

## Amla (*Emblica officinalis* Gaertn) fruit extract prevents high-sugar diet-induced metabolic syndromes by preserving glucose and lipid homeostasis

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### Article history:

Received: 18 January 2023

Received in revised form: 23 April 2024

Accepted: 4 January 2025

Available Online: 19 March 2025

### Keywords:

Amla,  
Phytochemicals,  
High-sugar diet,  
Diabetes,  
Obesity

### DOI:

[https://doi.org/10.26656/fr.2017.9\(2\).034](https://doi.org/10.26656/fr.2017.9(2).034)

### Abstract

Obesity and diabetes mellitus are two interrelated metabolic conditions that share a number of pathophysiological mechanisms which are frequently observed to develop various physical complications. Consumption of excess sugar has been reported to promote the development of obesity and type 2 diabetes (T2DM) both directly and indirectly. Sugar-induced elevated blood glucose level leads to develop various syndromes such as hyperinsulinaemia, insulin resistance, hypertension, hypertriglycerolaemia, dislipidaemia, and a decrease in serum HDL-cholesterol concentration. Hence, the current study aimed to evaluate the beneficial effect of amla (*Emblica officinalis*) fruit extract (AE) on mitigating the detrimental effect of high-sugar and sugary food consumption. Mice were treated with or without amla extract (20 mg/kg BW) in both normal and high-sugar diet (HSD) fed mice for 8 weeks. Amla extract exerted a remarkable effect to hamper the hyperphagia due to HSD consumption in mice. The excessive body weight gain caused by HSD was effectively prevented by the administration of AE. Furthermore, oral administration of AE also improved glucose tolerance in HSD-fed mice. The group that received AE experienced a significant difference in liver weight, white adipose tissue (WAT), and brown adipose tissue (BAT) weights, but not in heart or kidney weight in comparison to that of the HSD-fed group. The HSD+AE group significantly lowered total cholesterol and triacylglycerides. These findings suggest that amla has the potential to ameliorate the metabolic dysregulation caused by high-sugar diet consumption.

## 1. Introduction

Over the past century, both in developed and developing countries, obesity and type 2 diabetes have become the most common metabolic diseases. Furthermore, 280 million people had diabetes in 2010 which is about 6.2% of the global population and it has been speculated that this number will rise to almost 7.5% of the world's population by 2030 (Riobó Serván, 2013). An imbalance in energy intake and expenditure causes obesity which is one of the serious health issues and is linked to the emergence of chronic disorders including type 2 diabetes and cardiovascular diseases. Insulin resistance, characterized by the impaired insulin sensitivity of peripheral tissues such as muscle and fat cells to the action of insulin in obese individuals, is a pathophysiologic factor of type 2 diabetes mellitus

(T2DM). A number of mechanisms, including increased production of adipokines or cytokines like tumor necrosis factor-resistin and retinol-binding protein 4, have been postulated to link obesity and insulin resistance, which predispose to diabetes (Deng and Scherer, 2010). Free fatty acids (FFAs), particularly those released by visceral fat, have a direct effect on insulin signaling. It causes the liver and skeletal muscles to increase their FFA oxidation to produce energy while inhibiting the activity of glycolytic enzymes and thus reducing the ability to absorb and process glucose (Boden and Shulman, 2002).

Diabetes is a chronic disorder of the metabolism of carbohydrates, fats, and proteins marked by elevated fasting and post-meal blood sugar levels (Kastorini and

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Panagiotakos, 2009). The main causes of the rapidly increasing incidence of T2DM in both developed and developing nations are dietary practices and a sedentary lifestyle (Sami *et al.*, 2017). In addition to other factors, sugar consumption has grown to be a major concern in relation to the emergence of metabolic and cardiovascular disorders. Furthermore, high-sugar consumption has been suggested as a potential risk factor for the development of metabolic syndromes such as diabetes and obesity, as well as hypertriglyceridemia, low HDL levels, and insulin resistance (Rippe and Angelopoulos, 2016). However, due to the side effects of anti-diabetic and anti-obesity drugs recent studies have focused on screening natural sources that have been reported to treat diabetes and obesity with minimal side effects. Traditional medicine has made extensive use of a wide range of natural remedies, including crude extracts and isolated chemical constituents from plants, to treat chronic ailments including diabetes and obesity.

Amla (*Emblica officinalis* Gaertn.) belongs to the family Euphorbiaceae and grows well in tropical and subtropical regions, particularly in India, China, Malaysia, Bangladesh, Indonesia, and the Malay Peninsula. Amla is a significant nutritional source of vitamin C, minerals, and amino acids. In addition, it includes phenolic compounds, tannins, phyllembelic acid, phyllemblicin, rutin, curcuminoides, and emblicol. The tannins present in amla include emblicanin A, emblicanin B, phyllaemblicin B, punigluconin and pedunculagin. Amla fruit extract has been reported to have hypolipidemic, anti-diabetic, and anti-inflammatory properties, as well as to prevent the growth of tumors, the development of stomach ulcers, and retroviruses like HIV-1 (Anila and Vijayalakshmi, 2002; Kim *et al.*, 2010). Amla extract has also been demonstrated to possess antioxidant effects. It has also been reported that the aqueous extract of amla is a powerful inhibitor of the generation of lipid peroxide and a scavenger of hydroxyl and superoxide radicals *in vitro* (Jose and Kuttan, 1995). In a prior study, authors demonstrated that ethyl acetate (EtOAc) extract of amla, which contains a high concentration of polyphenols, reduces the effects of oxidative stress on renal dysfunction and age-related hyperlipidemia. To the best of our knowledge, no research has been published that examines whether amla extract combats the physiological symptoms developed by a high-sugar diet. The present study was conducted to evaluate the effect of the ethanol extract of amla on metabolic syndromes induced by a high-sugar diet.

## 2. Materials and methods

### 2.1 Experimental layout

The experimental study was conducted on twenty-

eight adult male mice (Swiss Albino strain; 6 weeks old, bodyweight 35-37 g) purchased from the Animal Resources Facility of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). The mice were provided with a normal diet and *ad libitum* water to be acclimatized to the new environment. Animals were housed in a well-ventilated room at 28±2°C and relative humidity of 70-80% with natural daylight (during the months of February to April 2021). During the rearing period, animals were also habituated for handling every day to minimize the stress response that may occur during the experiment.

Thereafter, mice were divided randomly into four different treatment groups. The first group received a normal diet, the second group received the normal diet with amla extract (20 mg/kg BW), the third group was provided with high-sugar diet (30% sucrose) and finally, the fourth group was provided with high-sugar diet and amla extract (20 mg/kg BW). The normal diet contained 40% wheat, 20% wheat bran, 5.5% rice polishing, 10.0% fish meal, 6.0% oil cake, 0.39% gram, 0.39% pulses, 0.38% milk, 1.5% soybean oil, 0.095% molasses, 0.095% salt, and 0.1% embavit (Ulla *et al.*, 2017). The high-sucrose diet (HSD) was composed of 30% sucrose in a normal diet (Gibson *et al.*, 2013; Santoso *et al.*, 2019). The amla extract was administered orally at a dose of 20 mg/kg body weight (Kim *et al.*, 2010) to the experimental mice. The control groups were administered with vehicle (water) to maintain equal stress. The amount of administration was adjusted according to the body weight of the individual mouse. The diet treatment was performed for eight weeks continuously. All the protocols for animal care and use of this study were in accordance with the international guiding principles for biomedical research involving animals developed by the Council for International Organizations of Medical Sciences and have been approved (AWEEC/BAU/2021/17) by the Animal Welfare and Experimentation Ethics Committee of Bangladesh Agricultural University.

### 2.2 Preparation of ethanolic extract of amla fruit

Fresh amla fruits were procured from the local market of Mymensingh, Bangladesh. The fruits were properly cleaned, cut into slices, and dried in the sun before being dried in an oven at 45°C for 24 hrs to eliminate the moisture completely. Fruits were properly dried before being finely processed in a grinder. The powdered fruits were stored in a sterile container and tightly closed until used for ethanol extraction. With slight modifications, the method described by Algariri *et al.* (2013) was used to prepare the ethanolic extract of the amla fruit. In a nutshell, 100 mL of ethanol was added to 5 g of the powdered amla fruit and the mixer

was placed in a shaker for 24 hrs. The residue was again extracted with 100 mL of ethanol following filtration using filter paper (Whatman No. 1). The supernatant from two portions of the filtrate was combined and was evaporated in a vacuum rotatory evaporator at a maximum temperature of 45°C. A yellowish ethanolic extract of the amla fruit was obtained by freeze-drying the concentrated solvent. To get the necessary amount of extract for oral administration, this process was repeated.

### 2.3 Measurements

#### 2.3.1 Weekly food and water intake

Food and water intake by the individual mouse was measured weekly for 8 consecutive weeks. In brief, the measured food pellets were supplied to each mouse in individual cages at 10:00 AM. After one week, the weight of the remaining food was measured at 10:00 AM. The food intake was calculated by subtracting the remaining food weight from the initial weight of the food. In the case of water intake measurement, a similar procedure was followed but the water was supplied in bottles with nozzles.

#### 2.3.2 Body weight

The body weight of each mouse was measured with the help of a sensitive digital balance (eki300-2n electronic scale, A and D Company Ltd., Korea) at 7 days interval up to the end of the experiment.

#### 2.3.3 Intraperitoneal glucose tolerance test

Fasting blood glucose was measured by using a standardized automated blood glucose test meter (Glucolider™ Enhance Blood Glucose Meter, HMD Biomedical Inc., Taiwan) at the end of the experiment after approximately 6 h of fasting (food was deprived while drinking water was provided in the clean cages during fasting). The test was performed by following a protocol previously described by Maejima *et al.* (2015). A single dose of glucose (2 g/kg BW) was injected intraperitoneally for each mouse. After that, the tip of the tail was cut using a sterilized surgical blade with minimum injury. The first small drop of blood was discarded. Then the small drop of blood (approximately <5 µL) was taken on the test strip of the blood glucose meter and the concentration of the blood glucose was recorded for individual mice. The concentration of blood glucose was recorded at 0, 15, 30, 60, and 120 mins for each mouse after intraperitoneal (ip) glucose administration. The area under the curve (AUC) data was subsequently calculated from the blood glucose levels in ipGTT experiment.

#### 2.3.4 Blood samples collection and preparation of serum

At the end of the feeding trial, after 18 h of fasting, blood samples were collected from the posterior vena cava of the animals according to the method described previously (Hoff and Rlagt, 2000). Mice were anesthetized inside the airtight container containing cotton soaked with chloroform. A V-shaped cut was made in the abdominal cavity and the intestine was gently shifted over to the left. After that, the liver was pushed forward and the posterior vena cava (between the kidneys) was identified. A 26 gauge needle and a 1 ml syringe were inserted to collect blood from the posterior vena cava. The blood was collected in a 1.5 ml Eppendorf tube containing EDTA which acts as an anticoagulant. Then the blood-containing tubes were centrifuged at 4000 rpm for 10 mins at 4°C (Gyrozen 1580R Multi-Purpose High-Speed Refrigerated Centrifuge, Gangnam-gu, Seoul, South 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.

#### 2.3.5 Measurement of organs weight

After collecting the blood samples, the internal organs such as the liver, kidney, and heart were harvested and trimmed. Then, the organs were washed in saline solution and placed on filter paper to remove the saline solution on the surface of the organs. After that, the weight of the organs was taken using a digital balance (eki300-2n electronic scale, A and D Company Ltd., Korea).

#### 2.3.6 Hematoxylin and eosin (H and E) staining of liver tissues

Upon blood sample collection, mice were dissected and the liver was removed, weighed, and then kept in 10% formalin solution for 24 hrs. The samples were then used for histological examination by following the protocol of paraffin-embedded tissue processing (Qin *et al.*, 2018). Briefly, the fixed tissues were dehydrated using graded ethanol and then cleaned in xylene before being embedded in paraffin. Furthermore, the tissues were cut into slices (5 µm) using a microtome and stained with Hematoxylin and Eosin. The histologically prepared and stained slides of the liver tissue were examined under a microscope (Olympus CX21; Olympus America Inc.) and sections were photographed (Magnification, 40×).

#### 2.3.7 Determination of lipid profile parameters

Total cholesterol (TC) level, triacylglyceride (TAG) level, and HDL cholesterol level were determined by

CHOD-PAP method (Richmond, 1973), GPO-PAP method (Cole *et al.*, 1997) and CHOD-PAP method (Henry *et al.*, 1974), respectively. 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 (Friedewald *et al.*, 1972) as follows:

$$\text{LDL cholesterol (mg/dl)} = \text{Total cholesterol} - \text{HDL cholesterol} - (\text{Triglycerides}/5)$$

#### 2.4 Statistical analysis

All statistical analyses were performed using Prism 5 (GraphPad Software, CA). All data were depicted as mean  $\pm$  standard error mean (SEM). An analysis of variance (ANOVA) followed by Turkey's posthoc test was carried out to justify the significant differences among groups of treatment. The  $p < 0.05$  was set as a significant value for all analyses.

### 3. Results

#### 3.1 Effect of amla fruit extract on weekly food intake of mice

The purpose of the present experiment was to determine the beneficial effects of amla fruit extract on the food intake of mice fed a regular diet and a high-sugar diet. The experiment was carried out for 8 weeks. At the beginning of the experiment, the groups - normal diet (ND), normal diet with amla extract at a dose of 20 mg/kg BW (ND+AE), high-sugar diet (HSD), and high-sugar diet with amla extract at a dose of 20 mg/kg BW (HSD+AE)- did not consume significantly different amounts of food (Figure 1). From the 5<sup>th</sup> week to the end of the feeding experiment, AE treatment in the case of

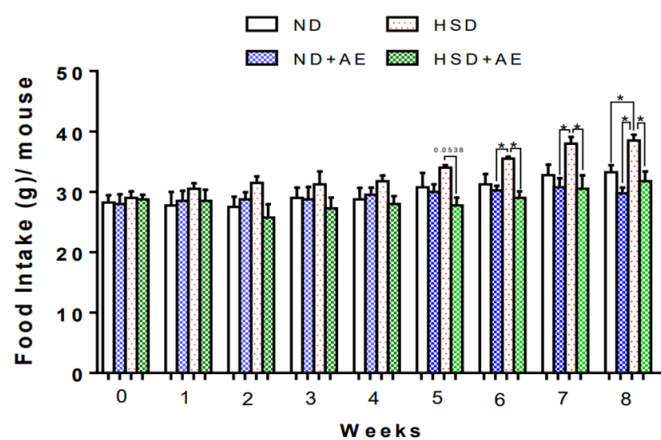


Figure 1. Amla fruit extract ameliorates food intake in Swiss Albino male mice. Food intake was measured weekly for a period of 8 weeks. Bars represent mean $\pm$ SEM,  $n \geq 4$  for each group. \* $p < 0.05$  by one-way ANOVA followed by Tukey's post-hoc test. ND: Normal diet, HSD: High-sugar diet, AE: Amla extract.

ND-fed mice decreased food consumption relative to the control group (ND), although the difference was not statistically significant. HSD-diet fed mice showed a tendency to increase food intake in comparison to the ND group from the first week to the completion of the feeding trial and it was significant at the 8<sup>th</sup> week. Administration of AE decreased the food intake in mice as compared with HSD-fed control mice from the 1<sup>st</sup> week but it was not significant until the 6<sup>th</sup> week of age ( $38.50 \pm 0.96$  g for HSD vs  $31.75 \pm 1.65$  g for HSD+AE at 8<sup>th</sup> week) suggesting the anti-hyperphagic properties of amla extract.

#### 3.2 Effect of amla fruit extract on weekly water intake in mice

Mice were allowed unlimited access to water in a bottle with a nozzle. Over the course of eight weeks, water intake per mouse was assessed once per week. The water intake was estimated by deducting the volume of residual water from the initial amount of water delivered. All groups consumed nearly the same amount of water one week before starting the feeding trial (Figure 2). The HSD and HSD+AE treated groups, however, showed an increasing tendency in their water consumption from the 1<sup>st</sup> week of the experiment to the end ( $46.50 \pm 1.19$  g for HSD vs  $48.75 \pm 2.72$  g for HSD+AE at the 8<sup>th</sup> week of treatment). Although the HSD and HSD+AE groups consumed more water than the ND group overall, this difference was not statistically significant. Furthermore, despite the fact that the water intake of the ND+AE and HSD+AE treated groups was comparatively higher than their corresponding control groups the differences were not statistically significant.

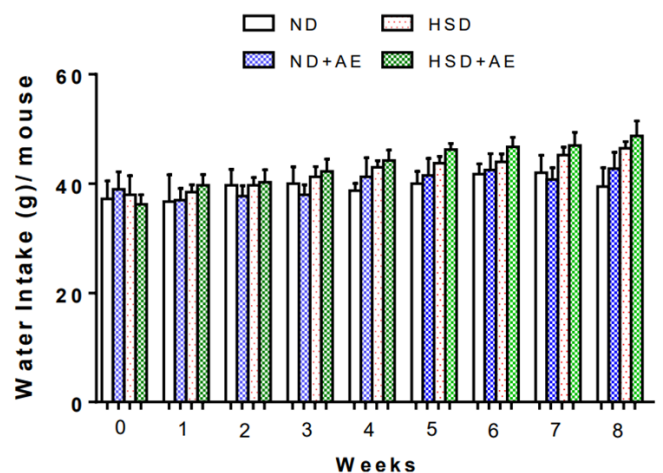


Figure 2. Amla fruit extract slightly increased water intake in Swiss Albino male mice. Water intake per mouse was measured weekly for a period of 8 weeks. Bars represent mean $\pm$ SEM,  $n \geq 4$  for each group. ND: Normal diet, HSD: High-sugar diet, AE: Amla extract.

#### 3.3 Effect of amla fruit extract on body weight of mice

The body weight of the mice was recorded

throughout the feeding experiment to explore whether the amla fruit extract contributes to inhibiting the development of obesity. During the onset of the experiment, there was no discernible variation in the body weights (ranging from 35 to 37 g) of the mice in the groups. Both the normal diet (ND) and high-sugar diet (HSD) groups of mice had lower body weights following AE treatment. In the 7<sup>th</sup> and 8<sup>th</sup> weeks of the feeding trial, AE administration in the case of ND-fed mice dramatically decreased body weight as compared to the control group (Figure 3A). The ND+AE group tended to decrease mice's body weight compared to the HSD group from the 4<sup>th</sup> week to the 8<sup>th</sup> week of the experiment. From the 1<sup>st</sup> week to the end of the feeding trial, mice supplemented with a high-sugar diet gained somewhat more body weight than mice in the ND group, however, this difference was not statistically significant (Figure 3B). With the exception of the 6<sup>th</sup> week, AE supplementation in the HSD group reduced the body weight of mice compared to animals fed only HSD, and a significant difference was seen from the 5<sup>th</sup> week

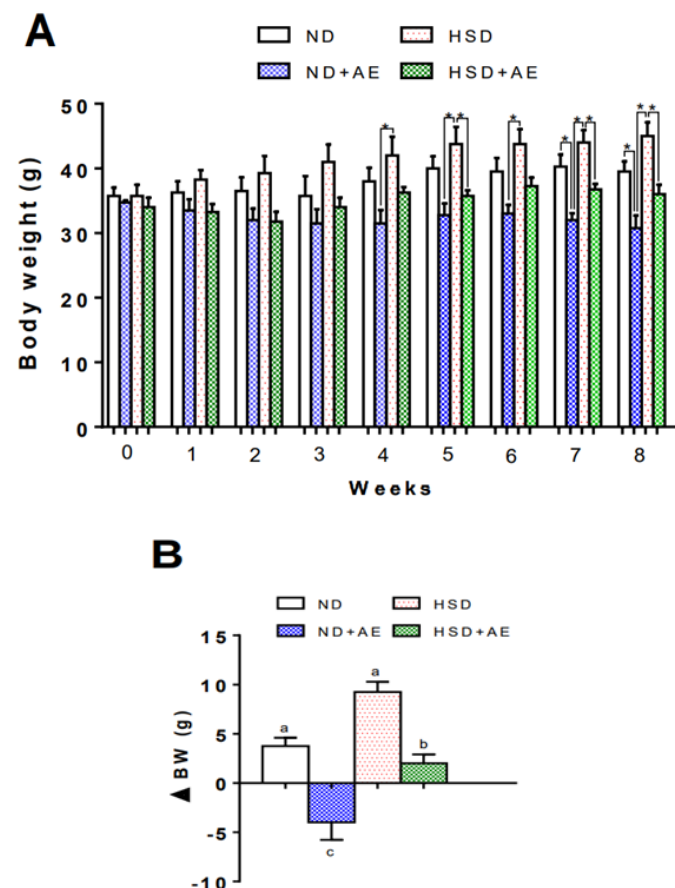


Figure 3. Amla fruit Extract reduced body weight gain in ND and HSD-fed mice: A) body weight of mice was measured every week, and B) body weight changes in mice after eight weeks of feeding trial. Bars represent mean±SEM, n≥4 for each group. \*p<0.05 by one-way ANOVA followed by Tukey's post-hoc test. Bars with different notations are statistically significantly different. ND: Normal diet, HSD: High-sugar diet, AE: Amla extract.

through the completion of the trial (45.00±1.20 g for HSD vs 36.00±1.47 g for HSD+AE at the 5<sup>th</sup> week of the treatment).

### 3.4 Effect of amla fruit extract on glucose tolerance in mice

The glucose tolerance test (GTT) was performed to evaluate how efficiently amla fruit extract contributes to utilizing excess glucose and maintaining blood glucose homeostasis. To assess the blood glucose concisely, a tiny drop of blood was obtained from the tail vein and placed on the test strip of the blood glucose meter. The values were recorded at different time intervals. According to the results of the ipGTT, mice fed a high-sugar diet had blood glucose levels that were higher at 0 min (178.85±11.68 mg/dL for ND vs 211.75±24.69 mg/dL for HSD) and took longer time to return to baseline than mice in the control group (Figure 4A). While the blood glucose levels were almost similar among the groups after the administration of intraperitoneal glucose (2 g/kg BW), the HSD group showed a fast increase at the 15 mins time point in comparison to the other groups. However, at all the periods (15, 30, 60, and 120 mins)

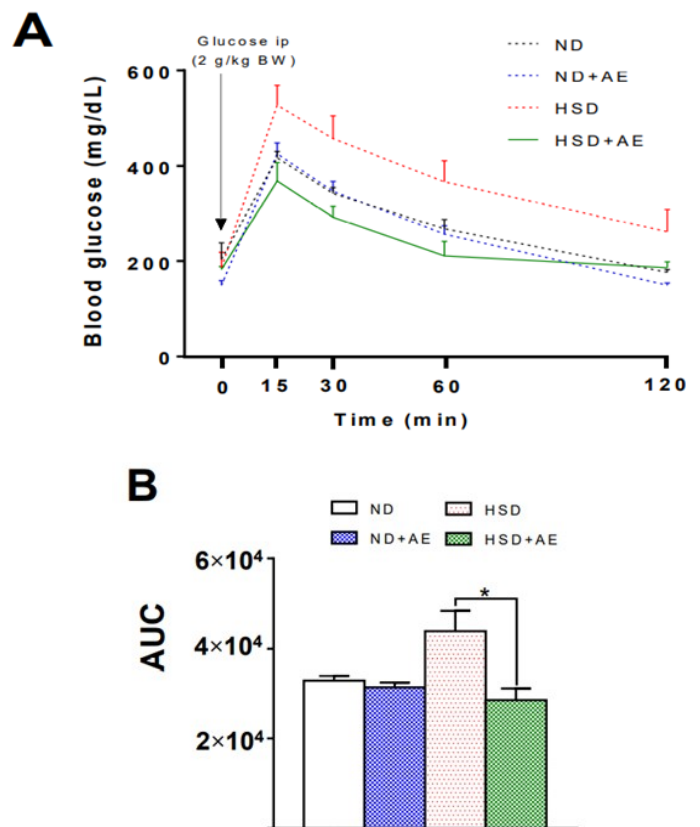


Figure 4. Amla fruit extract improves glucose tolerance in HSD-fed mice: (A) ipGTT done at the end of the study after an intraperitoneal injection of glucose (2 g/kg BW), (B) The corresponding area under the curve (AUC) derived from the ipGTT graph. Bars represent mean±SEM, n≥4 for each group. \*p<0.05 by one-way ANOVA followed by Tukey's post-hoc test. ND: Normal diet, HSD: High-sugar diet, AE: Amla extract.

following glucose injection, the AE-administered group tended to have lower glucose concentration than the HSD control group. The area under the curve (AUC) corresponding to the GTT graph revealed that there is no significant difference between the ND group and the ND+AE group (Figure 4B). On the contrary, the AUC value for the HSD+AE group was significantly lower than it was in the HSD control group.

### 3.5 Effect of amla fruit extract on organ weight of mice

Animals were sacrificed after 8 weeks of the feeding experiment, and organs such as the liver, heart, kidney, white adipose tissue (WAT) and brown adipose tissue (BAT) were isolated and weighed. An electric balance (Eki300-2n electronic scale. A and D Company Ltd. Korea) was used to measure the weight of the organs. Although it was not statistically significant, the AE treatment reduced liver weight in the ND+AE group relative to the ND control group. The findings demonstrated that, in comparison to the ND group, HSD increased liver weight. When compared to the HSD-fed group, the liver weight after AE administration ( $53.20 \pm 1.57$  mg/g for HSD vs  $43.43 \pm 1.44$  mg/g for HSD+AE) was considerably lower (Figure 5A). The weights of the heart and kidneys did not substantially differ across the groups at the end of the experiment. White adipose tissue (WAT) and brown adipose tissue (BAT) were enlarged in mice on high-sugar diets (Figure 5B). AE supplementation in the ND group (ND+AE) resulted in a reduction of the weights of WAT and BAT though the values were not statistically significant as compared with the control group (ND). However, administration of AE in HSD significantly decreased the weights of WAT and BAT ( $23.99 \pm 1.16$  mg/g for HSD vs  $18.08 \pm 1.70$  mg/g for HSD+AE in case of WAT;  $1.27 \pm 0.15$  mg/g for HSD vs  $0.51 \pm 0.15$  mg/g for HSD+AE in case of BAT) in mice.

### 3.6 Effect of amla fruit extract on lipid accumulation in the liver of mice

Hematoxylin and eosin (H and E) staining is frequently used in liver histology to identify metabolic disorders that have a relationship to the liver cell. Animals were sacrificed after 8 weeks of feeding trial and the livers were isolated. To study the liver histology, a slice of the liver was prepared after weighing the liver. This study revealed that a high-sugar diet causes fat to accumulate in vacuoles, indicating hepatic fat deposition in the liver of mice fed an HSD, which separates the cells. Moreover, compared to mice fed ND, the liver of HSD-fed mice has higher hepatic fat deposition with different-sized fat cells (Figure 6C). The fat formation was not seen in the liver tissues of the HSD-fed mice that received AE administration (Figure 6D).

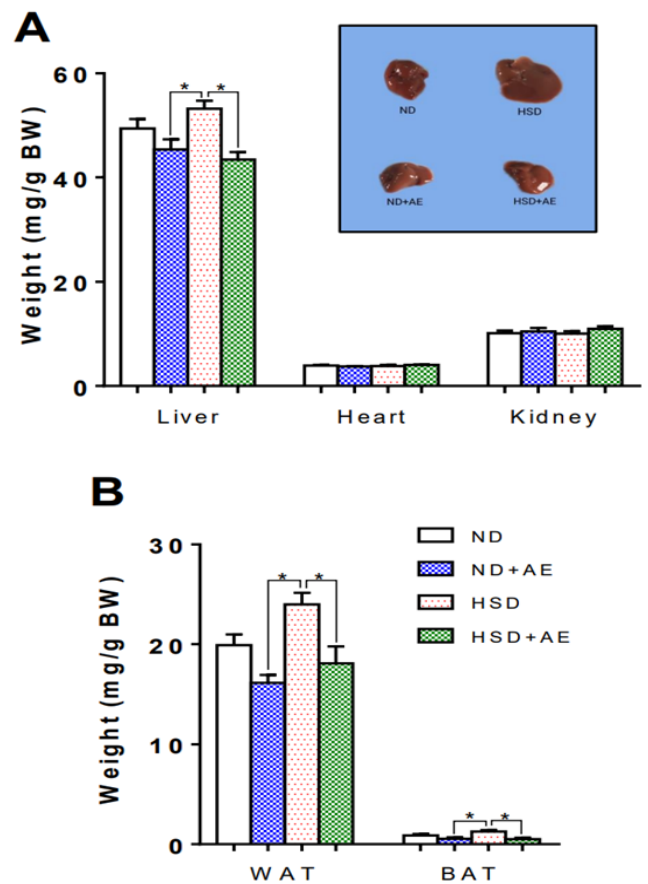


Figure 5. Amla fruit extract administration reduced liver, WAT and BAT weights in HSD-fed mice. Organ weights such as A) liver (representative photographs in the right panel), heart and kidney and B) white adipose tissue (WAT) and brown adipose tissue (BAT) were measured at the end of the experiment. Bars represent mean  $\pm$  SEM,  $n \geq 4$  for each group. \* $p < 0.05$  by one-way ANOVA followed by Tukey's post-hoc test. ND: Normal diet, HSD: High-sugar diet, AE: Amla extract.

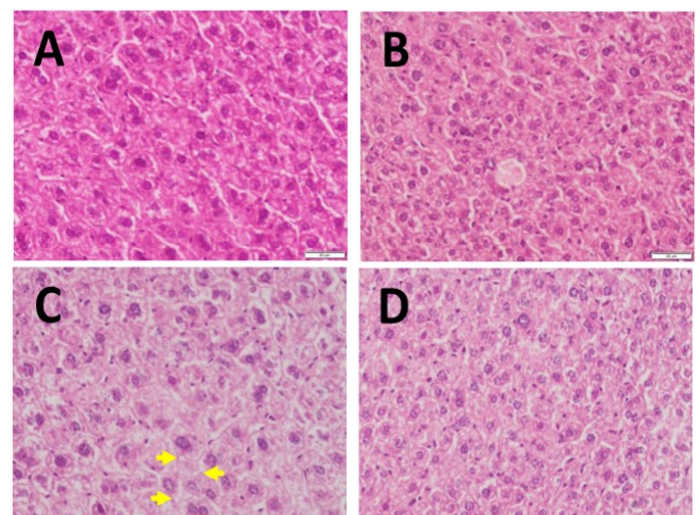


Figure 6. Amla fruit extract inhibited the HSD-induced lipid accumulation in the liver of mice. Histological changes such as fat accumulation (indicated by yellow arrows) in liver tissue were observed by hematoxylin and eosin (H and E) staining in ND (A), ND+AE (B), HSD (C) and HSD+AE (D)-fed mice after 8 weeks of treatment. Images were photographed with a 40 $\times$  objective. ND: Normal diet, HSD: High-sugar diet, AE: Amla extract.

### 3.7 Effect of amla fruit extract on blood lipid profile in mice

Blood samples were taken at the end of the feeding experiment, and serum samples were separated through centrifugation and kept in the freezer at  $-20^{\circ}\text{C}$  until further evaluation. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation, while total cholesterol (TC), triacylglycerides (TAG), and high-density lipoprotein cholesterol (HDL-C) were all measured using different reagents (Friedewald *et al.*, 1972). In comparison to the control group (ND), our findings showed that mice fed HSD had increased levels of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL) (Figure 7). In comparison to the HSD control group, the administration of AE in the HSD group dramatically reduced the TC ( $146.50 \pm 13.14$  mg/dL for HSD vs  $103.85 \pm 7.37$  mg/dL for HSD+AE) and TG ( $135.95 \pm 10.3$  mg/dL for HSD vs  $101.28 \pm 8.06$  mg/dL for HSD+AE) levels. Although mice treated with AE exhibited lower LDL levels than mice fed with HSD, the difference was not statistically significant. Serum HDL concentration was also not significantly different among the groups.

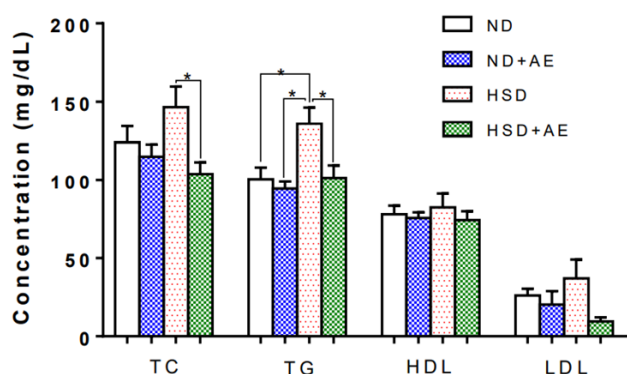


Figure 7. Amla fruit extract decreased the concentration of total cholesterol and triglycerides in HSD-fed mice. Blood lipid profile including total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL-C) and low-density lipoprotein (LDL-C) were measured. Bars represent mean  $\pm$  SEM,  $n \geq 4$  for each group. \* $p < 0.05$  by one-way ANOVA followed by Tukey's post-hoc test. ND: Normal diet, HSD: High-sugar diet, AE: Amla extract.

## 4. Discussion

Several high-quality systematic reviews and meta-analyses have assessed the relation of sugar-sweetened beverages with incidents of type 2 diabetes and obesity (Basaranoglu *et al.*, 2015; Hydes *et al.*, 2021). Although numerous side effects have been reported, different types of disease management practices are prevailing to fight against metabolic diseases. Nowadays, research works are in motion emphasizing the use of traditional plant products and their derivatives to fight against common

metabolic syndromes. Given its lower toxicity and fewer side effects when compared to synthetic medications, phytotherapy has a promising future in the management of diabetes. Thus, providing an alternative supplemental phytotherapy that could preclude the deleterious effect of high-sugar consumption is considerably needed. Thus, the present study was conducted to evaluate the possible effect of ethanolic extract of amla fruit (AE) on different physiological and biochemical parameters of mice treated with high-sugar.

In comparison to the ND group, the HSD supplementation significantly increased food intake. High-sugar diets have been reported to cause rapid weight gain as well as abnormalities in blood pressure, glycemia, insulin sensitivity, and lipid and uric acid levels in rats (Lean and Te Morenga, 2016). The fructose component of sucrose, not glucose, was found to be the primary cause of these effects. Recent evidence suggests that sugar metabolism in regions of the central nervous system (CNS) that control food intake and energy expenditure, fructose metabolism also differs from that of glucose. It is known that the initial steps of hepatic fructose metabolism use a different set of enzymes that allow this sugar to bypass the rate-limiting step catalyzed by phosphofruktokinase (PFK) in the glycolytic pathway. Similar enzymes of fructose metabolism are found in regions of the CNS that play an important role in monitoring energy balance and satiety control (Funari *et al.*, 2005; Shu *et al.*, 2006). These findings are consistent with a recent report that centrally administered fructose provokes feeding (Miller *et al.*, 2002). In contrast, the central administration of glucose causes satiety (Miller *et al.*, 2002; Wolfgang *et al.*, 2007). Previous studies also showed that glucose administered by i.p. injection rapidly enters and is metabolized by the brain, increasing the level of hypothalamic malonyl-CoA (Wolfgang *et al.*, 2007), which is known to suppress food intake (Wolfgang and Lane, 2006). The administration of amla extract significantly reduced food intake in the HSD group but did not affect food intake in the ND group as the values were insignificant. Phytochemicals such as gallate, ellagic acid have been reported to improve satiety by increasing non-adrenaline levels, which activates the sympathetic nervous system and regulates appetite and hunger. Amla being a rich source of gallic acid, ellagic acid, saponins and other compounds may counteract the hormonal regulation stimulated by high-sugar diet consumption. In this study, water intake appeared to be slightly higher in mice fed amla fruit extract in both ND and HSD groups. Additionally, the HSD-fed group also showed an increase in water intake than the normal diet-fed group. Nazish and Ansari (2018) reported that HFD treatment slightly increased the water intake and the combination of aqueous extract

of *E. officinalis* (20 mg/kg) and HFD had no effect on the water intake of female Wistar rats as the results were not significant. Similarly, A remarkable increase in water intake in AE-treated groups may be due to the difference in the extraction method used for this study.

Both ND+AE and HSD+AE groups have decreased body weights after receiving continuous AE administration compared to ND and HSD groups, respectively. When compared to the ND group, AE administration showed a strong and quick reduction in body weight of the HSD group. In a murine model of high-fat diet (HFD)-induced obesity, Nazish and Ansari (2018) discovered that AE dose-dependently reduced body weight change in comparison to rats fed an HFD alone. Sato *et al.* (2010) also reported that aqueous extract of amla significantly inhibited body weight gain as well as adipose tissue weights in mice fed high-fat diet (HFD). The glucose utilization of the four groups of experimental mice varied significantly. According to this finding, there is no substantial difference between the ND group and the ND+AE group's blood glucose levels. However, the blood glucose concentration of the HSD group was higher than that of the ND group which was consequential. The clear and consistent values of the GTT graph showed that the glucose levels at each time point in the HSD-fed group were remarkably higher in comparison to that of the HSD+AE group. In a study, healthy and diabetic human volunteers taking 2 or 3 grams of *E. officinalis* powder per day showed a substantial drop in fasting and 2 h post-prandial blood glucose levels on the 21<sup>st</sup> day when compared to their initial level (Akhtar *et al.*, 2011). An *in vitro* study demonstrated that the activity of the main phytochemicals found in amla (such as ellagic acid and ascorbic acid) reduced the activity of key enzymes involved in glucose digestion (especially amylase and glucosidase). For instance, daily doses of up to 3 g of amla powder extract reduced blood glucose levels in diabetic patients after 21 days of the trial (Akhtar *et al.*, 2011). Bioactive compounds present in amla seem to play a key role in the management of diabetes, particularly in assisting in the restoration of glucose and insulin levels.

No significant differences were observed in the weights of the heart and kidney, but the weight of the liver was significantly greater in HSD fed group compared to AE treated group. Sugar-like fructose intake has been shown to stimulate *de novo* lipogenesis in animals, as well as to block hepatic  $\beta$ -fatty acid oxidation (Lanaspa *et al.*, 2012; Softic *et al.*, 2016). Similarly, studies in humans have also shown that fructose stimulates *de novo* lipogenesis and blocks fatty acid oxidation in the liver (Cox *et al.*, 2012; Faeh *et al.*,

2005; Lim *et al.*, 2010; Softic *et al.*, 2016; Stanhope *et al.*, 2009). Acutely fructose stimulates thermogenesis and metabolic rate (Mizobe *et al.*, 2006; Schwarz *et al.*, 1992), but chronically fructose has been shown to reduce resting energy expenditure (Cox *et al.*, 2012). Liver X receptor (LXR) and farnesoid X receptor (FXR) are key regulatory molecules involved in lipid homeostasis. They are steroid receptors found in many tissues, especially the liver, intestine and kidney. They regulate important steps in cholesterol and fat metabolism. A previous study observed that treatment with amla was highly effective in preventing fructose-induced fatty liver in both ovariectomized and sham-operated, ovary-intact rats. This could be due to its effect in increasing the protein levels of liver LXR $\alpha$  in all the groups with a prominent increase in fructose-fed rats. Thus, amla may not increase LXR $\alpha$  indiscriminately but rather increase it in proportion to the need (Koshy *et al.*, 2015).

Adipose tissue is not only the store of excess energy but also an endocrine organ in the regulation of lipid homeostasis (Galic *et al.*, 2010). The weight of the white adipose tissue (WAT) was observed to reduce significantly in ND+AE and HSD+AE groups when compared to ND and HSD control groups, respectively. An increasing tendency was found in the weight of WAT in the HSD-fed group compared with the ND-fed group, though the differences were not significant. Similar results were also observed in the case of brown adipose tissue (BAT). The reduction of body weight gain can be correlated with the reduction of fat mass, indicating that reduced fat mass may lead to reduced body weight (Lee *et al.*, 2008). Hepatic steatosis mainly occurs due to the accumulation of fat droplets in hepatocytes. In the present study, fat droplets in hepatocytes were observed in the sections of the liver from the HSD-fed group. On the other hand, amla extract administration helped to keep the structure of the liver cells normal as observed in the normal diet-fed group. Amla administration maintained the normal hepatocytes and changed the fatty liver (Pallavi *et al.*, 2016). However, it was reported that simple carbohydrates stimulated fat accumulation in the liver (Basaranoglu *et al.*, 2015).

In the present study, the total cholesterol level (TC) in the HSD group was remarkably higher than that in the ND group which was significantly attenuated by the administration of amla extract. When compared to ND, TAG level also increased significantly in the HSD diet group. AE administration significantly reversed the increase in TAG level in the HSD-fed group. In groups receiving AE supplements, LDL concentrations were decreased compared to their respective control groups though the differences were not significant. The HDL levels, however, did not differ significantly among the

groups. TAG was especially positively associated with increased sugar intake in a number of studies (Kell *et al.*, 2014; Lee *et al.*, 2014; Van Rompay *et al.*, 2015; Welsh *et al.*, 2011). Additionally, increased TG/HDL-C ratio (Welsh *et al.*, 2011) and LDL-C (Loh *et al.*, 2017) have been observed during increased sugar intake. A meta-analysis of randomized controlled trials concluded that higher sugar intakes were significantly associated with increased TAG, TC, and LDL-C concentrations in adults (Te Morenga *et al.*, 2014). The possible mechanism of the blood cholesterol-lowering effect of AE may be due to the inhibition of hepatic cholesterol biosynthesis which enhances the uptake of cholesterol from circulation. However, mice treated with amla extract in this experiment did not exhibit any serious toxic symptoms.

Overall, the results obtained demonstrate that AE administration may modulate parameters that appeared to be altered in metabolic syndromes such as body weight, glycemia, and lipid profile in high-sugar diet intake. It seems to be possible that the dietary percentages of AE lower than that used in the present study will also exhibit health benefits in preventing risk factors associated with cardiometabolic disorders. The development of a new source of phytoconstituents from an underutilized agricultural commodity like amla could also offer an important way to mitigate the negative impacts of high sugar.

## 5. Conclusion

Medicinal plants, having great elementary and therapeutic importance, are a gift to mankind to acquire a healthy lifestyle. Amla extract significantly reduced food intake and body weight in mice fed a high-sugar diet, improved glucose tolerance, decreased liver, WAT and BAT weight, and reduced lipid accumulation in the liver, total cholesterol and triglyceride concentration in blood. To counteract the development of diabetes and obesity due to the consumption of a high-sugar diet, amla fruit extract effectively maintained a normoglycemic state as well as body weight and food intake. In order to manage metabolic dysregulations, amla fruit could be a dietary supplement.

## Conflicts of interest

The authors declare no conflict of interest.

## Acknowledgments

This research project was conducted with financial support provided by the University Grant Commission (UGC), Bangladesh. For the financial management of the research grant, the authors acknowledge the support and

cooperation provided by the Bangladesh Agricultural University Research System (BAURES).

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