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Ameliorative potential of aqueous leaves extract of *Syzygium cumini* (L) associated metabolic alterations in Alloxan induced diabetic rats

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Abstract
In the present study oral administration of aqueous extract of *Syzygium cumini* to *Alloxan monohydrate* induced diabetic rats secluded the rats from the changes induced in carbohydrate and lipid metabolism. The increase in the *glycosylated* hemoglobin is a sign of succession in diabetes. In addition during diabetes there is an enhancement in the cholesterol and triglyceride contents. The Supplementation of *Syzygium cumini* leaves aqueous extract (200 mg/kg bw) brought the levels of glucose (98±8.4) and lipids (LDL: 32.14±2.71 b and VLDL: 25.71±1.86 b) to almost normal by demonstrating anti-hypoglycemic and anti-lipidemic properties. The reduction in *HDL* cholesterol in diabetic rats can be used as a marker in the evaluating the severity of diabetes.

Keywords: *Syzygium cumini,* Alloxan, Monohydrate, Haemoglobin, Glucose levels, Sugar, Lipid profile.

1. Introduction
Diabetes is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion and/or insulin action. The worldwide prevalence of diabetes mellitus is estimated to be 2.8% [14]. A recent study by the World Health Organization (WHO) estimated as 170 million people in 2010, is predicted to increase to 366 million people by the year 2030. The majority of this diabetic population will emerge from developing countries [15]. Many synthetic oral hypoglycemic agents like Sulphonylureas, biguanides, thiazolidinediones, meglitinide derivatives and α-glucosidase inhibitors are presently in use but they all have several side effects [4]. Most of the plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., that are frequently implicated as having antidiabetic effect [10]. This necessitates the use of herbal preparations, plant decoctions or infusions, for their little side effects, easy availability and cost effectiveness. Hypoglycemic activity of the plants is mainly due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. Despite the availability of various classes of antidiabetic agents, diabetes mellitus remains a major cause of mortality and morbidity globally [6, 13]. As a result, there has been a considerable effort to search for more effective drugs. This has resulted in a renewed interest in research that investigates the health benefits of herbs and natural products including *Syzygium cumini* in the management of diabetes mellitus.
India has more than 40 million diabetic individuals which represents nearly 20% of total diabetes population worldwide. DM affects approximately 4% of the population worldwide and is expected to increase by 5.4% in 2025 [5, 8]. A number of currently existing antidiabetic agents have number of unfavorable effects on the body [5]. Therefore, regulation of diabetes without any side effects is still a difficult task for health care researchers [18]. Consequently, the exploration for more successful and safer hypoglycemic agents with lesser side effects has unremitting to be a momentous area of study. Much diabetes related metabolic alterations are reported [9, 2]. Still though antidiabetic action of crude extracts and purified bio- active components of many plants are identified, investigated related to the curative activity of medicinal plants with reference to the diabetes linked altered metabolic functions are very scanty. Therefore in this investigation *S. cumuni* leaves has been chosen to

study the crude extract effect in the renovation of enzyme activities which are involved in the carbohydrate metabolism in Alloxan induced alterations in diabetic albino rats.

Syzygium cumini (*L*) (Myrtaceae) is a medicinal plant locally Telugu name as "neredu" and it is also called as Eugenia jambolona, Jamun, Black plum and Indian black berry. It is a large ever green tree up to 30 m high, the leaves measuring about 10 to 15 cm long and 4 to 6 cm wide. These are entire, ovate-oblong, sometimes lanceolate and also acuminate, coraceous, tough and smooth with shine above. It is widely distributed throughout India. It has been valued in Ayurveda and Unani system of medication for possessing variety of therapeutic [7]. In present study, we evaluate the hypoglycemic activity of *Syzygium cumini* leaves.

The present work was premeditated with leaves as the test materials which are usually shredded or thrown away as a waste during autumn season or other reasons. Literature survey revealed that the leaves of *S. cumini* have not been studied for different parameters regarding antihyperglycemic activity. Keeping above in view, the present investigation was conducted to study the effect of ethanolic leaves extract of *S. cumini* on blood glucose levels on test in Alloxan induced diabetic mices

2. Materials and Methods

2.1 Animals

Male albino rats (Wistar strain, weighing 180-200 g) were purchased and housed under standard husbandry conditions (30 ± 2 ºC, 60-70% relative humidity and 12 hr day night cycle) and allowed standard pelleted rat feed and water ad libitum.

2.2 Plant material and extraction preparation

The *Syzygium cumini* leaves were harvested and shade dried for 20 days. Then grinded mechanically and 200 g of coarse powder was extracted by using water in soxhlet apparatus. Extract was concentrated to semi-solid water free material and final extract yield was 9.5%.

2.3 Collection of plant material

Fresh leaves of *S. cumini* Linn were collected in June 2013 from Botanical garden, Acharya Nagarjuna University. The leaves were washed neatly and air dried at room temperature for 10 days and fine powdered with an auto mix blender. This powder was kept in a deep freezer until the time of use.

2.4 Induction of Experimental Diabetes

Experimental diabetes in rat was induced by intraperitoneal (i.p.) administration of aqueous alloxan monohydrate in acetate buffer (0.15 M, pH 4.5) in fasting mice by method of (Ozbek *et al*., 2004). Total dose of Alloxan (80 mg/kg b.wt.) was administered. After 48 h animals showing blood glucose level above 200 mg/dl (diabetic) were selected for study.

2.5 Experimental design

Animals were divided in to six groups of six animals each. Group I served as a control: Group II had normal + Scumini (100 mg/ kg bw) rats; group III had normal + Scumini (200 mg/kg bw) and Group IV acts as diabetic control, V as diabetic + S cumini (100 mg/ kg bw) and VI comprised the diabetic + Scumini (200 mg/kg bw) rats treated with *Syzygium cumini* aqueous leaves extract from 100 mg/Kg bw/day and 200 mg/Kg bw/day respectively for 6 weeks, by oral incubation method. Rats were sacrificed at the end of 6 weeks and the blood samples were collected to analyze the effect of *S. cumini* leaves extract on biochemical parameters. Collection and processing of blood for estimation of glucose and other biochemical parameters. Total hemoglobin was estimated by the cyanomethaemoglobin method Drabkin and Austin (1932) and glycosylated hemoglobin (HbA1C) was estimated by the method Nayak and Patabiraman, 1981; Bannon (1982). Serum total cholesterol, triglycerides and serum HDL- cholesterol were using commercial kits (Dialab, Austria).

2.6 Toxicity studies

The aqueous extract was administered orally to different groups of rats (n=6) in doses ranging from 100 mg- 1 g/kg of bw/day to 2-5 g/kg of bw/day. The rats were observed for any lethal effects.

2.7 Statistical analysis

Statistical analysis was performed using the SPSS software package, version 9.05. The values were analyzed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMART). All the results were expressed as mean ± SD for six rats in each group and $p<0.05$ was considered as significant.

3. Results

The yield of aqueous roots extract of (Sc) was found to be 9.5% (w/v). The *S cumini L* leaves aqueous extract treated rats appeared as normal. No toxic effect was reported with the effective dose of aqueous extract and there were no death in all the groups. The application of aqueous roots extract of *Syzygium cumini* on the change of body weight, plasma glucose, hemoglobin and glycosylated hemoglobin is mentioned in Table 1 and Table 2.

In diabetic rats there are significant decrease in the levels of glycogen and glycosylated hemoglobin was observed when compared to the untreated normal rats Oral administration of aqueous leaves extract of *S cumini* significantly increased the levels of glycogen and restored the normal levels of glycosylated hemoglobin in diabetic treated rats. In Table 3 and 4 serum lipids of normal and diabetic rats were mentioned. Total cholesterol, triglycerides and LDL cholesterol levels were significantly increased in diabetic rats with significant decrease of HDL cholesterol levels in comparison with untreated control rats. Oral administration of aqueous leaves extract of SS showed significant effect in the restoration of the normal levels of above mentioned lipids. Thus *S. cumini* aqueous leaves extract is able to protect the system from diabetic induced damage by altering both carbohydrate and lipid metabolism.

Table 1: Effect of *S. cumini* leaf extract (100 and 200 mg/kg bw) on glucose and changes of body weight in control and Alloxan- diabetic rats.

Group	Glucose mg/dl	Change in Body weight/gm
Control	71±7.6	+23.9±5.4
Normal + SC (100 mg/kg bw)	84±7.6	+24.0±4.3
Normal + SC (200 mg/kg bw)	76±7.1	+26.0±5.4
Diabetic control	210±13.0	-24.0±8.1
Diabetic + SC (100 mg/kg bw)	96±8.1	-11.3±7.3
Diabetic + SC (200 mg/kg bw)	97±8.3	-8.±7.0

Each value is mean ± SD for 6 rats in each group a: $p<0.05$ by comparison with normal rats.
b: $p<0.05$ by comparison with Alloxan diabetic rats. Non significant.

Table 2: Effect of *S.cumini* leaf extract on Hemoglobin (Hb), Glycosylated hemoglobin (HbA_1C), and Hepatic glycogen levels in control and Alloxan – diabetic rats.

Groups	Hb (mg/dl)	HbA_1C (mg/g of Hb)	Hepatic Glycogen (gm/100g wet tissue)
Normal	15.1±1.11	0.62±0.06	4.18±0.30
Normal + SC (100 mg/kg bw)	13.9±1.05[b]	0.51±0.02 [b]	4.02±0.31 [b]
Normal + SC (200 mg/kg bw)	13.7±1.06 [b]	0.48±0.03 [b]	4.19±0.33 [b]
Diabetic control	6.0±0.51[a]	1.22±0.08 [b]	1.32±0.09 [b]
Diabetic + SC (100 mg/kg bw)	14.2±1.04 [b]	0.56±0.05 [b]	3.82±0.31 [b]
Diabetic + SC (200 mg/kg bw)	13.9±1.06 [b]	0.62±0.03[Ab]	3.56±0.34[b]

Each value is mean ± SD for 6 rats in each group. a: $p<0.05$ by comparison with normal rats.
b: $p<0.05$ by comparison with Alloxan diabetic rats. Non significant.

Table 3: Effect of *S. cumini* - leaf extract on tissue total cholesterol levels in control and Alloxan – diabetic rats.

Groups	Total cholesterol (mg/g wet tissue)	
	Liver cholesterol	Triglycerides
Normal	7.12±0.61	6.12±0.54
Normal + SC (100 mg/kg bw)	6.75±0.56 [b]	6.01±0.52 [b]
Normal + SC (200 mg/kg bw)	6.17±0.61 [b]	6.22±0.49 [b]
Diabetic control	15.12±1.07 [a]	13.78±1.01 [a]
Diabetic + SC (100 mg/kg bw)	8.13±0.61 [b]	8.12±0.69 [b]
Diabetic + SC (200 mg/kg bw)	7.84±0.57 [b]	7.88±0.58 [b]

Each value is mean ± SD for 6 rats in each group. a: $p<0.05$ by comparison with normal rats.
b: $p<0.05$ by comparison with Alloxan diabetic rats. Non significant.

Table 4: Effect of *S cumini* - leaf extract on serum HDL, LDL and VLDL levels in control and Alloxan –diabetic rats.

Groups	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	VLDL-cholesterol (mg/dl)
Normal	45.16±3.61	23.67±1.67	19.72±1.21
Normal + SC (100 mg/kg bw)	48.27±3.91 [b]	22.61±1.45 [b]	20.12±1.68 [b]
Normal + SC (200 mg/kg bw)	52.12±4.12 [b]	24.72±1.71 [b]	19.21±1.32 [b]
Diabetic control	22.68±1.81 [a]	79.66±4.95 [a]	47.51±3.79 [a]
Diabetic + SC (100 mg/kg bw)	41.67±3.12 [b]	41.56±3.12 [b]	28.91±2.12 [b]
Diabetic + SC (200 mg/kg bw)	60.12±3.01 [b]	32.14±2.71 [b]	25.71±1.86 [b]

Each value is mean ± SD for 6 rats in each group. a: $p<0.05$ by comparison with normal rats.
b: $p<0.05$ by comparison with Alloxan diabetic rats. Non significant

4. Discussion

Syzygium cumini is a plant that has been used in popular medicine for the treatment of the diabetes. They are prepared as an aqueous or ethanolic extract, by infusion or as a juice of the plant [12]. Alloxan can specifically destroy the beta (b) cells of the pancreatic islets, inducing loss of the cell turgor, nuclear pincnosis, cytoplasmatic vacuolization, mitochondrial edema and fragmentation, leading to cell death [3, 11]. The aim of the present study was to assess the anti diabetic effect of ethanolic leaf extract of *S. cumini* against alloxan induced diabetic rats. The continuous treatment of the leaf extract for a period of 21 days produced a significant decrease in the blood glucose levels in diabetic rats. On the other hand, the characteristic loss of body weight, as revealed in the present work in Alloxan induced diabetic rats, is due to the increased muscle wasting and loss of tissue proteins in diabetes. It shows that the administration of *S. cumini* leaf extract improve the body weight in diabetic rats by protective effect in controlling muscle wasting (i.e., reversal of gluconeogenesis and glycogenolysis). It may be due to the improved insulin secretion and glycemic control [16]. Hence, Alloxan is believed to destroy the beta cells of the islets and this leads to deficiency in circulating insulin levels leading to many pathological alterations. In the diabetic rat's pancreas, the islets number is reduced and there are individual variations in number of islets. When these rats treated with *S. cumini* leaf extract resulted in normalization of islets with increased secretory granular were observed.

The present investigation was to evaluate the efficiency of the aqueous leaves extract *of S. cumini* on alloxan-induced metabolic changes diabetic rats. Decreased Hb content was observed in diabetic rates might be due to increased formation of glycosylated Hb. Generally total hemoglobin levels is much below the normal levels in diabetic subject by Chandaliam (2002) and HbA1c levels has been reported to be increased in patients with diabetes mellitus [22]. It was reported that during diabetes mellitus, the excess of glucose present in the blood reacts with hemoglobin to form HbA1C. The levels of HbA1C are always monitored as a reliable index of glycemic control in diabetes Gabbay (1976). Elevated levels of HbA1C and reduced levels of Hb observed in our study reveals that diabetes animals had prior high blood glucose levels. Administration of aqueous leaves extract of *S cumini* (200 mg/ Kg bw/day) had brought back the elevated HbA1C levels to near normal levels. It has already been reported that decreased liver glycogen content was due to insulin deficiency and associated glycogenolysis process (Vats *et al.,* 2004) [20]. The possibility of restoration of glycogen content in Alloxan -induced diabetic rats by the administration of SS aqueous leaves extract may be due to increased insulin secretion and reactivation of glycogen Synthase enzyme system. Hypercholesterolemia and hypertriglyceridemia in Alloxan - induced diabetic rats are well documented Insulin deficiency leads to increased serum lipids because of increased lipolysis was investigated by (Shirwaikar *et al.,* 2004; Ravindra Babu *et al.,* 2012) [16, 19] .

The elevated levels of serum total cholesterol, triglycerides and LDL cholesterol were significantly decreased after treatment with SS leaves extract. Similar findings were also reported with the methanolic extract of the Talinum triangulare.

5. Conclusion

From this study it can be concluded that the administration of aqueous extract of *Syzygium cumini* leaves is beneficial in normalizing the alterations in carbohydrate metabolism during diabetes

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