Jackfruit Seed Powder Supplementation Attenuates High-Sugar Diet-Induced Hyperphagia and Hyperglycemia in Mice †

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Abstract: High-sugar diets (HSD) are strongly associated with the development of obesity, diabetes, and other metabolic diseases. Diets that are rich in dietary fiber have been reported to have substantial health benefits. Jackfruit seed powder (JSP) is a good source of dietary fiber and could be a possible candidate to fight against metabolic diseases. JSP supplementation showed a significant reduction in HSD-induced hyperphagia and also in body weight gain. The addition of JSP significantly improved glucose tolerance and reduced LDL cholesterol. Overall, JSP consumption could play a vital role in the management of metabolic disorders caused by HSD.

Keywords: jackfruit seeds; hyperphagia; hyperglycemia; hyperlipidemia; high-sugar diet

1. Introduction

Artocarpus heterophyllus Lam., which is commonly known as jackfruit, is a tropical climacteric fruit belonging to Moraceae family [1]. This fruit is grown in different parts of Asia, Africa, and South America [2]. Jackfruit grows in warm and moist regions [3]. It is most widely cultivated in Bangladesh, Burma, Malaysia, Indonesia, Thailand, and on a smaller scale in Brazil and Australia [4]. Jackfruit is the national fruit of Bangladesh, and is known as “kathal”. The jackfruit ranks third in area of cultivation and second in production among the fruits of Bangladesh. Jackfruit has been reported as an abundant source of protein, potassium, thiamine, niacin, calcium, sodium, magnesium, and vitamin B6 [5]. Commonly, the pulps of mature and ripe jackfruits are eaten by people. Jackfruit seeds are normally discarded or sometimes kept for consumption. As jackfruit is highly seasonal and seeds have a shorter shelf life, these seeds go to waste during the seasonal glut. In rural areas, seeds are dried and roasted to consume as snacks. Though the nutritional properties of jackfruit seeds have not yet been fully explored, it is a good source of protein, starch, and dietary fiber. The protein concentration of jackfruit seeds may vary from 5.3 to 6.8% [6]. Jackfruit seeds contain lignans, isoflavones, saponins, and other phytonutrients, which have a wide range of health benefits [7–9]. Jackfruit seeds are also a rich source of many minerals such as N, P, K, Ca, Mg, S, Zn, Cu, etc. [10]. Because of their high fiber content, the seeds can lessen the risk of heart disease, prevent constipation, and encourage weight loss [11]. Resistant starch, found in jackfruit seeds, helps to regulate blood sugar and maintain gut health [12]. Antimicrobial activity in jackfruit seeds helps to prevent foodborne illnesses [10,13]. A large number of fruits and seeds are produced during part of the year in Bangladesh. As the seeds are recalcitrant, they germinate immediately after maturity. Therefore, fresh seeds cannot be kept for long time. As a result, a large amount of...
the seeds remain unused. However, seed flour can be an alternative product to be used in some foodstuffs [14]. To best of our knowledge, no study has yet been done to evaluate the physiological significance of jackfruit seed consumption.

Diabetes mellitus is a disease of modern times which is characterized by a disorder of carbohydrate, fat, and protein metabolism [15,16]. Whereas obesity is known as the accumulation of excess body fat resulting from a chronic imbalance of energy due to excess consumption of nutrients, inadequate physical activity, or other factors. Excess energy intake combined with low energy expenditure induces lipid accumulation not only in adipose tissue but also in liver, muscle, and other internal tissues. High-sugar diets (HSD) accelerate body weight gain [17–19]. A previous study reported that high consumption of sugar develops insulin resistance and hyperglycemia in rats [20]. Furthermore, hepatic steatosis (grade 1) and increase in triglyceride concentration due to HSD have also been reported [20]. The phytonutrients such as lignans, saponins, and isoflavones present in the jackfruit seeds play beneficial roles in human health [21]. Seeds make up around 10% to 15% of the total fruit weight and are a good source of dietary fiber [22]. Dietary fiber has been shown to improve blood glucose control by trapping ingested carbohydrates inside the viscous gel formed after digestion. The proteolytic activities of different animal pancreatic preparations were reported to be inhibited effectively by jackfruit seed extract [23]. Consumption of soluble dietary fiber reduces postprandial glucose responses after carbohydrate-rich meals, as well as lowering total and LDL cholesterol levels [24]. In a previous report, authors mentioned that resistant starch present in jackfruit seeds may control blood sugar and keep the gut healthy [12]. Therefore, we hypothesize that supplementation of jackfruit seed flour may positively contribute to metabolic disorders through glucose and lipid homeostasis.

Lesser-known and underutilized agricultural commodities which have beneficial effects on human health have been focused on by the research community in recent years. Jackfruit seeds also contain resistant starch, which helps to maintain blood glucose homeostasis and keep a healthy gut. Jackfruit seeds contain a number of phytochemicals which have antioxidant and anticancer activity [25]. However, no study exists on the impact of jackfruit seed powder supplementation on glucose and lipid homeostasis. Therefore, this study was undertaken to evaluate the potential benefits of jackfruit seed powder supplementation to maintain glucose and lipid homeostasis.

2. Materials and Methods
2.1. Collection of Jackfruit Seeds and Powder Preparation

Mature and ripe Jackfruits were collected from the local market of Mymensingh, Bangladesh. The fruits were opened and the required seeds inside the pulps were collected. The collected seeds were washed properly, sliced, and dried under the sun. After proper sun drying, the seeds were kept in an oven at 60 °C for 24 h to remove the moisture completely. Dried pieces were finely ground with a grinding machine and stored in polythene bags until further use.

2.2. Food Formulation and Diet Paradigms

Normal food formulation includes wheat, wheat bran, polished rice, fish meal, oil cake, gram, pulses, milk, soybean oil, molasses, salt and embavit (vitamin) at different proportions as shown in Table 1 [26]. Three diet paradigms deployed in this study were normal food formulation, 30% (w/w) sucrose, and 30% (w/w) sucrose in combination with 20% (w/w) JSP. The justification of the dose refers to previous studies [27,28]. To examine the potential benefits of JSP to ameliorate the deleterious effects of high-sugar diets, the animals were randomized into three groups. All the animals of the first group received normal food formulation (denoted as ND: normal diet), the mice of the second group (denoted as HSD: high-sugar diet) received a normal diet supplemented with 30% sucrose, and the third group (denoted as HSD+JSP: high-sugar diet + jackfruit seed powder) received normal diet supplemented with 30% sucrose and 20% JSP. The diets were provided ad libitum for
animals and changed daily to ensure their quality. Each treatment group consisted of at least four mice housed in individual cages. The treatment using diet paradigms was carried out for 8 weeks continuously.

Table 1. Composition of normal food formulation used in this study (for 100 g).

<table>
<thead>
<tr>
<th>Ingredients of Normal Lab Diet</th>
<th>Percent</th>
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<tbody>
<tr>
<td>Wheat</td>
<td>40%</td>
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<tr>
<td>Wheat bran</td>
<td>20%</td>
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<tr>
<td>Polished Rice</td>
<td>5.5%</td>
</tr>
<tr>
<td>Fish meal</td>
<td>10.0%</td>
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<tr>
<td>Oil cake</td>
<td>6.0%</td>
</tr>
<tr>
<td>Gram</td>
<td>0.39%</td>
</tr>
<tr>
<td>Pulses</td>
<td>0.39%</td>
</tr>
<tr>
<td>Milk</td>
<td>0.38%</td>
</tr>
<tr>
<td>Soybean Oil</td>
<td>1.5%</td>
</tr>
<tr>
<td>Molasses</td>
<td>0.095%</td>
</tr>
<tr>
<td>Salt</td>
<td>0.095%</td>
</tr>
<tr>
<td>Embavit (vitamin)</td>
<td>0.1%</td>
</tr>
</tbody>
</table>

2.3. Experimental Animals

Six week-old Swiss albino male mice were obtained from the Animal Resources Facility of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) and adapted for 10 days in order to acclimatize them with the new environment. Animals were housed in a well-ventilated room at 28 ± 2°C and a relative humidity of 70–80% with natural daylight. Normal food and water were available ad libitum before the start of feeding experiments. Animals were divided into three groups and each group contained at least 4 mice. During the rearing period, animals were also habituated for handling every day to minimize the stress response that may occur in the experiment. All protocols used in this study were approved on February 2020 (AWEEC/BAU/2020_30) by the Animal Welfare and Experimentation Ethics Committee of Bangladesh Agricultural University, Bangladesh guided by the Council for International Organizations of Medical Sciences international guiding principles of biomedical research involving animals.

2.4. Measurement of Food Intake

Food intake by the individual mouse was measured weekly at 10:00 am for 8 weeks according to the following formula:

Food intake = Initial food weight − remaining food weight

2.5. Measurement of Body Weight

The body weight of each mouse was measured with the help of an electric balance (eki300-2n electronic scale, A&D company Ltd., Korea) at 7-day intervals up until the end of the experiment. Change in body weight (ΔBW) of each mouse was also calculated at the end of the feeding experiment.

2.6. Intraperitoneal Glucose Tolerance Test

The intraperitoneal glucose tolerance test (ipGTT) was conducted at the end of treatment by following the standard procedure as described in another report [29]. Mice were fasted for approximately 4 h by transferring the mice to clean cages with no food or feces in the hopper or bottom of the cage. Access to drinking water was ensured at all times. The tip of the tail was scored using a fresh or sterilized scalpel blade. The first small drop of blood was discarded. A small drop of blood (<5 µL) was placed on the test strip of the blood glucose meter. Blood glucose level was measured using a standardized automated blood glucose test meter (GlucoleaderTM Enhance Blood Glucose Meter, HMD Biomedical Inc., Hsinchu County, Taiwan). A single dose of glucose (2 g/kg BW) was injected intraperi-
toneally for each mouse. The concentration of blood glucose was recorded for each mouse at 0, 15, 30, 60, and 120 min after ip glucose administration. The area under curve (AUC) data was subsequently calculated from the blood glucose levels in ipGTT.

2.7. Blood Samples Collection and Preparation of Serum

At the end of 8 weeks, blood samples were collected from the posterior vena cava using the method described previously [30]. The mice were placed one by one into an air-tight container containing cotton soaked with chloroform. The abdominal cavities of anesthetized mice were opened by making a V-cut through the skin and abdominal wall 1 cm caudal to the rib cage. The intestines were shifted over to the left and the liver was pushed forward. The widest part of the posterior vena cava (between the kidneys) was located. A 26-gauge needle and a 1 mL syringe were used. The needle was carefully inserted into the vein and blood was drawn slowly until the vessel wall collapsed. The blood was collected in a 1.5 mL Eppendorf tube containing EDTA which acted as an anticoagulant. Then, the blood-containing tubes were centrifuged at 4000 rpm for 10 min at 4 °C (Gyrozen 1580R Multi-Purpose High-Speed Refrigerated Centrifuge, Gangnam-gu, Seoul, Korea). After centrifugation, the supernatant serum without unwanted blood cells was collected in a new tube. Serum samples were stored at −20 °C until lipid profile assay.

2.8. Measurement of Organ Weight

After collecting the blood samples, the internal organs such as the liver, heart, kidney, white adipose tissue (WAT), and brown adipose tissue (BAT) were harvested and trimmed to remove additional tissues. The organs were cleaned in saline solution and placed on a filter paper to remove the saline on the surface. Then, the organ weights were measured using a digital balance (eki300-2n electronic scale, A&D company Ltd., Seoul, Korea).

2.9. Determination of Lipid Profile Parameters

Lipid profile studies involved analysis of parameters such as total cholesterol (TC) level determined by the CHOD-PAP method [31], triglyceride (TG) level determined by GPO-PAP method [32], and HDL cholesterol level determined by CHOD-PAP method [33]. The HumaTex febrile antigen test kit (Human Diagnostic, Wiesbaden, Germany) was used and the absorbance of all the tests was determined using Humalyzer, Model No-3000 (Human GmbH, Wiesbaden, Germany). Serum LDL cholesterol concentrations were calculated using the Friedewald equation [34] as follows:

\[
LDL \text{ cholesterol (mg/dL)} = \text{Total cholesterol} - \text{HDL cholesterol} - \left( \frac{\text{Triglyceride}}{5} \right)
\]

2.10. Statistical Analysis

All statistical analyses were performed using Prism 5 (GraphPad Software 7.0, CA). All data were displayed as mean ± SE. An analysis of variance (ANOVA) followed by Tukey’s post hoc test was employed to justify the significant differences among treatment groups. The \(p < 0.05\) was set as a significant value for all analyses.

3. Results

3.1. Effect of Jackfruit Seed Powder on Food Intake of Mice

We carried out food intake measurements throughout the experimental period of 8 weeks. There was no significant difference in weekly food intake among the groups at the beginning of the experiment (Figure 1). However, 30% sucrose supplementation and 30% sucrose and 20% Jackfruit seed powder (JSP) supplementation in the food influenced the food intake per mouse from the 2nd week of the treatment \((38.00 \pm 3.34 \text{ g for ND, } 42.50 \pm 4.57 \text{ g for HSD, } 36.00 \pm 1.73 \text{ g for HSD + JSP})\). Although the high-sugar diet increased the food intake compared to the normal diet, it was significant only after the 6th week of the treatment. The high-sugar diet (HSD) supplementation showed an increase in food intake in contrast to the normal diet (control), which was reversed by the addition of
JSP. JSP supplementation significantly reduced food intake in comparison to the high-sugar diet group (30% sucrose) from the 3rd week (40.00 ± 3.51 g for ND, 48.25 ± 4.39 g for HSD, 36.25 ± 3.99 g for HSD + JSP) to the 8th week (42.50 ± 3.95 g for ND, 57.00 ± 1.22 g for HSD, 40.25 ± 3.01 g for HSD + JSP) of the experiment (Figure 1). The food intake was comparable between the control group and the HSD + JSP group.

Figure 1. JSP supplementation attenuated HSD-induced hyperphagia. ND: Normal Diet; HSD: High-Sugar Diet; and JSP: Jackfruit Seed Powder. *p < 0.05 vs. ND; #p < 0.05 vs. HSD by one-way ANOVA followed by Tukey’s post hoc test. Bars represent mean ± SEM. n ≥ 3 for each group.

3.2. Effect of Jackfruit Seed Powder on Body Weight of Mice

We also measured the body weight of each mouse to reveal the effectivity of jackfruit seed powder (JSP) in mitigating the development of HSD-induced obesity. The results showed that body weight tended to be lower in the JSP-supplemented group (37.50 ± 1.55 g for ND, 41.75 ± 3.94 g for HSD, 33.25 ± 0.75 g for HSD + JSP at 2nd week) compared to the normal diet (control) - sugar diet (HSD) supplementation showed an increase in food intake per mouse from the 2nd week of the treatment (38.00 ± 3.34 g for ND, 42.50 ± 3.95 g for HSD, 36.25 ± 3.99 g for HSD + JSP) of the experiment (Figure 1). The food intake was statistically insignificant (p > 0.05) within the ND group but significant with in the HSD group (Figure 2B). The body weight gain in HSD + JSP mice was statistically insignificant (p > 0.05) within the HSD group but significant within the ND group (Figure 2B).

3.3. Effect of Jackfruit Seed Powder on Glucose Tolerance in Mice

An Intraperitoneal Glucose Tolerance Test (ipGTT) was performed after 8 weeks of the feeding experiment and results are presented in Figure 3. After glucose (2 g/kg BW, ip) challenge, HSD-fed mice were unable to utilize glucose properly to establish homeostasis and develop glucose intolerance. There was an elevation in blood glucose concentration in HSD-fed mice compared to the control mice (187.40 ± 10.85 vs. 333.25 ± 24.43 mg/dL) after 15 min of glucose (2 g/kg BW, ip) challenge. Jackfruit seed powder (JSP) supplementation in HSD-fed mice (221.83 ± 20.94 mg/dL) showed remarkable reduction in blood glucose concentration compared to HSD-fed mice. The blood glucose level in HSD + JSP-supplemented mice also quickly returned to the baseline in comparison to that of the HSD group. Increase in the AUC of HSD group (vs. ND group) was reversed by the supplementation of JSP and was significantly different compared to the HSD group (Figure 3B).

3.4. Effect of Jackfruit Seed Powder on Organ Weight of Mice

At the end of the feeding experiment, vital organs were obtained from euthanized animals and wet weights were measured. In comparison to the control group, the actual liver wet weight showed an insignificant increase (p > 0.05) in the HSD mice (Figure 4A).
JSP supplementation significantly attenuated the weight of the liver in the HSD-treated mice (2.52 ± 0.13 g BW for ND, 3.12 ± 0.23 g for HSD, and 2.21 ± 0.30 g for HSD + JSP). There were no significant differences in heart and kidney weight of the mice (Figure 4A). As shown in Figure 4B, HSD-treated mice showed an increase in white adipose tissue (WAT) which was significantly reduced by JSP supplementation (0.50 ± 0.04 g BW for ND, 0.57 ± 0.02 g for HSD, and 0.40 ± 0.02 g for HSD + JSP). Although it was statistically insignificant, the weight of brown adipose tissue (BAT) tends to increase in HSD-fed mice compared to the control group (Figure 4B). JSP supplementation normalized the BAT weight in HSD-fed mice.

Figure 2. JSP supplementation counteracted the body weight gain in HSD-fed mice. (A) Body weight was monitored weekly, (B) Body weight gain was determined at the end of treatment. ND: Normal Diet; HSD: High-Sugar Diet; and JSP: Jackfruit Seed Powder. # p < 0.05 vs. HSD by one-way ANOVA followed by Tukey’s post hoc test. Bars represent mean ± SEM. n ≥ 3 for each group.
Figure 3. JSP supplementation with HSD improved glucose tolerance. (A) Blood glucose content was measured at 0, 15, 30, 60, and 120 min after i.p. glucose (2 mg/kg BW) administration; (B) The area under the curve (AUC) for glucose tolerance test was quantified. ND: Normal Diet; HSD: High-Sugar Diet; and JSP: Jackfruit Seed Powder. * $p < 0.05$ vs. ND; # $p < 0.05$ vs. HSD by one-way ANOVA followed by Tukey’s post hoc test. Bars represent mean ± SEM. $n \geq 3$ for each group.
Figure 4. JSP supplementation significantly reduced liver weight and the weight of WAT. (A) Weight of Liver, Heart, and Kidney; (B) Weight of white adipose tissue (WAT) and brown adipose tissue (BAT). ND: Normal Diet; HSD: High-Sugar Diet; and JSP: Jackfruit Seed Powder. # p < 0.05 vs. HSD by one-way ANOVA followed by Tukey’s post hoc test. Bars represent mean ± SEM. n ≥ 3 for each group.

3.5. Effect of Jackfruit Seed Powder on Lipid Profile Parameters

Blood lipid parameters were measured from serum collected after the feeding experiment. HSD-fed mice showed an insignificant increase in serum total cholesterol (TC) and triglyceride (TG) concentrations in comparison to the normal diet (ND)-fed group (Figure 5). JSP supplementation significantly attenuated the rise in serum TC and TG concentrations that were observed in the HSD-fed group (Figure 5). No significant difference was found in serum HDL-cholesterol among the groups. In the HSD + JSP group, there was a decrease in LDL-cholesterol concentration (30.27 ± 9.79 g for HSD and 8.15 ± 5.24 g for HSD + JSP), which was statistically insignificant and was observed after eight weeks of the experiment (Figure 5).
High-sugar diets are associated with the development of metabolic dysregulations including diabetes and obesity [40,41]. In this study, as expected, mice fed with HSD exhibited a slight increase in fasting blood glucose as observed at 0 min of glucose tolerance test (GTT). This finding might indicate an impaired blood glucose homeostasis toward diabetic development [42]. As observed in ipGTT, HSD-fed mice showed an impaired resistance to glucose (2 g/kg BW) challenge. However, supplementation of JSP with HSD remarkably improved glucose tolerance in mice. A previous study showed that the consumption of diets containing high sugar may increase an excessive body weight gain which accelerates obesity development in rodents [20]. Previous studies reported that consuming high-sugar drinks and fast foods frequently could significantly increase the risk of obesity and diabetes in humans [37,38]. Our findings also demonstrated that the HSD-fed mice exhibited a tendency in body weight gain after 8 weeks of treatment, and it was significantly hampered by the 20% JSP supplementation in their diet. However, at the end of treatment, despite the fact the final body weight of HSD-fed mice was higher, it was statistically comparable with control group. In support of our findings, a previous study also demonstrated that mice fed a solid sugar diet remained insignificantly different in their body weight when compared with a control group [39].

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inclusion of soluble fiber in food of diabetic mice significantly reduced glucose basal levels in comparison to mice that were fed without fiber [43]. The area under the curve at 8 weeks was significantly lower in the control group compared to that of the HSD group. Again, JSP supplementation significantly lowered the area under the curve in the HSD + JSP group, which was similar to the control group. In contrast to our findings, methanolic extract of jackfruit seeds has been reported to increase the blood glucose levels compared to control animals in glucose-loaded mice [44]. Previous and current findings may differ due to the way of administration as we incorporated JSP into the diet instead of methanolic extract of seeds.

These results showed that the actual liver wet weight of mice in the HSD group was insignificantly higher than that of the control group. However, actual liver wet weight of the HSD + JSP group was significantly lower than that of the HSD group, indicating that JSP prevented liver enlargement. We did not find any significant difference in heart and kidney weight of the mice. The weight of WAT in the HSD group was relatively higher than that in the control group. However, JSP supplementation significantly decreased the weight of WAT in HSD + JSP mice. Mice in the HSD group showed a tendency to increase the weight of BAT than those in the control group. However, JSP slightly reduced the weight of BAT in HSD-fed mice. JSP supplementation resulted in the lowering of fat deposits and improvement of insulin sensitivity in high-sugar diet-fed mice. One of the limitations of this study is that we could not identify the specific chemical constituent of the jackfruit seed responsible for the above-mentioned physiological functions.

Maintaining healthy levels of lipids circulating in the blood stream is important to prevent cardiovascular diseases. High-sugar diets can induce fatty liver or hepatic steatosis in mice. In this study, HSD consumption increased plasma total cholesterol (TC) and triglycerides (TG) levels in blood, but it was insignificant. However, JSP supplementation significantly prevented the rise of plasma TC and TG in HSD-fed mice. Although there was a decreasing tendency in plasma LDL-C level in the HSD + JSP group, plasma HDL-C levels were comparable among the groups. The hypocholesterolemic action of JSP may be attributed to the presence of flavonoids and phenolic compounds that enhanced lipid metabolism [45]. Besides this, JSP also contains an appreciable quantity of non-digestible carbohydrates that have been reported to be associated with lowering plasma cholesterol [46]. Indifference in HDL-C level may be due to absorption of intestinal cholesterol and enhanced cholesterol turnover to bile acids by bioactive compounds present in jackfruit seeds [47]. Further investigation is needed to completely understand the beneficial effects of jackfruit seed powder consumption in maintaining metabolic homeostasis.

5. Conclusions

The supplementation of jackfruit seed powder with the high-sugar diet was effective in maintaining a normoglycemic state as well as preventing excessive body weight gain and food intake against the development of diabetes and obesity caused by high-sugar diets. The addition of jackfruit seeds in diets may help to improve blood lipid profile that may attenuate the deleterious effect of high sugar consumption. Despite their nutritional value, most of the time jackfruit seeds are wasted/discarded or spoiled rapidly. Therefore, consumption of jackfruit seeds and utilization of its flour at household and commercial levels may be enhanced as a supplemental diet to overcome metabolic dysregulation and gain better health.

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