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RESEARCH ARTICLE

ANTI-HYPERGLYCEMIC ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *SYZYGIUM CUMINI* (L) IN DIABETIC INDUCED RATS

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ABSTRACT

Syzygium cumini is a plant that has been used in popular medicine for the treatment of the diabetes. It is originated in India, and it can be found in most Brazilian states, principally on the noth and north-east. This work verified the effect of *S.cumini* ethanolic leaf extract up on the alloxan induced diabetes rats. The animals were divided in to four groups, Control (C), Control + *S.cumini* treatd(CT), Diabetes (D), Diabetes + *S.cumini* treatd (DT). Diabetes was induced in male wistar albino rats by intra-peritoneal injection with cold aqueous alloxan monohydrate (80 mg/kg body wt). Then the blood glucose levels were increased significantly. Ethanolic leaf extract of *S.cumini* was given to the diabetic rats in daily dose of 450mg/ kg of body weight (21 days). Then in the diabetic rats of blood glucose levels decreased highly significant ($p<0.005$). And the body weights of the experimental animals there is no more weight gain in controls after 21 days of treatment with *S.cumini* ethanolic leaf extract (180-195±5).Where as diabetic rats treated with leaf extract for 21 days resulted in 12-15 % increased in the body weight. No side effects appear in the whole study. Hence this work suggested that *S.cumini* ethanolic leaf extract effectively treated diabetes

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% (Sarah *et al*., 2004). 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 (Shaw *et al*., 2010).Many synthetic oral hypoglycemic agents like Sulphonylureas, biguauanides, thiazolidinediones, meglitinide derivatives and α-glucosidase inhibitors are presently in use but they all have several side effects (Edwin *et al*., 2006). Most of the plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., that are frequently implicated as having antidiabetic effect (Malviya *et al*., 2010). This necessitates the use of herbal preparations, plant decoctions or infusions, for their little side effects, easy availability and cost effectiveness. Hypoglyceamic 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 (Kokil *et al*., 2010, Roglic *et al*., 2010). 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.

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 varity of therapeutic (Kirtikas and Basu, 1975). 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

MATERIALS&METHODS

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.

Preparation of plant leaves extract

1000 g of dry leaves powder was suspended in 3 liters of Ethanol stirred magnetically and kept for overnight (24h) at room

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temperature. The extract was collected successfully by using a Soxhlet apparatus and the process was repeated for three consecutive times with the residual powder, each time collecting the extract. The collected extract was pooled and passed through a filter paper. The filtrate upon evaporation at 40ºC at low-pressure (Rotavapor apparatus) yielded 15% of semi solid extract. It was stored in a refrigerator at 0ºC- 4ºC until used in the diabetic studies.

Phytochemical Study

A portion of extract was subjected to phytochemical analysis (Ethanol) in order to see the presence of Alkaloids, Flavonoids, Glycosides, Phinols, Saponins, Sterols, Tannins and Carbohydrates.

Selection of experimental animals

Adult male albino Wistar rats weighing approximately 180±20g were collected from National Institute of nutrition, Hyderabad in Andhra Pradesh. Our work approved by the ethical committee of the pharmaceutical sciences of the Acharya Nagarjuna University. The animals were kept in polycarbonate cages and maintained in an animal room with a 12h daylight cycle, at a temperature of 22º ± 20ºC and humidity up to 45-64%. During the total experimental period, animals were fed with a balanced commercial diet made in India, pranav agro industries Ltd. Sangli, India.

Experimental design

24 rats were divided into 4 groups each contained 6 and used as follows;

Group 1: Cantrol Rats,
Group 2: Control Rats treated with *S.cumini* extract for 21 days,
Group 3: Diabetic treated Control Rats,
Group 4: Diabetic rats administered with *S.cumini* extract daily for 21 days.

Throughout the experimental period, the body weight, food and fluid intake were monitored. At the end of 21 days, all the rats were killed by cervical dislocation. Blood was collected in heparin-coated tubes and centrifuged at 1,000g for 15 min at 4ºC.

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.

Estimation of blood glucose levels

Blood glucose levels were estimated by using Accu Chek Glucometer (Sensor Comfort) regularly for the experimental period of 21 days before and after giving the leaf extracts of *S.cumini*.

Statistical analysis of data

Statistical analysis such as Mean, Standard Deviation (SD), Standard Error of Mean (SEM) and Paired T-Test were performed with 'MINITAB 11. 1232 Bit'. For between group comparisons 'Two Sample T-Test' was performed. Differences were considered statistically significant at a $p<0.05$ and a $p<0.001$.

RESULTS

Diabetes is attributed to the diminished production of insulin or mounting resistance to its action. Chronic hyperglycemia during diabetes causes glycation of body proteins, which in turn leads to secondary complications affecting the eyes, kidneys, nervous system and arteries (Sharma & Misra, 1993). In this experiment aqueous alloxan monohydrate in acetate buffer (0.15 M, pH 4.5) injected by intraperitonial (i.p). Then the diabetic mice were used to assess the effects of *S.cumini* leaf extracts for a period of 21 days in alloxan –induced diabetic rats. These diabetic mice were treated after 48 hours of alloxan injection (i.p). In this period no detectable irritation or restlessness was observed after drug administration.

Blood glucose levels

Intra-peritonial injection of Alloxan increased the blood glucose levels up to four times of the normal range (i.e., 80-300mg/dl). However, the administration of ethanolic extraction of *S.cumini* to diabetic induced group decreased their blood glucose levels by 10-33% in 10 days, 33-52 % in 15 days and 40-82% in 21 days. In our findings the blood glucose levels decreased after feeding ethanolic leaf extracts of *S. cumini* was highly significant ($p<0.005$) which indicates this antihyperglycemic effect of the ethanolic extracts. And control rats are treated with leaf extract showed the decrease in blood glucose level which indicates the hypoglycemic effect of the ethanolic extract (Table 1).

Body weights

In this study the body weights of the experimental animals there is no more weight gain in controls after 21 days of treatment with S.cumini ethanolic leaf extract (180-195±5). The rats with high blood glucose sugar (Alloxan induced) 20-45% loose in the body weight observed, where as diabetic rats treated with leaf extract for 21 days resulted in 12-15 % increased in the body weight. (Table1 2)

Table 1 Anti-hyperglycemic effects (mg/dl) of *S. cumini* leaves extracts normal, diabetic and leaves extract treated rats (Mean ± SD values) $p < 0.005$

Groups of experimental animals	Day 1	Day 5	Day 10	Day 15	Day 21
Control	80.6 ± 0.8 (6)	81.4 ± 1.7 (6)	80.3 ± 1.7 (6)	81.8 ±1.6 (6)	82.6±1.4 (6)
Control + *S. cumini* treated	92.1±1.1 (6)	92.4 ± 1.3 (6)	88.5 ±1.4 (6)	80.7±1.3 (6)	76.6±1 (6)
Diabetic	250.4±8.2 (6)	259.5±5.1 (6)	261.9 ±7 (6)	270.6±4.0 (6)	315.1±3.2 (6)
Diabetic + *S. cumini* treated	291.5±2.5 (6)	291.0±7.1 (6)	263.2±2.8 (6)	242.7±3.0 (6)	209.2±4.8 (6)

DISCUSSION

Syzygium cumini is a plant that has been used in popular medicine for the treatment of the diabetes. It is originated in India, and it can be found in most Brazilian states, principally on the noth and north-east. (Branganca 1996) the bark, the fruit, the seed, as well as the leaves are utilized in the treatment of diabetes. They are prepared as an aqueous or ethanolic extract, by infusion or as a juice of the plant (Pepato, M. T. *et al.,* 2001). 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.(Drews, G. *et a*l.,2000 ; Mathus, C.E and Leiter E.H 1999). 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 gluconiogenesis and glycogenolysis). It may be due to the improved insulin secretion and glycemic control (Shiwaikar et al., 2004). 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.

Table 2 Changes in body weights (g) of normal, diabetic and leaves extract treated rats (Mean ± SD values) p< 0.05

Groups of experimental animals	Day 1	Day 5	Day 10	Day 15	Day 21
Control	187.15±1.5 (6)	189.7±1.5 (6)	191.8±1.9 (6)	194±1 (6)	195.2±1.2 (6)
Control + *S. cumini* treated	183.13±1.5 (6)	185.4±1.3 (6)	182.5±1.4 (6)	180.6±1.7 (6)	172.5±3.9 (6)
Diabetic	197.6±2.6 (6)	194.5±2.6 (6)	192.0±2.2 (6)	185.9±1.3 (6)	175.2±1.50 (6)
Diabetic + *S. cumini* treated	194.0±1.3 (6)	192.3±1.8 (6)	190.9±1.5 (6)	191.6±1.2 (6)	194.5±1.6 (6)

CONCLUSION

In this pilot study Alloxan monohydrade is to destroy the beta cells of the pancreas islets. So alloxan induced rats shows diabetes (i.e., increased blood glucose levels significantly). After that these rats treated with leaf extract of *S.cumini* blood glucose levels significantly decreased. And body weight is slightly increased in treated rats. No side effects appear in the whole study. Hence this work suggested that *S.cumini* ethanolic leaf extract partially treated diabetes.

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