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Jamun (*Syzygium cumini*) seed and fruit extract attenuate hyperglycemia in diabetic rats

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ABSTRACT

Objective: To evaluate the potential of both jamun (*Syzygium cumini*) seed and fruit extracts against hyperglycemia.
Methods: Male Sprague Dawley rats were used to evaluate hypoglycemic potential of jamun extracts. Purposely, jamun fruit and seed's ethanolic extracts based diets were provided to normal and high sucrose diet induced hyperglycemic/diabetic rats for sixty days. The serum glucose and insulin levels were monitored at monthly intervals to evaluate hypoglycemic effect of jamun extracts.
Results: The results of instant research depicted that both seed and fruit extracts reduce the blood glucose level significantly and also regulate the insulin levels in hyperglycemic rats. It was noted that jamun fruit extract attenuated serum glucose levels to 5.35% and 12.29% in normal and hyperglycemic rats, respectively; while insulin levels were improved by 2.82% and 6.19%, correspondingly. Whereas, jamun seed extract reduced glucose to 7.04% & 14.36% and showed 3.56% & 7.24% higher insulin levels in normal & hyperglycemic rats, respectively.
Conclusions: The present research revealed that both jamun fruit and seeds have potent prophylactic role against hyperglycemia. In this respect, diet based regimen may be tailored using jamun fruit/seed and their extracts to alleviate hyperglycemia.

1. Introduction

Diabetes mellitus is a chronic disease characterized by high blood glucose level. Globally, it is known as major cause of morbidity and mortality. This impact has been mainly marked in developing countries like Pakistan. Diabetes leads to cardio-vascular diseases, particularly coronary heart disease [1]. Plant based diet including fruits, vegetables, herbs and spices are regarded as valuable source of phytonutrients with positive effects on individual's health. The ability of several plant based foods to lessen the threat of chronic disorders has been linked with the presence of non-nutritive secondary metabolites known as phytochemicals, possessing various biologically active moieties [2]. These secondary metabolites are less effective as bioactive ingredients as compared to pharmaceuticals; meanwhile these are consumed in sufficient quantity as a part of the regular diet. They possibly exert significant long-term health promoting effects in human body. The bioactivity of these phytonutrients has been associated to their antioxidant characteristics as they scavenge free radicals, which are responsible for the development of several severe degenerative disorders like LDL oxidation, DNA oxidation, inflammation and ageing [3]. Such types of health promoting products cannot be accurately categorized as food. Therefore, a new hybrid term 'nutraceuticals' has been coined between nutrients and pharmaceuticals [4].

A nutraceutical is described as a food or part of food that has proven to show therapeutics effects against different diseases together with the avoidance and cure of ailment [5]. A huge number of herbal medicines have been listed with hypoglycemic properties throughout the world. Jamun is one of the fruit of ancient times which are usually practiced by medical practitioner for the treatment of diabetes. In the same way, Sharma *et al.* [6] reported the antidiabetic potential of

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jamun seeds. Jamun, botanically named as *Syzygium cumini*, belongs to family Myrtaceae. Its fruit is generally acknowledged to be very high quality for its curative function chiefly against diabetes because of its effect on pancreas [7]. Jamun holds antihypercholesterolemic properties and helps in regulating the blood lipid profile due to presence of bioactive components [8]. Studies accomplished in last twenty years have explored that jamun have got good complex of naturally present antioxidant compounds [9]. Recently, studies have shown that jamun fruit and seed contain significant quantity of antioxidant compounds such as phenolic acids, flavonoids and anthocyanins. These bioactive compounds are helpful in preventing different metabolic syndromes [10]. The present research was carried out to evaluate the antihyperglycemic properties of jamun fruit and seed.

2. Materials and methods

2.1. Raw material procurement

The jamun fruits were obtained from the local market. The reagents (analytical and HPLC grade) and standards were purchased from Merck (Merck KGaA, Darmstadt, Germany) and Sigma–Aldrich (Sigma–Aldrich Tokyo, Japan). Male Sprague Dawley rats used in the bio evaluation trials were obtained from National Institute of Health (NIH), Islamabad. The Diagnostic Kits used for various analyses were from Sigma–Aldrich, Bioassay (Bioassays Chemical Co., Germany) and Cayman Chemicals (Cayman Europe, Estonia).

2.2. Sample preparation

Jamun fruit was washed and then fruits and seeds were separated. The fruits and seeds were then dried in tunnel drier at 40 °C for 24 h. The dried fruits and seeds were ground to a fine powder using a grinder (PHILIPS 600W Type HR 2068). The resultant powders were packed in air tight glass jars and stored in laboratory cabinet at room temperature of 25–30 °C for further investigations.

2.3. Preparation of jamun extracts

The jamun extracts were prepared using binary solvent *i.e.* aqueous ethanol (50% v/v). About 50 g sample was added in volumetric flasks followed by the addition of solvent. Afterward, the volumetric flasks were placed in orbital shaker operating at 280 rpm and 50 °C at temperature for a time length of 45 min. Later on, all extracts were filtered. The filtrate was then evaporated using Rotary Evaporator (Eyela, Japan) at 40 °C under reduced pressure for the removal of solvent. The extracts were then stored in sealed bottles for future use [11].

2.4. In vivo studies

The present study was carried out on 40 Sprague Dawley rats procured from National Institute of Health (NIH), Islamabad for model feeding trials. International guidelines for the use of laboratory animals were followed during rodent feed trial. All the rats were housed in well ventilated metal cages and provided normal diet for two weeks before the experiment for acclimatization and to ensure the normal growth and behavior. They were fed on normal diet and tap water *ad libitum*. At the commencement of study, some rats were slaughtered to get baseline values. For efficacy trials, the research was carried out in two categories; normal and hyperglycemic. In all studies, three groups of rats were planned to have five rats in each. One group was given control diet, while other two groups were fed on respective test diets respectively, during sixty days trial period. During entire study span, the animal room was maintained at a temperature of (23 ± 2) °C and relative humidity of 55% ± 5%, with 12 h light/dark cycle. At the culmination of the study, the overnight fasted rats were slaughtered to probe the effect of respective treatments on the selected parameters including glucose & insulin levels as well as hematological studies.

In Study I, for an initial period of one week, basal diet was given to the rats to acclimatize them to the environment. Later, the diets containing jamun fruit and seed extracts were fed for sixty days. The experimental diet consisted of flour (82%), corn oil (10%), casein (4%), mineral mix (3%) and vitamin mix (1%).

In Study II, high sucrose diet containing 40% sucrose was provided to the normal rats to determine the effect on serum glucose and insulin levels. Simultaneously, the effect of fruit and seed extracts containing diets on the induced trait in relevant groups of rats was assessed.

Nutraceutical (SE) and Nutraceutical (FE) diets were prepared by adding 3% extracts of seed and fruit in normal diet, respectively.

2.5. Serum glucose and insulin levels

For each Study, the collected sera were evaluated for glucose concentration by GOD-PAP method as described by Kim *et al.* [12] whereas insulin level was assessed following the method of Ahn *et al.* [13].

2.6. Statistical analysis

The data obtained from entire study were subjected to statistical analysis to determine the level of significance as described by Steel *et al.* [14]. Analysis of variance was calculated by ANOVA test and means were interrupted by Duncan's Multiple Range test.

2.7. Ethical approval

Ethics approval was given by the head of the National Institute of Food Science and Technology, University of Agriculture Faisalabad (UAF), Pakistan, by reviewing the suggestions of Animal Experimentation Ethics Committee, UAF. Animal experiments were conducted accordance with the instructions for the care and use provided by the committee and instructed by the university.

3. Results

3.1. Glucose

Statistical analysis of responses regarding serum glucose indicated significant variations due to treatments and time intervals. Means values for serum glucose levels in normal and hyperglycemic rats are presented in Table 1. In Study I, highest

Table 1
Effect of diet and intervals on serum glucose (mg/dL) of rats in different studies.

Studies	Diet	Study intervals (Days)			Means
		0	30	60	
Study I	Control	91.25 ± 3.83	91.61 ± 3.84	91.88 ± 3.85	91.58 ± 3.84[a]
	Nutraceutical (FE)	90.48 ± 3.80	87.79 ± 3.68	85.63 ± 3.65	87.97 ± 3.68[b]
	Nutraceutical (SE)	89.62 ± 3.67	86.11 ± 3.52	83.31 ± 3.49	86.24 ± 3.51[b]
	Means	90.45 ± 3.82[a]	88.50 ± 3.57[ab]	86.94 ± 3.53[b]	–
Study II	Control	139.17 ± 6.40[ab]	147.06 ± 6.78[a]	148.65 ± 6.83[a]	144.96 ± 6.47[a]
	Nutraceutical (FE)	138.46 ± 6.36[abc]	129.23 ± 5.94[bcd]	121.41 ± 5.58[d]	129.70 ± 5.97[b]
	Nutraceutical (SE)	137.02 ± 6.31[abc]	126.27 ± 5.67[cd]	117.34 ± 5.39[d]	126.88 ± 5.82[b]
	Means	138.22 ± 6.35[a]	134.19 ± 6.07[ab]	129.13 ± 5.78[b]	–

Data with different alphabetical letters in a column or row have significant difference ($P < 0.05$). Study I: Normal rats; Study II: Hyperglycemic rats; FE: Jamun Fruit Extract enriched diet; SE: Jamun Seed Extract enriched diet.

value for glucose (91.58 ± 3.84) mg/dL was measured in control diet group while Nutraceutical (SE) and Nutraceutical (FE) diet groups showed lower levels, (86.24 ± 3.51) mg/dL and (87.97 ± 3.68) mg/dL, respectively. Sixty days of study led to an increase in glucose level of control group subjects from (91.25 ± 3.83) to (91.88 ± 3.85) mg/dL. Nutraceutical (SE) diet showed a reduction from (89.62 ± 3.67) to (83.31 ± 3.49) mg/dL while Nutraceutical (FE) from (90.48 ± 3.80) to (85.63 ± 3.65) mg/dL over sixty days. Likewise, in Study II, hyperglycemic control diet group showed elevated glucose level (144.96 ± 6.47) mg/dL. However, lower levels were observed in Nutraceutical (SE) (126.88 ± 5.82) mg/dL and Nutraceutical (FE) (129.70 ± 5.97) mg/dL diet groups. During the study, control group serum glucose increased from (139.17 ± 6.40) mg/dL at 0 day to (148.65 ± 6.83) mg/dL after 60 days. With Nutraceutical (SE) diet, there was reduction from (137.02 ± 6.31) to (117.34 ± 5.39) mg/dL while it was reduced in the Nutraceutical (FE) diet group from (138.46 ± 6.36) to (121.41 ± 5.58) mg/dL over sixty days period. From study, it may be concluded that the jamun seed extract treatment [Nutraceutical (SE)] resulted in highest glucose reduction by 14.36% in the hyperglycemic rats (Study II) while 12.29% reduction was observed in the same study on the provision of jamun fruit extract [Nutraceutical (FE)]. However, in the control group, 7.04% and 5.35% decrease was recorded by provision of Nutraceutical (SE) and Nutraceutical (FE) diets respectively.

3.2. Insulin

With regard to insulin, the data explicated that diet affected insulin level momentously while the interval was non-significant in both studies. It can be observed from means that in Study I the control group showed (8.74 ± 0.30) while (8.33 ± 0.31) and (8.19 ± 0.28) μU/mL for the Nutraceutical (FE) and Nutraceutical (SE) diet groups, respectively. During trial interval, diet with jamun fruit caused non-momentous elevation in insulin levels of rats with an increase from (8.21 ± 0.31) to (8.44 ± 0.32) μU/mL in Nutraceutical (FE) while (8.05 ± 0.28) to (8.33 ± 0.29) μU/mL in Nutraceutical (SE) group. It is obvious that percent increase in insulin level was 2.82% and 3.56% for Nutraceutical (FE) and Nutraceutical (SE) groups, respectively (Table 2). The means for serum insulin in Study II depicted an increasing trend with respect to the base line values. In this study, (12.20 ± 0.52) μU/mL was recorded for hyperglycemic rats for the control diet group while (11.52 ± 0.53) and (11.97 ± 0.50) μU/mL for Nutraceutical (SE) and Nutraceutical (FE) groups respectively. During the study period, maximum increase was recorded for Nutraceutical (SE) diet as (11.10 ± 0.49) to (12.90 ± 0.55) μU/mL (7.24%). However, $\text{nutraceuticals}_{FE}$ showed lesser increment from (11.59 ± 0.54) to (12.31 ± 0.57) μU/mL (6.19%) over sixty days period.

4. Discussion

Diabetes mellitus is a metabolic syndrome of complex and multiple etiologies; chronic hyperglycemia and long-term complications are often associated [15]. The overall prevalence of diabetes mellitus around the globe is approx. 6% out of which 90% is type II diabetes [16]. The therapy includes lifestyle management, weight control, regular exercise and use of antiglycemic nutraceutics & drugs at extreme conditions [17]. In this regard, jamun has widespread therapeutic applications and the current study proposed its hypoglycemic effect.

Earlier, Grover *et al.* [18] probed the effect of alcoholic extract of jamun on blood glucose parameters. Male Wistar rats were made diabetic through injection of alloxan. After

Table 2
Effect of diet and intervals on serum insulin (μU/mL) of rats in different studies.

Studies	Diet	Study intervals (Days)			Means
		0	30	60	
Study I	Control	8.73 ± 0.30	8.74 ± 0.31	8.76 ± 0.29	8.74 ± 0.30[a]
	Nutraceutical (FE)	8.21 ± 0.31	8.35 ± 0.32	8.44 ± 0.32	8.33 ± 0.31[b]
	Nutraceutical (SE)	8.05 ± 0.28	8.21 ± 0.29	8.33 ± 0.29	8.19 ± 0.28[b]
	Means	8.33 ± 0.30	8.43 ± 0.31	8.51 ± 0.29	–
Study II	Control	12.34 ± 0.53	12.19 ± 0.52	12.09 ± 0.51	12.20 ± 0.52[a]
	Nutraceutical (FE)	11.59 ± 0.54	12.02 ± 0.56	12.31 ± 0.57	11.97 ± 0.56[a]
	Nutraceutical (SE)	11.10 ± 0.49	11.55 ± 0.53	11.90 ± 0.55	11.52 ± 0.53[b]
	Means	12.68 ± 0.50[b]	11.92 ± 0.51[ab]	12.10 ± 52.00[a]	–

Data with different alphabetical letters in a column or row have significant difference ($P < 0.05$).

induction of diabetes, three doses of jamun seed extracts were given to rats at 50, 100 and 200 mg/kg per day. Rats were treated for a period of three weeks. Significant reductions in blood glucose in diabetic rats were observed when treated with jamun extract. The outcome is in harmony with results. It was concluded that alcoholic extract of jamun seed imparted momentous reduction in blood glucose level in diabetic rats. According to Tripathi and Kohli [19], the effect of ethyl acetate and methanolic extract of jamun was observed on normal and streptozotocin-induced diabetic rats. Jamun seed extract was given to rats at two different concentrations (200 & 400 mg/kg) for 15 days in comparison to glibenclamide; an antidiabetic drug. It was concluded that jamun seed extract significantly lowered serum glucose. The results of the present study are consistent with the reports of Shankar [20] who recorded 65% decrease in glucose level of jamun seed fed rats. They inferred that jamun exerts positive influence on the insulin secretion from the pancreatic β-cells thereby reducing the glucose levels. Sharma *et al.* [21] evaluated the hypoglycemic potential of jamun seed using ethanolic extract on the alloxan-induced diabetic rabbits. On the provision of ethanolic extract of jamun seed to the diabetic rabbits, decline of 42.85% was observed in the blood sugar concentration.

Findings of Safdar *et al.* [22] are analogous to the current results. They evaluated the hypoglycemic effects of jamun pulp on diabetic individuals for 12 days. They observed a positive relationship between the intake of jamun fruit and consequent decrease in the abnormal glucose levels of the individuals. Decrease up to 9.1% was recorded in serum glucose level. The current findings are also in accordance with Sharma *et al.* [6], they explored that administration of jamun fruit ethanolic and water extract to diabetic rabbits for 15 days showed 14.8% and 37.1% reduction in serum glucose, respectively.

Previously, Sharma *et al.* [23] analyzed the hypoglycemic worth of jamun seed on rabbits. The authors provided supplemented diets to male rabbits for 15 days and recorded a substantial increase in insulin levels *i.e.* 38.4% in mild diabetic and 44% in severe diabetic animals. Furthermore, Sharma *et al.* [24] studied the ethanolic extract of jamun seed in diabetic animals which showed 26.9% increase in insulin levels. Previously, researchers demonstrated the effect of jamun pulp in diabetic rats and recorded 26.3% increase in insulin after the treatment of fruit extract for 15 days [6]. The antihyperglycemic effect of jamun seed was documented and results showed momentous effect on the insulin release [21].

Conclusively, jamun fruit and seed hold nutraceutical worth to address various diet related malfunctions especially hyperglycemia. In the current research, jamun seed and fruit extracts proved effectual in the regulation of blood glucose and insulin parameters. Likewise, hyperglycemia and hyperinsulinemia were also managed by the provision of jamun seed extracts. Results exhibited that fruit has lower potential than seed in reducing the sugar levels of diabetic rats. It can be concluded that jamun is potential source of naturally occurring bioactive components, thus regulating the blood glucose profile and may be used as curing therapy in diabetes.

Conflict of interest statement

All authors declare no conflicts of interest.

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