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Research Article

SYZYGIUM CUMINI DEPARTS THE NEGATIVES OF THE ALLOXAN INDUCED DIABETES IN SWISS ALBINO MICE

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ABSTRACT

Background: Because of the unfavorable side effects and higher cost of synthetic anti-diabetic medicines, an alternative approach to manage diabetes mellitus from an herbal source would be safe and within the affordability of the common people. Hence we investigated the hypoglycemic and pancreatic islets restoration effect of *Syzygium cumini* seed extract using glibenclamide as positive control.

Methods: Six (06) normal control mice and eighteen (18) alloxan induced diabetic mice grouped as diabetic control, *Syzygium cumini* and standard drug, were used for this experiment. In comparison to a reference drug-glibenclamide (@600µg/kg body weight), *Syzygium cumini* seed ethanolic extract (@500mg/kg body weight) was given orally once daily for thirty days to counteract alloxan induced alteration in blood glucose level and pancreatic cells morphology. Fasting blood glucose (FBG) level was evaluated at 15 days intervals, whereas mice were ethically sacrificed to collect pancreas at the completion of the experiment and processed for histological examination.

Results: Research results revealed that in both the *Syzygium cumini* and standard drug treated groups, FBG level were significantly lower than the diabetic control group ($P < 0.05$). Histologically, smaller islets and necrosis, present in the pancreas of diabetic mice were returned to normal following treatment with *Syzygium cumini* seed extract and standard anti-diabetic drug.

Conclusion: *Syzygium cumini* seed extract could be recommended as anti-diabetic for humans and animals especially in developing and under developed countries where this plant is readily available and affordable.

INTRODUCTION

A deficit in insulin secretion and/or action results persistent hyperglycemia which is a common characteristics of diabetes mellitus (DM).^[1] People of Bangladesh are the worst sufferers of such life-long disease with a prevalence of 8%.^[2,3] Treatments for diabetes consist of glycemic level control by exercise therapy, diet therapy and pharmacotherapy including oral hypoglycemics and insulin.^[4] However, synthetic anti-diabetics have drawbacks like insulin resistance, fatty liver as well as their higher cost are not affordable by the low income people.^[5,6] In such

scenario, plants such as *Syzygium cumini* have shown potentiality for treatment of DM in terms of cost effectiveness and minimal side effects.

Syzygium cumini of Myrtaceae family, has conventionally been used for decreasing the danger of degenerative diseases including diabetes mellitus. Although almost all segments of the *S. cumini* plants has anti-diabetic property, seeds was preferred for the presence of flavonoids, glycosides, mycaminose, tannins, saponins and vitamins, which helps by various modes and mechanisms in regulating the

blood sugar level in diabetic animal. Pancreatic beta (β) cells produce insulin which regulates glucose homeostasis by glucose uptake rate increment, hepatic glucose output reduction and glycogenesis induction.^[7] In diabetic animals, degenerated islets results in β -cells number reduction and inadequate insulin secretion.^[8] Phytochemicals from *S. cumini* stop further destruction of the remaining β cells, regenerate damaged pancreatic islets and increase insulin release.

Therefore this experiment was designed to evaluate the hypoglycemic and pancreatic islets restoration effects of *Syzygium cumini* seed in alloxan- induced diabetic mice.

MATERIALS AND METHODS

Extract preparation: *Syzygium cumini* fruits were procured from local market for seed collection. Seeds were cut, air dried and then ground to make fine powder. 95% ethanol was used for phytochemicals extraction. After filtration, the solvent were concentrated by rotary evaporator and finally by lyophilizer for extract preparation. 500 mg *Syzygium cumini* seed extract were dissolved in 0.5 ml dimethylsulfoxide to prepare the desired solution.^[2,9]

Animals and experimental procedures: Twenty-four (24) ICDDR,B bred 3-4 weeks old Swiss albino mice were kept in standard environmental condition with adequate food and water after their purchase. The mice were categorized into 4 groups containing six (06) mice each: Group-A: Normal Control (NC), mice received only fresh water; Group-B: Diabetic Control (DC), diabetic mice received no treatment only fresh water; Group-C: *Syzygium cumini* seed extract treated (SC), diabetic mice were administered with *Syzygium cumini* seed ethanolic extract @ 500mg/kg body weight orally once daily for 30 days;

Group-D: Standard drug treated (GL), Glibenclamide @ 600 μ g/kg body weight were administered in diabetic mice orally once daily for 30 days. After an overnight fast, single dose of alloxan monohydrate (@150 mg/kg body weight) was injected intra-peritoneally for diabetes induction. After 72 hours, fasting blood glucose level (FBG) was measured by a glucometer and mice with a FBG value of 11.1 mmol/L or above were considered as diabetic.^[10] At 15 (fifteen) days interval, FBG values were measured and pancreas was collected after the mice were ethically sacrificed at the end of the experiment for routine histological examination.

Histo-morphological analysis: The gross examination of the pancreas was done by eye estimation. Following that, pancreas was fixed in 10% formalin for 48 h, dehydrated and paraffin embedded. The paraffin block were cut at 7 μ m thickness using Leica RM2135 rotary microtome and stained with haematoxylin & eosin.^[11]

RESULTS AND DISCUSSION

Hematological study: The changes in the fasting blood glucose (FBG) level during experimental period in different groups were given in Table 1. In the diabetic mice, intra-peritoneal injection of alloxan monohydrate results 4 times increase of FBG level. After 30 days of administration, *S. cumini* seed (SC) showed 30.11% decrease whereas standard anti-diabetic drug had 44.50% decrease in blood glucose level. The presence of phyto-chemicals such as mycaminose, jambosine, jambolin or antimellin, saponins, flavanoids, phenols could be the reason for significant hypoglycemic activity of *S. cumini* seeds. The individual and/or synergistic action of these phytochemicals might be responsible for such anti-diabetic activity.

Table 1: Changes in FBG level during experimental period in different groups

Group	Day 0 (mmol/L)	Day 15 (mmol/L)	Day 30 (mmol/L)
Normal Control (NC)	05.7 \pm 0.35	05.8 \pm 0.32	05.9 \pm 0.30
Diabetic Control (DC)	18.7 \pm 0.61	19.0 \pm 0.65	19.2 \pm 0.56
<i>Syzygium cumini</i> seed extract (SC)	18.6 \pm 0.77*	15.7 \pm 0.72*	13.0 \pm 0.58*
Standard drug- Glibenclamide (GL)	19.1 \pm 0.67*	13.5 \pm 0.56*	10.6 \pm 0.44*

Results are Mean \pm SEM (standard error of mean) of 6 mice in each group. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test was performed as the test of significance. The difference was considered significant when * $p < 0.05$ compared to the diabetic control group

Histopathological study: Among the experimental groups, there was no significant difference in the color, size and shape of the pancreas. Alloxan, a beta cytotoxin, was responsible for necrosis of islets of Langerhans in the diabetic pancreas (Figure 1). Regeneration of pancreatic islets with normal

proportion and regular arrangement of alpha (α) & beta (β) cells were observed in herbal extract and standard drug administered mice after 30 days of treatment. Restoration of histological architecture was much prominent in the glibenclamide treated pancreas with increased number of α & β cells in

large sized islets. Diabetogenic action of alloxan was reversed through beta-cell regeneration and increase insulin releasing ability of flavonoids, alkaloids,

saponins and tannins present in the *S. cumini* seeds.^[12]

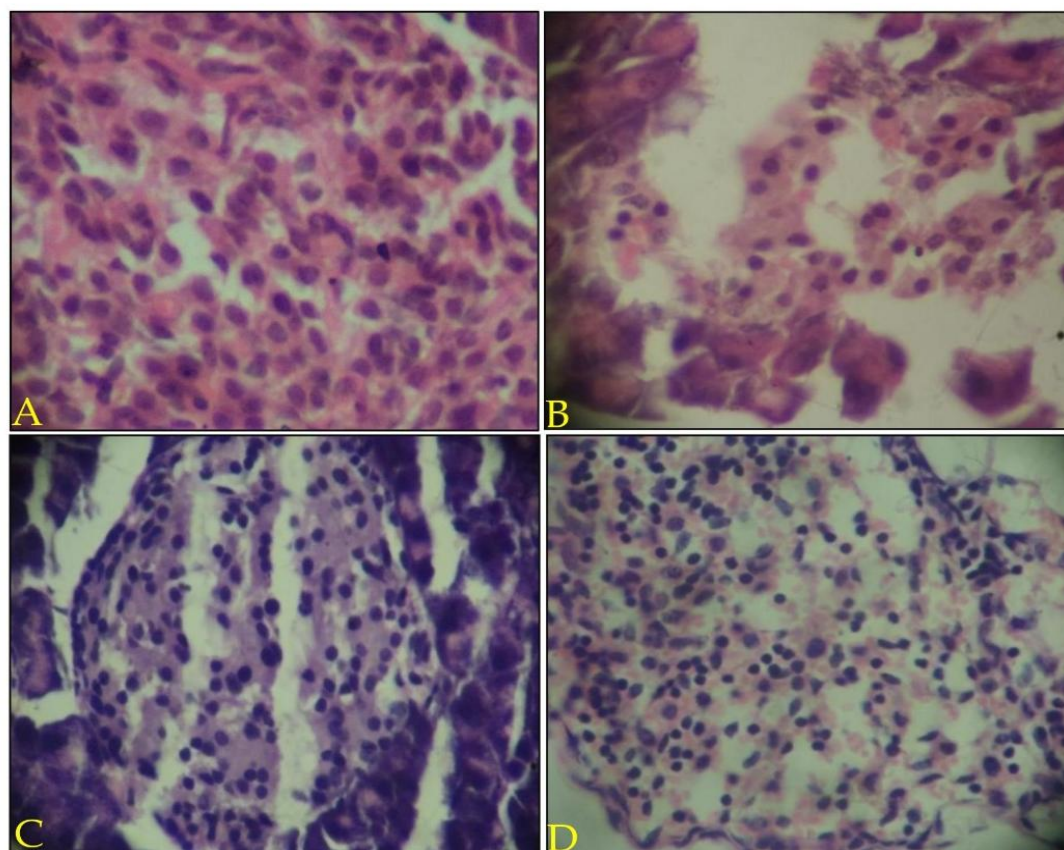


Figure 1. Histological architecture of the pancreatic islets of Langerhans in the normal control group (A) showing regular arrangement of α & β cells; diabetic control group (B) showing considerable necrosis; *S. cumini* seed ethanolic extract treated group (C) showing regeneration of the islets; glibenclamide treated group (D) with increased number of α & β cells in large sized islets (40X; H & E stain).

CONCLUSIONS

It may be concluded that, anti-diabetic influence of *Syzygium cumini* seed extract were shown through significant blood glucose level reduction and histological improvement of islets and β -cells number after 30 days of administration. Therefore, this seed extract could be recommended as natural hypoglycemic agent as substitutes to synthetic ones.

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