Original Article

Anti-Diabetic Effect of Spirulina and Karela in Streptozotocin Induced Diabetic Rats

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Abstract:

Background: Diabetes mellitus (DM) is a significant public health issue affecting millions of individuals worldwide. Its macrovascular and microvascular complication are debilitating. Anti diabetic drugs available in the market have several common adverse effects and they also pose a financial burden to many diabetic patients. On the contrary the herbal alternatives might be a better option due to lack of such adverse effects, having other beneficial effects and are a cheaper choice of treatment. **Objective:** The study was undertaken to evaluate the anti-diabetic effect of Spirulina and aqueous extract of karela compairing with an oral antidiabetic drug glimepiride. **Method:** Total 35 healthy young albino rats of both gender were used for the experiment. Diabetes was induced by intraperitoneal injection of Streptozotocin@ 50mg/kg and experiment was carried out for a period of 3 weeks. Rats were divided into 5 groups and 7 rats on each. The groups were (i) Normal control (ii) Diabetic control which receives pellet as supplement (iii) Diabetic with oral administration of Spirulina (iv) Diabetic with oral administration of Karela (v) Diabetic with oral administration of Glimepiride. **Result:** The study showed that spirulina and karela reduces blood glucose level significantly compared to diabetic control group with the increases of body weight. **Conclusion:** The results of this study showed that oral administration of spirulina and extract of karela fruits may be a good alternative antidiabetic agent.

Keywords: Diadetes mellitus, Spirulina, Karela, Streptozotocin induced Rat.

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Introduction:

Diabetes Mellitus is the most common endocrine disease and also a chronic and slowly progressive disease which has been recognized as a global epidemic. The term diabetes describes a group of metabolic disorders characterized and identified by the presence of hyperglycaemia in the absence of treatment. The heterogeneous aetio-pathology includes defects in insulin secretion, insulin action, or both and disturbances of carbohydrate, fat and protein metabolism.[1] There are two major types of diabetes mellitus type 1 and type 2 in which type 1 diabetes accounts for only 5-10%. Type 2 diabetes- This form of diabetes which accounts for 90–95% of those with diabetes.[2] Characteristic symptoms of type 2 diabetes are excessive thirst and frequent urination (polyuria), leading to the intake of large volume of water (polydipsia).[3] The disease is associated with reduced quality of life and increased risk factors for mortality and morbidity.

It is an incurable disease but, normal life can be led through controlled diet and with the use of specific anti-diabetic drug and maintaining discipline. Although, oral hypoglycemic agents and insulin are the mainstay of treatment of diabetes, they have prominent side effects and fail to significantly alter the course of diabetic complications. The common side effects

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associated with oral hypoglycemic agents are hypoglycemia, weight gain, gastrointestinal disorders and impaired liver function in addition to the cost of treatment. So that it was attempted to develop an alternative source of drug for controlling diabetes mellitus. Plant sources of hypoglycemic agents are easily available, cost-effective and presumably devoid of any side effects. Among the indigenous plants that are noteworthy for their hypoglycemic and antihyperglycemic principles include Methi, neem, telakucha, karolla, nayantara, spirulina. According to WHO, medicinal plants would be the best source to obtain variety of drugs.[4] So it was considered important to explore herbal medicinal agents in plant kingdom and develop natural resources that are readily available, cheaper around our green belt and have less side effects.

Spirulina is a photosynthesizing cyanophyte (blue green algae) that grows vigorously in strong sunshine under high temperatures and highly alkaline conditions. In 1967, Spirulina was established as a "Wonderful future food source" in the International Association of Applied Microbiology.[5] The United Nations world food conference declared Spirulina as "the best for tomorrow". Spirulina maxima is a cyanobacteria characterized by a high protein content (60–70%). It also contains carotenoids, vitamin E, phycocyanine and chlorophyll. The Spirulina has ability as a potent anti-viral, anti-cancer, anti-diabetic and decreases body weight loosing agent is gaining attention as a neutraceutical and a source of potential pharmaceutical.[6]

On the other hand Bitter gourd or karela or bitter melon (Momordica charantia) member of Cucurbitaceae family is a commonly consumed vegetable in Southern & Eastern Asia and some of the clinical trials have reported potential benefits during diabetes.[7] However, most of these studies were with inadequacies in their study design. In some cases, fresh aqueous extract of whole fruit appeared to be more effective than dried powder or dietary consumption as a vegetable.[8] Also, contradictory claims have been reported on the hypoglycaemic effects of bitter gourd and data are not conclusive to recommend its use in the management of diabetes. It is well known that dietary fibre rich diets are beneficial in the management of diabetes. Bitter gourd reduces the amount of glucose that is released into the blood by inhibiting the enzymes that break down disaccharides into two monosaccharides. The blood glucose lowering effects of Momordica charantia were closely associated with its inhibitory activity against disaccharidase.[9] This effect is important for the treatment of both Type I and Type II diabetic patients and helps to prevent high blood sugar levels after meals.

Diabetes mellitus (DM) ranked as one of the four principal non-communicable diseases (NCD) and the seventh leading cause of disability. About 537 million people had diabetes in the world and number will be rise to 642 million in 2040. Globally, 1 per 11 adults has diabetes and 11.5% of global health expenditure is spent on diabetes.[10] Both type I and type 2 diabetes are increasing worldwide in people, especially youth.[11] So it is one of the major public health problem. The disease is associated with reduce quality of life and increased risk factors for mortality and morbidity. According to the IDF statistics, presently every seven seconds someone is estimated to die from diabetes or its complications, with 50% of those deaths (4 million in total per year) occurring under the age of 60 years.[12] This is against the background of a global diabetes prevalence of 9.3% of the world population in 2019, standardized for the age group 20-79 years.

The present study was designed to evaluate the effect of Spirulina & karela on fasting blood glucose levels in streptozotocin induced diabetes in albino rats. Experimental diabetes mellitus was induced in laboratory animals by injecting drugs Streptozotocin. Using 50 mg/kg Streptozotocin dose can begin an autoimmune process that results in the destruction of the Langerhans islets beta cells.

Methods:

This was an experimental study. The study was conducted in the animal house of Department of Pharmacology, Dhaka Medical College, Dhaka. The study was carried out on the year of 2021.

Experimental animals & design: A total number of 35 healthy rats of both gender weighting 150-200 grams were selected for the study. Rats were collected from science laboratory (BCSIR), Dhaka. They were acclimatized for 7 days in well ventilated room with optimum temperature and relative humidity before starting experiment and kept in alternating 12 hours' light and 12 hours' dark cycle in the animal house of the Department of Pharmacology, DMC, Dhaka. Rats of different groups were kept in different metallic cages. They were allowed to feed on standard laboratory diet and to drink water ad libitum. Group A (Normal control group), Group B (Diabetic control group), Group C (Diabetic group treated by spirulina), Group D (Diabetic group treated by karela), Group E (Diabetic group treated by Glimepiride).

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Induction of diabetes in rats: Albino rats weighting 180-200 grams were used for inducing diabetes. The animals were injected by streptozotocin at the dose of 50mg/kg body weight intravenously. Streptozotocin induces diabetes within 3 days by destroying the beta cells.

Plant material: Karela was bought from local market and identified and authenticated by National Herbarium, Dhaka. The extract of karela was prepared in our Laboratory. The dose of karela was 200mg/kg body weight. Another plant material Spirulina was procured from Spirulina Biotech Division, BCSIR (Science Laboratory, Dhaka, Bangladesh) in the form of powder. The prepared solution was given orally once to animals throughout the experimental period by intragastric tube at 15 mg/ kg body weight.[6]

Determination of body weight: Body weight of all treated groups and control group of rats were recorded before treatment (on day 1) and at the end 21th day. A properly calibrated and standardized electronic balance was used for taking body weight of the rat and expressed as gram(g).

Determination of blood glucose: Blood was collected from each of the animal at day 1 and day 4 by aseptically cutting the tail at the tip with a sharp sterile blade and on day 21 by cardiac puncture. Blood glucose level were estimated by glucometer. Glucolab blood glucose meter manufactured by Infopiaco. Ltd, korea was used. Test results were demonstrated in mmol/L. For diagnosis of diabetes mellitus we follow the WHO guideline.

Sacrifice of animals: The animals were sacrificed under ether anesthesia. The abdomen was opened and the whole of the pancreas was dissected out. The pancreas was then examined thoroughly by naked eye and was placed in a container of 10% formalin for fixation sectioning.

Histological method: Tissues were kept in 10% formalin for five days. The tissues were sectioned into pieces which were gradually dehydrated in ascending concentration of ethyl alcohol. Tissues were stained by hematoxylin eosin stain. Histology of pancreas were observed from the slides stained in hematoxylin-eosin stain.

Statistical analysis: Statistical analysis was done by SPSS 22 software. Data were expressed as Mean +/- Standard deviation. Student's paired and unpaired 't' test were used to compare the results. The test results was considered as statistically significant at Probability (p) value < 0.05 at 95% CI .

Results:

The effect of Spirulina and extract of Karela on body weight of streptozotocin induced diabetic rats is shown in table-I. Body weight was measured at the beginning and at the end of study. The average body weight of Group A (Normal control) were 192.14±9.34gm and 203.05±8.91gm at day 1 and day 21. On the other hand, Group B (Diabetic control) showed 196.65±8.54gm and 154.56±3.65gm respectively. Here body weight significantly decrease in Group B. Measuring, others group body weight increase in group C, D and E. But significant increase in body weight occurs in Group C and Group E.

Table-I: Effect of Spirulina and Karela on body weight (gm) in streptozotocin induced diabetic rats.

Group	Body weight(gm)		P value
	Day 1(onset)	Day 21(End)	
A	192.14 ± 9.34	203.05 ± 8.91	0.0451
B	196.65 ± 8.54	154.56 ± 3.65	<0.001*
C	190.76 ± 4.37	200.81 ± 3.44	<0.001*
D	188.07 ±8.83	196.86 ± 8.51	0.0822
E	198.66 ± 8.25	211.15 ± 6.27	0.008*

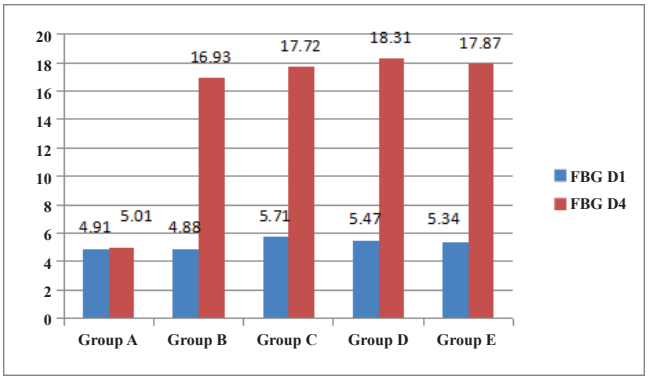

Figure-1: Blood glucose level of different group after induction of diabetes by streptozotocin at day 4.

Figure 1 shows raising blood glucose level in different group after induction of diabetes by streptozotocin @50mg/kg at day 4. Blood glucose level was significantly raise in all group(P<0.05) except group A.

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Table-II: Effect of Spirulina and Karela on blood glucose level in streptozotocin induced diabetic rats

Group	FBG D4	FBG D21	P value
A	5.01 ± 0.68	5.12±0.27	0.698
B	16.93±1.72	17.63±2.07	0.504 ns
C	17.72±1.81	9.18±1.46	< 0.001 s
D	18.31±1.96	8.55±1.26	< 0.001 s
E	17.87±2.16	7.61±1.04	< 0.001 s

The effects of spirulina and karela on rats blood glucose levels are presented in Table II. Comparison of pre and post treatment blood glucose level of different group was done. The results indicated, pre-treatment with streptozotocin induced hyperglycemia in group C, D and E was reduced by treatment with spirulina 15mg/kg bwt, karela 200 mg/kg bwt and glimepiride 800mcg/kg bwt.

Histopathology:

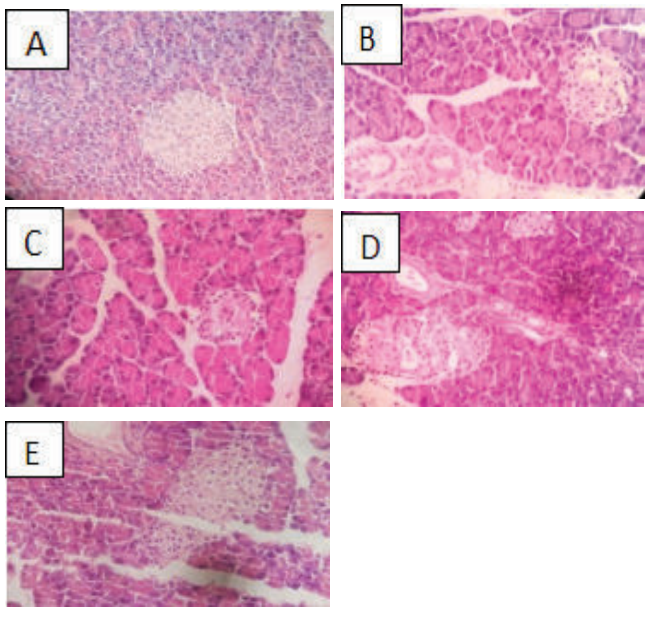

Figure-2: Photomicrograph of rat pancreas stained by haematoxylin and eosin (A) Normal control (B) diabetic control (C) Spirulina treated (D) karolla treated (E) Glimepiride treated.

In (A) light micrograph of the pancreas of normal rat consist of exocrine and endocrine portion. The endocrine portion consist of Islets of Langerhans(IL) (B) Pancreas of untreated diabetic rat after 3 weeks of experiment showed necrosis and vaculation. (C) Pancreas of diabetic rat treated with Spirulina showing little damage. (D) Pancreas of diabetic rat treated with Karela showing degenerative change with increased number of cells at Islets.(E) Pancreas of glimepiride treated rat showing mild damage with well number of cells at Islets.

Discussion:

The result of effect of spirulina and extract of karela on body weight of streptozotocin induced diabetic rat shown in Table1. In our study streptozotocin induced rat showed severe loss in body weight which has been reported by Erejuwa et al., 2010.[13] This may be due to decrease production of protein synthesis in all tissues due to decrease production of adenosine triphosphate or absolute and relative deficiency of insulin. Administration of spirulina increased body weight significantly (P< 0.05) which was also reported by other study.[14] However diabetic rat treated with spirulina increase body weight may be explained by increased insulin secretion or increased food consumption. On the other hand karela also increase body weight at 200mg/kg body weight compairing initial weight but it was not significant at our study. It may be due to variation of dose where some study use 400mg/kg.

From Figure 1 it is evident that there was an increase of blood glucose after administration of streptozotocin and it was significant (P< 0.05) among all group except group A. The antihyperglycemic effect of spirulina, karela and standard oral drug glimepiride presented in Table 2. In the present study, it was observed that oral administration of spirulina could reverse the above mention diabetic effects. Layem and Reddy also found similar effect in their stydy at India.[6] The possible mechanism may be potentiation of the pancreatic secretion of insulin from islets ß—cell or due to enhanced blood glucose to the peripheral tissue.

The result obtained in this study demonstrated karela decrease blood glucose level in streptozotocin induced diabetic rat. The observed reduction in blood glucose was statistically significant. The decrease in blood glucose produced by the extract at this dose is also comparable to glimepiride , a standard hypoglycemic drug.Miura et al,2001 conducted a study that showed significant reduction in blood glucose of diabetic animal after the administration of karela fruit juice that is similar to our study.[15] Another study shows karela at 200mg/kg not enough to reduce blood glucose significantly.[16] However our study showed that karela has insulinomimetic effects and possible mechanism by decreased intestinal absorption of glucose but this is not experimentally established. We suggest more study to establish the mechanism by which the plant exerts its antidiabetic effect.

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Histopathological findings of our study showed atrophy and necrotic changes in cell structure on diabetic rats. But the pancreas treated with spirulina and karela showed minimal necrosi and increase number of cell in the islets of Langerhans.

Conclusion:

Our results suggest that spirulina and extract of karela supplementation demonstrated significant positive effect on streptozotocin induced diabetic rats. Therefore, spirulina and karela could be an effective alternative therapy for diabetic patients.

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