wu JH, Tung YT, Lee TH, Chien SC, Kuo YH. Triterpenoids and Aromatics from *Derris laxiflora*. *J Nat Prod.* 2008;71(11):1829-1832.
- Abaira C, Colwell JA, Nuttall FQ, Sawin CT, Nagel NJ. Veterans Affairs Cooperative Study on glycemic control and complications in type II diabetes (VA CSDM). *Diabetes care.*1995; 18(8):1113-1123.
- Trease GE, Evans WC, A text book of pharmacognosy Academic press, London,198; 22-40.
- Litchfield JT, Wilcoxon FA, Simplified method of evaluating dose effect experiments. *J Pharmacol Exp Ther.* 1949;96:99-133.
- Sunarwidhi AL, Sudarsono S, Nugroho AE. Hypoglycemic Effect of Combination of *Azadirachta indica* A. Juss. and *Gynura procumbens* (Lour.) Merr. Ethanolic Extracts Standardized by Rutin and Quercetin in Alloxan-induced Hyperglycemic Rats. *Adv Pharm Bull.* 2014;4(2):613-8.

**Cite this article as:** Chattu M, Attyam G Evaluation of anti Diabetic Activity for ethanolic extract *Syzygium cumini* leaf in Alloxan induced diabetic rats. *Res Pharm Health Sci.* 2015;1(1):27-31.