EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 02
209-213
Year: 2017

AN EXPERIMENTAL STUDY TO EVALUATE THE ANTIDIABETIC EFFECT OF *SYZYGIUM CUMINI* LINN. SEED EXTRACT IN ALBINO RATS

Dr. Dick B. S. Brashier[1]*, Dr. Sandeep Vihan[-2] and Dr. Htet Wai Moe[3]

[1]Professor, Department of Pharmacology, Armed Forces Medical College, Pune-411040, India.
[2]MD (Pharmacology), Military Hospital, Mizoram-796310, India.
[3]Resident, Department of Pharmacology, Armed Forces Medical College, Pune-411040, India.

***Corresponding Author: Dr. Dick B. S. Brashier**
Professor, Department of Pharmacology, Armed Forces Medical College, Pune-411040, India.

Article Received on 28/11/2016 **Article Revised on 18/12/2016** **Article Accepted on 08/01/2017**

ABSTRACT
Diabetes mellitus is a chronic disorder causing considerable morbidity and mortality worldwide. Various herbs have been found to be useful in treating diabetes. *Syzygium cumini* Linn. Or Jamun tree is one such plant. Its extract has been identified as having antidiabetic properties. Present study was conducted with the aim of evaluating antidiabetic effects of aqueous extract of seed of *Syzygium cumini* Linn. in lab animals. This study was done employing normal euglycemic rats and alloxan induced diabetes in rats. *Syzygium cumini* Linn. Extract did not have any effect on the blood glucose levels of euglycemic rats either on acute or chronic administration. *Syzygium cumini* Linn Extract did not have any effect on ALT, AST, Serum bilirubin & creatinine levels in euglycemic rats. *Syzygium cumini* Linn extract, when given over 4 weeks, to alloxan induced diabetic rats, led to a dose dependent and time dependent reduction in blood glucose levels. *Syzygium cumini* Linn. extract did not have any effect on ALT, AST, Serum bilirubin & creatinine levels in alloxan induced diabetic rats. *Syzygium cumini* Linn. Extract has no effect on the blood glucose levels of euglycemic animals. *Syzygium cumini* Linn. Extract can reduce blood glucose levels in alloxan induced diabetic rats, in a dose dependent and time dependent manner. This study clearly demonstrates the efficacy of *Syzygium cumini* Linn. Extract in animal models of diabetes mellitus.

KEYWORDS: *Syzygium cumini*, Aqueous extract, Antidiabetic activity, Albino rats.

INTRODUCTION
Diabetes mellitus is a major public health problem in the developed as well as developing countries.[1] It can be classified into four categories, Type 1 (Insulin dependent Diabetes mellitus), Type 2, (Non-Insulin dependent Diabetes mellitus), type 3 (others- Non pancreatic disease, drug-induced) and type 4 (gestational diabetes).[2] Among 4 types of DM, type 2 DM accounts for 90% of all DM cases.[3] Type 2 DM is characterized by relative deficiency of insulin. The major cause of type 2 DM is insulin resistance (IR) or lack of responsiveness in the target tissue towards insulin. Insulin resistance is present in most of the patients of type 2 DM.[4,5] Despite a wide array of treatment options, therapy of DM still leaves a lot to desire**.** Available treatment options do not mimic physiological behavior of β cells.[6] IR can only partially be reversed and insulin supplementation is parenteral and painful. Because of these limitations search for new treatment options continue with ideal treatment still remaining elusive.[7]

Syzygium cumini Linn. (Syn. *Eugenia jambolan* Linn.) or Jamun tree is a widely found and cultivated tree in India which is known to have a lot of medicinal properties including antidiabetic, anti-hypertensive, carminative, astringent, anti-diarrhoea & diuretic effects.[8] This plant occupies a special place in traditional medicine and is a prominent component of many Indian household concoctions.[9,10] Therefore, this study was undertaken to evaluate antidiabetic effects of *Syzygium cumini* Linn.in euglycemic, and alloxan induced diabetes.

MATERIALS AND METHODS
Animal collection
Healthy young adult (approximately 04 weeks old) wistar albino rats of either sex, weighing between 150-250 g, were used as experimental animals in this study. They were housed in clean cages and were maintained on standard laboratory diet and water *ad-libitum*. After a five-day acclimatization period, the rats were used for the study. Institutional animal ethics committee (IAEC) approved the experimental protocol and the animals were taken care as per guidelines of CPCSEA department of animal welfare, Government of India.

Preparation of *Syzygium cumini* Linn. Extract
Seeds of *Syzygium cumini* were dried and crushed to a coarse powder form with the help of a grinder. Aqueous

extract was prepared with the help of a Soxhalet apparatus. This extract was dried by putting it in flat petri dishes till it was reduced to a crust at the bottom of the petri dish. This brownish- black crust was subsequently weighed and used for the study.

Antidiabetic activity

Phase I

Phase I of the study was conducted to evaluate the acute and chronic effects of extract on blood glucose levels in euglycemic animals. Total 36 animals were divided into 3 sets (A, B and C) of 12 animals each. Each set was further sub-divided into test and control groups having 6 animals each. Blood glucose level was evaluated at 1 and 4 hours in set A, 2 and 6 hours in set B and 16 and 24 hours in set C after *Syzygium* extract administration. All of these three sets of animals were given three doses of *Syzygium* extract i.e. 200mg/kg, 400mg/kg and 800 mg/kg. A gap of 30 days was maintained between these three doses. To see the chronic effects, 18 euglycemic rats were divided into 3 groups of 6 animals each. They were then treated for next 4 weeks as follows: Group I, II and II were given *Syzygium* extract in the dose of 200 mg/kg, 400 mg/kg, and 800 mg/kg respectively. Blood glucose levels were checked every week. Biochemical parameters such as AST, ALT, serum bilirubin and creatinine were checked every alternate week.

Phase II

In this phase of the study, 42 euglycemic rats (blood glucose between 70 – 110 mg/kg) were fasted for 18 hours and at the end of this period were given 150 mg/kg i.p. alloxan monohydrate (day 0). After 10 days, total 30 animals, which had blood glucose levels more than 180 mg/ were selected and divided into 5 groups of 6 animals each. Group I was given normal saline orally. Group II, III and IV were given p.o. 200 mg/kg, 400 mg/kg, and 800 mg/kg respectively of *Syzygium* extract throughout the study. Group V was given p.o. 10 mg/kg glibenclamide suspension throughout the study. Treatment with drugs was continued for 4 weeks. All the drugs were given orally using an infant feeding tube, as a single dose in the morning. Blood glucose was measured before starting the treatment (day 10) and weekly thereafter up to the end of the treatment period (i.e. on days 17, 24, 31 and 38). In addition to this, serum creatinine and liver function tests (AST, ALT and serum bilirubin) were also estimated on days 0, 10, 24 and 38. Blood was collected from the orbital plexus under ketamine anesthesia (40 mg/kg i.v.). Blood glucose and other parameters were estimated by standardized biochemical methods.

Statistical analysis

Hypoglycemic effects of the extract on euglycemic animals were analysed by two tailed, unpaired student's t test. $P < .05$ was considered to be statistically significant. Effects of the extract in alloxan induced diabetic rats were analysed by one way ANOVA and followed by post hoc Tukey's test. $P< .05$ was considered to be statistically significant.

RESULTS

Results of phase I

Effects of *Syzygium cumini* Linn. Extract on blood glucose of euglycemic animals

In phase I of the study, *Syzygium* extract had no effect on the mean blood glucose levels when given in the doses of 200, 400 and 800 mg/kg, from 1-24 hours (**table 1)**. It shows that Syzygium extract per se does not possess any hypoglycemic activity in normal animals at all doses. After chronic administration to euglycemic rats for 4 weeks, *Syzygium* extract also did not produce any significant change in blood glucose levels when given at various doses from 200-800 mg/kg (**table 2)**.

Effects of *Syzygium cumini* Linn. extract on renal function tests and liver function tests of euglycemic animals

There was no significant change in the AST and ALT levels in euglycemic animals who were administered *Syzygium cumini* Linn. extract (800mg/kg) (**table 3)**. Serum bilirubin and creatinine levels also did not show any significant change **(table 4).**

Table: 1 acute effects of *Syzygium cumini* Linn. extract on changes in blood glucose levels (mg/100ml) in euglycemic rats

	After 1 hr	**After 2 hrs**	**After 4 hrs**	**After 6 hrs**	**After 16 hrs**	**After 24 hrs**
Control (Normal saline)	1.67 ± 0.77	1.16 ± 0.54	1.83 ± 1.08	1.33 ± 0.73	1.33 ± 0.63	0.66 ± 0.44
Syzygium extract (200 mg/kg)	0.5 ±0.31	1.16 ± 0.60	1.16 ± 0.60	3 ± 1.41	0.66 ± 0.44	2.166 ± 1.044
Syzygium extract (400 mg/kg)	2.83 ± 2.25	1.33 ± 0.63	3.5 ± 2.67	2.5 ± 1.19	1.0 ±0.51	3 ±1.39
Syzygium extract (800 mg/kg)	0.5 ± 0.31	1.33 ± 0.63	1.16 ± 0.60	2.5 ± 1.19	0.66 ± 0.44	2.166 ± 1.044

All values show mean change from 0 hr in blood glucose levels.

N=6 in each group, *= p<.05, **= p<.01, ***= p<.001 as compared to control group of the same dose range by Student's t test.

Table 2: chronic effects of *Syzygium cumini* Linn. Extract on blood glucose levels (mg/100 ml) of euglycemic rats

	0 Day	7th Day	14th day	21st Day	28th Day
Syzygium extract (200 mg/kg)	89 ±1.52	87.83 ±1.40	86.66±1.33	85.33 ±1.45	84.5 ±1.17
Syzygium extract (400 mg/kg	86.16 ±3.27	85.6 ±3.43	85 ±3.49	84.33 ± 3.39	83.16 ±3.22
Syzygium extract (800 mg/kg)	79.16 ± 1.93	78.5 ±1.72	77 ± 1.78	76.33 ± 2.02	75.16 ± 1.90

N=6 in each group, * = p<.05, ** = p<.01, *** = p<.001 as compared to the baseline values (on day 0) in the same group by paired t test.

Table: 3 Effect of chronic administration of *Syzygium cumini* Linn. extract (800 mg/kg) on liver enzymes in euglycemic rats

	0 Day	14th Day	28th Day
AST (IU/L)	53.66 ± 1.30	55.33 ± 1.90	55.66 ±1.74
ALT (IU/L)	22 ± 0.73	22.66 ± 0.98	23.33 ± 0.98

N=6 in each group, * = p<.05, ** = p<.01, *** = p<.001 as compared to the baseline values (on day 0) in the same group.

Table: 4 Effect of chronic administration of *Syzygium cumini* Linn. extract (800 mg/kg) on serum bilirubin and creatinine in euglycemic rats

	0 Day	14th Day	28th Day
S Creatinine (mg/dl)	0.576 ± 0.012	0.583 ± 0.009	0.58 ± 0.007
S Bilirubin (mg/dl)	0.356 ± 0.006	0.36 ± 0.005	0.36 ± 0.007

N=6 in each group, * = p<.05, ** = p<.01, *** = p<.001 as compared to the baseline values (on day 0) in the same group.

RESULTS OF PHASE II

Effects of *Syzygium cumini* Linn. extract on blood glucose of alloxan-induced diabetic rats

Treatment with all the three doses of *Syzygium cumini* extract (200,400 and 800 mg/kg) produced a significant reduction in the blood glucose level. (P value <.001 as compared to group I)The glucose lowering effect started at the end of 2 weeks and it increased till the end of the study in all the groups (**Table 5).** These results show that the reduction in blood glucose levels produced by *Syzygium cumini* extract progresses in a dose dependent manner. This effect also improves with time. The results are comparable to the standard, Glibenclamide. However, even after 4 weeks blood glucose levels failed to return to pre alloxan levels in all the groups.

Effects of *Syzygium cumini* extract on renal function tests and liver function tests of alloxan induced diabetic rats

There was no significant change in the AST and ALT levels in Groups I – V (T**able 6)**. Serum bilirubin and Creatinine levels did not show any significant change in any of the groups. **(Table 7).**

Table 5: Effect of chronic administration of *Syzygium cumini* extract on blood glucose (mg/100 ml) of alloxan induced diabetic rats

Treatment	0 Day	10th Day after Alloxan treatment	17th Day (1week of treatment)	24th day (2 weeks of treatment)	31st Day (3 weeks of treatment)	38th Day (4 weeks of treatment)
Group I (Alloxan 150 mg/kg IP only)	88.66 ± 0.66	197 ± 3.60	195 ± 3.60	193.33 ± 3.49	192 ± 3.38	190.66 ± 3.08
Group II (*S.cumini* 200 mg/kg)	88 ± 0.73	199 ± 2.67	186.16 ± 3.16	182.66 ± 2.10	177 ± 1.69*	168.66 ± 1.11***
Group III (*S. cumini* 400 mg/kg)	88.66 ± 0.66	202.33 ± 4.42	191 ± 6.08	167.33 ± 5.43***	153.66 ± 4.48***	139.66 ± 1.66***
Group IV (*S.cumini* 800 mg/kg)	88.66 ± 0.66	199 ± 2.67	178.66 ± 1.90	161.33 ± 0.84***	147.66 ± 2.80***	129.33 ± 0.98***
Group V (Glibenclamide 10 mg/kg)	88 ± 0.73	199.66 ± 2.80	181 ± 6.98	161.33 ± 3.92***	132 ± 2.47***	117.33 ± 1.52***

N=6 in each group, * = p < .05, ** = p < .01, *** = p<.001. As compared to group I at similar days.

Table: 6 Effect of chronic administration of *Syzygium cumini* Linn. extract on liver enzymes of alloxan induced diabetic rats

Treatment AST (IU/L)	0 Day (alloxan treatment)	10th Day after alloxan treatment	24th Day (2 weeks of treatment)	38th Day (4 weeks of treatment)
Group I (Alloxan only)	54.16±1.16	57.16±1.37	54.33±1.66	55.33±1.90
Group II (S.cumini 200)	53.33±0.98	55.33±1.33	55±1.34	58±2.58
Group III (S.cumini 400)	53±0.85	55.33±1.90	52.66±1.22	52.66±0.98
Group IV (S.cumini 800)	52.66±0.66	55.33±1.90	52.66±0.98	52.66±1.22
Group V (Glibenclamide 10 mg/kg)	55±0.85	55±1.12	54.33±1.20	53.66±1.40
ALT (IU/L)				
Group I (Alloxan only)	23.66±1.08	24±1.03	23.66±1.08	23.66±1.20
Group II (S.cumini 200)	22.33±0.95	22.33±0.95	22.33±0.95	22.33±0.95
Group III (S.cumini 400)	22±0.73	22.33±0.95	23.33±0.84	23±1.00
Group IV (S.cumini 800)	22.66±0.98	23±1.12	23.33±0.84	23.33±0.84
Group V (Glibenclamide 10 mg/kg)	22.33±1.08	23.33±0.98	23.33±0.98	23±1.12

N=6 in each group, * = p<.05, ** = p<.01, *** = p<.001 as compared to the baseline values (on day 0) in the same group. Evaluation using T Test, [a] = p < .05 as compared to Group I. Values between various groups evaluated using ANOVA.

Table: 7 Effect of chronic administration of *Syzygium cumini* Linn. Extract on serum bilirubin and creatinine of alloxan induced diabetic rats

Treatment BILIRUBIN (mg/100 ml)	0 Day (alloxan treatment)	10th Day after alloxan treatment	24th Day (2 weeks of treatment)	38th Day (4 weeks of treatment)
Group I (Alloxan only)	0.356 ± 0.006	0.356 ± 0.006	0.36 ± 0.007	0.36 ± 0.007
Group II (*S.cumini* 200)	0.368 ± .004	0.365 ± 0.004	0.386 ± 0.008	0.371 ± 0.004
Group III (*S.cumini* 400)	0.37 ± .007	0.368 ± .004	0.373 ± 0.01	0.353 ± 0.003
Group IV (*S.cumini* 800)	0.363 ± 0.004	0.37 ± 0.004	0.368 ± 0.005	0.363 ± 0.006
Group V (Glibenclamide 10 mg/kg)	0.363 ± 0.005	0.365 ± 0.005	0.365 ± 0.005	0.36 ± 0.004
CREATININE (mg/100ml)				
Group I (Alloxan only)	0.583 ± 0.012	0.586 ± 0.019	0.576 ± 0.012	0.573 ± 0.013
Group II (*S.cumini* 200)	0.586 ± 0.019	0.583 ± 0.012	0.6 ± 0.010	0.576 ± 0.06
Group III (*S.cumini* 400)	0.573 ± 0.011	0.576 ± 0.009	0.573 ± 0.006	0.576 ± 0.006
Group IV (*S.cumini* 800)	0.60 ± 0.007	0.596 ± 0.013	0.58 ± 0.013	0.57 ± 0.008
Group V (Glibenclamide 10 mg/kg)	0.586 ± 0.008	0.58 ± 0.008	0.57 ± 0.012	0.60 ± 0.008

N=6 in each group, * = p<.05, ** = p<.01, *** = p<.001 as compared to the baseline values (on day 0) in the same group. Evaluation using T Test, [a] = p < .05 as compared to Group I. Values between various groups evaluated using ANOVA.

DISCUSSION

Diabetes mellitus is a chronic disorder causing considerable morbidity and mortality worldwide. Search for newer and effective drugs continue due to rising trend of diabetes.[11] Various herbs have been found to be useful in treating diabetes. *Syzygium cumini* Linn or Jamun tree is one such plant.[12] Its extract has been identified as having antidiabetic properties. *Syzygium cumini* Linn. extract did not produce any significant alteration in blood glucose levels of euglycemic rats after either acute or chronic administration for 4 weeks. During this period all the doses (200mg/kg, 400mg/kg, 800mg/kg) have also not altered liver and kidney functions as there is no change in the serum bilirubin, AST, ALT and creatinine levels. In alloxan induced hyperglycemic rats, *Syzygium cumini* Linn extract lead to gradual reduction in fasting blood glucose levels. The effect was dose dependent and improved with time. The results are comparable to glibenclamide, a standard oral hypoglycemic agent. There was no significant change in renal function tests and liver function tests in all the groups.

Our result indicates that the seed extract does not affect the normal physiological functions governing glucose metabolism so no effect on glucose level in healthy animals but due to some mechanisms not known clearly, is able to correct the glucose metabolic defects due to alloxan administration. These results point towards a combination of mechanisms including insulin secretagogues/mimetic action as well as increasing the sensitivity of insulin. However, the lack of acute hypoglycemic effects cannot be explained and probably point towards a lack of any secretagogues or acute effect. This study also clearly brings out the effect of *Syzygium* cumini Linn. extract on various biochemical parameters

like liver and kidney function tests indicating safety of the extract. While there is a fair amount of evidence outlining the efficacy of *Syzygium* cumini Linn extract in animal models of diabetes, it's mechanism of action and isolation of bioactives is required to be elucidated by further research work.

CONCLUSION

Syzygium cumini Linn extract has no effect on the blood glucose levels of euglycemic animals. *Syzygium cumini* Linn extract can reduce blood glucose levels in alloxan induced diabetic rats, in a dose dependent and time dependent manner. This study clearly demonstrates the efficacy of *Syzygium cumini* Linn extract in animal models of diabetes. However, it's mechanism of action requires further investigation should be elucidated by further research.

REFERENCES

1. Gerrits EG, Landman GW, Bilo HJ. Mortality trends in diabetes mellitus. JAMA Intern Med., 2015; 175(3): 469.
2. Martha S Nolte, John H Karam. Pancreatic Hormones and Antidiabetic Drugs. In: Bertram G Katzung, editor. Basic and Clinic Pharmacology. 13th Ed, Mc Graw Hill, 2015; 683-703.
3. Davis SN. Insulin, oral hypoglycemic agents, and the pharmacology of the endocrine pancreas. In: Brunton LL, Lazo JS, Parker KL, editors. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 12th edition 2011. McGraw Hill, 1613–1645.
4. Simental-Mendía LE, Rodríguez-Morán M, Gómez-Díaz R, et al. Insulin resistance is associated with elevated transaminases and low aspartate aminotransferase/alanine aminotransferase ratio in young adults with normal weight. Eur J Gastroenterol Hepatol. 2016; 23-26.
5. Zhang L, Zhang M, Wang JJ Association of TCF7L2 and GCG Gene Variants with Insulin Secretion, Insulin Resistance, and Obesity in New-onset Diabetes. Biomed Environ Sci., 2016; 29(11): 814-817.
6. McEwan P, Bennett H, Qin L, Bergenheim K, Gordon J, Evans M. An alternative approach to modelling HbA1c trajectories in patients with type 2 diabetes mellitus. Diabetes Obes Metab, 2016; 17-19.
7. Davis SN. Insulin, oral hypoglycemic agents, and the pharmacology of the endocrine pancreas. In: Brunton LL, Lazo JS, Parker KL, editors. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 11th edition. McGraw Hill P, 2006; 1613–1645.
8. Gushiken LF, Beserra FP, Rozza AL, Bérgamo PL, Bérgamo DA, Pellizzon CH. Chemical and Biological Aspects of Extracts from Medicinal Plants with Antidiabetic Effects., 2016; 13(2-3): 96-112.
9. Coelho GD, Martins VS, do Amaral LV, Novaes RD, Sarandy MM, Gonçalves RV. Applicability of Isolates and Fractions of Plant Extracts in Murine Models in Type II Diabetes: A Systematic Review. Evid Based Complement Alternat Med., 2016; 43-45.
10. Baldissera G, Sperotto ND, Rosa HT, et al. Effects of crude hydroalcoholic extract of Syzygium cumini (L.) Skeels leaves and continuous aerobic training in rats with diabetes induced by a high-fat diet and low doses of streptozotocin. J Ethnopharmacol, 2016; 194: 1012-1021.
11. Martha S Nolte, John H Karam. Pancreatic Hormones and Antidiabetic Drugs. In: Bertram G Katzung, editor. Basic and Clinic Pharmacology. 13th Ed, Mc Graw Hill, 2015; 683-703.
12. Rao SS, Najam R. Efficacy of combination herbal product (Curcuma longa and Eugenia jambolana) used for diabetes mellitus. Pak J Pharm Sci., 2016; 29(1): 201-4.