INFLUENCE OF METHANOLIC EXTRACT OF *SYZYGIUM CUMINI* SEEDS ON THE ACTIVITY OF GLICLAZIDE IN NORMAL AND ALLOXAN-INDUCED DIABETIC RATS

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Summary

Since drugs from oriental medicine are used along with allopathic drug therapy in highly populated countries, such as India and China, and since optimal blood sugar control is needed in diabetes, herbal drug that influences blood glucose was studied for its effect on gliclazide response in normal and diabetic rats. In this study the safety of the herb-drug combination with respect to blood glucose was studied in animal models. Albino rats were divided into three groups of six each and were fasted for 18 h prior to the experiment with water *ad libitum*. Three groups were orally received, 500 mg/kg body weight of methanolic extract of *Syzygium cumini* seeds (SME), gliclazide 2 mg/kg body weight, and SME prior to the administration of gliclazide 2 mg/kg body weight, respectively, in normal and diabetic rats. Blood samples were collected from the retro-orbital plexus at regular intervals after drug administration and were analyzed for blood glucose by glucose oxidase/peroxidase (GOD-POD) method. Diabetes was induced by alloxan monohydrate 100 mg/kg body weight administered by i.p route. Gliclazide produced hypoglycemia/antihyperglycemia in normal and diabetic rats with peak activity at 2 h and 8 h. SME produced hypoglycemia/antidiabetic activity when given alone, and prolonged the effect of gliclazide in combination, in normal and diabetic rats without hypoglycemic convulsions. The blood glucose levels of gliclazide were not altered in the presence of SME. The study indicated that the combination can be used safely to obtain prolonged and sustained antidiabetic effect.

Key words: *Syzygium cumini*, gliclazide, hypoglycemia, diabetes, blood glucose

Introduction

Diabetes mellitus is a major metabolic disorder characterized by chronic hyperglycemia as a result of a relative or absolute lack of insulin or the actions of insulin. It is estimated that in the year 2010, more than 200 million people world wide will have diabetes mellitus, and 300 million people will subsequently have the disease in 2025 (1). Treatment of diabetes aims at maintaining blood glucose homeostasis, prevention of ketosis and secondary complications. The major mode of control over diabetes can be achieved by diet and exercise, insulin replacement therapy and by the use of oral hypoglycemic agents (2). Use of herbs for the treatment of diabetes has been a common practice in countries such as India and China since ancient times. In India and China, people often use both herbs and drugs together. In such a situation, the herb may interact with the drug, thereby enhancing or reducing the effects of the drug. Literature evidence suggests that some herbs interact with oral hypoglycemic agents (3).

Gliclazide is a widely used drug in the treatment of Type-2 diabetes owing to its selective inhibitory activity towards pancreatic K^+ATP channels (4). *Syzygium cumini* (Syn. Eugenia cumini, Eugenia jambolana, jambul, black plum) is a tree of the family Myrtaceae distributed in Asia. The leaves, barks, and seed extracts of *Syzygium cumini* have been reported to possess anti-inflammatory (5), antibacterial (6) and anti HIV activity (7) including antidiabetic activity (8). In our previous studies also the methanolic extract of *Syzygium cumini* seeds has been reported to have anti-arthritic (9), immunomodulatory (10) and cardioprotective (11) activities.

In the present study, the effect of methanolic extract of *Syzygium cumini* seeds (SME) on gliclazide activity was studied in rats (normal and diabetic) to evaluate the safety of the combination with respect to blood glucose.

Material and Methods

Animals

Albino rats of either sex, weighing between 300-340 g were used in the study. They were procured from National Institute of Nutrition, Hyderabad, India. They were maintained under standard laboratory conditions at an ambient temperature of 25 ± 2^{o}C and 50 ± 15% relative humidity with a 12-h light/12-h dark cycle. Animals were fed with a commercial pellet diet (Rayan's Biotechnologies Pvt Ltd., Hyderabad, India) and water *ad libitum*. The animal experiments were performed after prior approval of the study protocol by the Institutional Animal Ethics Committee of our institute. The study was conducted in accordance with the guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Chemicals and Drugs

Alloxan monohydrate was purchased from LOBA Chemie, Mumbai, India. Gliclazide was supplied by Aristo Pharma, Mumbai, India. Glucose kits (Span diagnostics) were purchased from the local pharmacy. All other chemicals used for this study were analytical grade.

Preparation of Methaolic Extract
The *Syzygium cumini* fruits were first washed well and pulp was removed from the seeds. Seeds were washed several times with distilled water to remove the traces of pulp from the seeds. The seeds were dried at room temperature and coarsely powdered. The powder was extracted with hexane to remove lipids. It was then filtered and the filtrate was discarded. The residue was successively extracted with methanol using cold percolation method. The percentage yield was 10.28% in methanol. The extract was stored at -70^{o}C.

Preliminary Phytochemical Screening
One gram of the methanol extract of *Syzygium cumini* (SME) was dissolved in 100 ml of its own mother solvent to obtain a stock of concentration 1% (v/v). The extract thus obtained was subjected to preliminary phytochemical screening.

Pharmacological Experiment

Acute Toxicity Studies
The acute oral toxicity study was carried out as per the guidelines of OECD (12). One tenth of the medium lethal dose [LD_{50}] was taken as an effective dose.

Study in Normal Rats
Normal rats were divided in to three groups.

Group-I	:	SME -500 mg/kg body weight
Group-II	:	Glicalzide-2 mg/kg body weight
Group-III	:	SME prior to the administration of glicalzide

Study in Diabetic Rats
Albino rats of either sex were treated with alloxan monohydrate (100 mg/kg body weight, i.p.). Alloxan monohydrate was dissolved in saline solution and was administered. Animals were treated with 10% dextrose orally to combat the early phase of hypoglycemia. Rats showing fasting blood glucose levels above 200 mg/dL were selected for the study. These rats were divided in to three groups.

Group-I	:	SME -500 mg/kg body weight
Group-II	:	Glicalzide-2 mg/kg body weight
Group-III	:	SME prior to the administration of glicalzide

Collection of Blood Samples
Blood samples were collected from the retro orbital plexus of each rat at 0, 1, 2, 3, 4, 6, 8, 10 and 12 h (after drug administration). Blood glucose levels were determined by using GOD-POD method (13).

Statistical Analysis

Data were expressed as mean ± SEM. The significance of blood glucose reduction produced by SME + gliclazide compared with gliclazide was determined by applying student's paired t-test. *P* values of <0.05 were considered to be statistically significant.

Results

Preliminary Phytochemical Screening

This investigation showed the presence of alkaloids, amino acids, flavonoids, glycosides, phytosterols, saponins, steroids, tannins and triterpenoids in the methanolic extract of *Syzygium cumini* seeds (SME).

Acute Toxicity Studies

From the acute toxicity study, the LD_{50} cut-off dose for SME was found to be 5000 mg/kg body weight. Hence one tenth of LD_{50} dose (500 mg/kg body weight) was selected as therapeutic dose for this study.

Effect in Normal Rats

SME produced hypoglycemia with peak effect at 6 h in Group-I. Glicalzide produced hypoglycemia with a peak effect at 2 and 8 h in Group-II. In Group-III, prior administration of SME enhanced gliclazide response with peak effects at 2 & 8 h and prolonged the hypoglycemic effect. The percentage values of blood glucose reduction are given in Table 1.

Effect in Diabetic Rats

SME produced peak antihyperglycemic effect at 6 h. Gliclazide produced an antihyperglycemic levels at 2 and 8 h. The combination produced peak antihyperglycemic effects at 2 and 8 h with significant variation at 2, 6 and 8 h. The percentage values of blood glucose reduction are given in Table 2.

Table 1 Mean percent blood glucose reduction in normal rats (n=6)

Time (hr)	SME (Group-I)	Gliclazide (Group-II)	SME + Gliclazide (Group-III)
1	07.46 ± 1.64	25.24 ± 1.62	35.56 ± 1.55*
2	11.20 ± 1.46	32.56 ± 1.46	40.36 ± 1.62*
3	17.30 ± 1.19	29.42 ± 1.82	33.24 ± 1.34*
4	23.52 ± 1.46	23.30 ± 1.64	29.40 ± 1.64*
6	29.30 ± 1.46	27.84 ± 1.56	37.52 ± 1.86*
8	21.26 ± 1.20	37.08 ± 1.82	42.22 ± 1.24*
10	13.84 ± 1.26	26.44 ± 1.26	32.46 ± 1.34*
12	09.32 ± 1.62	19.30 ± 1.46	25.56 ± 1.64*

Values were given as mean ± SEM (N=6)

SME: methanolic extract of *Syzygium cumini* seeds

* Significance at *P*<0.05, gliclazide response compared with combination

Table 2 Mean percent blood glucose reduction in diabetic rats (n=6)

Time (hr)	SME (Group-I)	Gliclazide (Group-II)	SME + Gliclazide (Group-III)
1	11.56 ± 1.48	29.36 ± 2.36	31.42 ± 2.24
2	26.62 ± 1.65	40.64 ± 1.64	44.26 ± 2.26*
3	29.32 ± 1.82	36.46 ± 1.52	37.56 ± 2.24
4	31.22 ± 1.68	30.64 ± 2.32	31.22 ± 1.24
6	37.42 ± 1.82	35.56 ± 2.36	40.46 ± 1.34*
8	32.20 ± 1.86	41.56 ± 2.35	46.85 ± 2.34*
10	25.61 ± 1.74	27.46 ± 1.64	28.46 ± 2.22
12	20.36 ± 1.22	23.26 ± 2.32	25.64 ± 1.36

Values were given as mean ± SEM (N=6)
SME: methanolic extract of *Syzygium cumini* seeds
* Significance at $P<0.05$, gliclazide response compared with combination

Discussion

Diabetes mellitus is a metabolic disease as old as mankind and its incidence is considered to be high (4-5%) (2). Treatment of this disorder taken three main forms (i) diet and exercise (ii) insulin replacement therapy (iii) the use of oral hypoglycemic agents. Several studies have found that adding fiber to the diet of diabetes improved plasma glucose.

Alloxan has been observed to cause a massive reduction of the β-cells of the islets of Langerhans and induce hyperglycemia. In our study we have found that SME decreases blood glucose in alloxan diabetic rats. The possible mechanism by which SME brings about its hypoglycemic action may be potentiating the insulin effect of plasma by increasing either the pancreatic secretion of insulin from the β-cells of the islets of Langerhans or its release from bound insulin. Gliclazide produced hypoglycemia/antihyperglycemia in normal/diabetic rats, respectively; with peak activity at 2 h and 8 h. Gliclazide is metabolized to several metabolites by hepatic cytochrome P4503A4 and 2C9 isozymes (4). A part of gliclazide is eliminated through the biliary route, which involves enterohepatic circulation in rats and humans. The reabsorption of gliclazide elimination through the biliary route might be responsible for a second peak in its hypoglycemic effect in rats. Since the SME did not alter the pattern of double-peak effect in rats, it does not seem to interfere with the metabolic pattern of glicalzide in rats. The extract might not have influenced biliary excretion of gliclazide, since double peak effects were found in the rat model in the presence, as well as absence, of the extract. The SME produced significant hypoglycemia when administered alone, and prolonged the effect of gliclazide by 1-12 h in normal and diabetic rats, without hypoglycemic convulsions.

It is well established that gliclazide produced hypoglycemia by pancreatic (insulin-release) and extra pancreatic (increase in glucose uptake) mechanisms. The possible mechanism(s) by which SME brings about its antihyperglycemic action may be through potentiation of pancreatic secretion of insulin from the intact β-cells of islets coupled with extra pancreatic

mechanisms like decreased glycogenolysis and enhanced glycogenesis by the liver and/or enhanced transport of blood glucose to peripheral tissues. The enhanced effect of gliclazide in the presence of SME might be due to combined effect of the two drugs on insulin release or increase in glucose uptake. However, no convulsions were seen in rats, even at peak hours of activity. Hence, their combination need not be contraindicated.

Conclusion

The results from our study are clearly indicating that the combination of gliclazide and methanolic extract of *Syzygium cumini* seeds can be used safely with respect to blood glucose to obtain a prolonged and sustained antidiabetic effect. Further studies are needed to establish its long-term safety in animals and humans.

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