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Anti-hyperglycemic and anti-hyperlipidemic effects of chia seed extract in alloxan-induced diabetic mice

RJ Moon[1], O Alam[1], MKH Kazal[1], MK Khatun[1], MR Haque[1], R Chacrabati[2], HA Kader[3] and C Goswami✉[1]

[1]Department of Biochemistry and Molecular Biology, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

[2]Interdisciplinary Institute for Food Security, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh

[3]Department of Biochemistry and Molecular Biology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706, Bangladesh

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Correspondence:
C Goswami ✉:
chayon.goswami@bau.edu.bd

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ABSTRACT

Diabetes is a chronic and debilitating metabolic disorder that gradually impairs the body's ability to regulate blood sugar levels, leading to complications that can affect multiple organs and systems, including the heart, kidneys, nerves, and eyes. Chia seed contains a high concentration of dietary fiber, omega-3 fatty acids, and essential amino acids, which may have hypotensive, hypoglycemic, and anticholesterolemic benefits. The objective of this research was to investigate whether administration of chia seed extract (CSE) may reduce the frequency of metabolic diseases in mice with or without diabetes. The experimental mice were divided into four groups such as normal control group, normal mice with 50 mg/ kg body weight of CSE, diabetic control group (no CSE) and diabetic mice with 50 mg/ kg body weight of CSE. In order to induce diabetes, 2% alloxan solution was used. The results showed that CSE administration led to a significant reduction in food intake in normal mice, but it did not exhibit a notable decrease in diabetic mice. While the extract did not affect the body weight of diabetic mice, a slight reduction was observed in the normal mice treated with chia. In comparison to the normal and diabetic mice, the blood glucose level was lower in chia-treated groups at all-time points of glucose tolerance test. Additionally, CSE showed a tendency to reduced LDL, TC, TG and HDL concentrations. Furthermore, CSE treated groups showed reduced liver and kidney weight in comparison to the normal and diabetic mice, but the weight of the heart was almost similar in all groups. The above findings suggest that CSE exhibits anti-hyperglycaemic, and lipid-lowering properties. These effects could potentially be used to reduce the risk of metabolic disorders and assist diabetic subjects in maintaining lipid and glucose homeostasis.

Introduction

Chia is a biannually growing plant belonging to the genus *Salvia*, family Lamiaceae, subfamily Nepetoideae, native to Mexico and Guatemala. Although the plant is primarily grown for its seeds, it is highly prized for its exquisite purple and white blooms. While the term chia comes from the Spanish word "Chía", which means "oily," the word Salvia is derived from the Latin word "Salvare", which means "the healer". Chia seeds contain 34.6% dietary fiber, while chia oil is mostly composed of α-linolenic (64% of total oil), linoleic (21% of total oil), oleic, stearic, and palmitic acids (Coorey *et al.,* 2014). It includes a high concentration of omega-3 (alpha-linolenic acid) and omega-6 (linoleic acid) fatty acids, which can aid in maintaining excellent health (Ayerza Jr and Coates, 2007). Both of the fatty acids are necessary for good health because they cannot be synthesized artificially and can only be obtained from external consumption. Even while linolenic acid makes up over 64% of the fat in chia, there is also a lesser

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amount of linoleic, oleic, and palmitic acid. Because of its high nutritional content and abundance of vital components like proteins, fiber, antioxidants, and phytochemicals, chia seeds are considered as a "superfood" that can help with a wide range of illnesses. It has about 30-34% dietary fiber, of which 85-93% are soluble and the remaining 7-15% are insoluble (Kulczyński *et al.,* 2019). Moreover, it has a high endogenous amino acid content and is a good source of protein. Chia seeds contain high amounts of protein, fat, carbohydrate, dietary fiber, ash, and dry matter ranging from 15 to 25%, 30 to 33%, 41%, 18 to 30%, 4 to 5%, and 90 to 93%, respectively, with a broad range of polyphenols (Ixtaina *et al.,* 2008). Tocopherols and phenolic compounds are only two of the many bioactive ingredients found in chia seeds. Natural antioxidants found in it also offer protection against a number of illnesses, including some malignancies and cardiovascular conditions.

Worldwide, diabetes is the most prevalent and deadly illness affecting people. Diabetes is a type of metabolic disorder that affects how glucose and other fuels for energy are metabolized. World Health Organization (WHO) estimated that there would be about 250 million cases of diabetes throughout the world by 2025 (Friedman, 2002). According to a 2015 survey carried out in Bangladesh, 7.1 million people had diabetes, 3.7 million cases went undiagnosed, and the illness was blamed for almost 13 million fatalities (Mohiuddin, 2019). According to Mostofa (2007), hyperglycemia is the main symptom of acute diabetes mellitus, and it is frequently accompanied by glucosuria and polyuria. Diabetes is becoming more common, and treating it is getting more expensive every day. Many different kinds of medications are used to treat diabetes. Only diabetes type 2, or non-insulin-dependent diabetes, can be treated with oral medicines. Patients with type 1 diabetes are dependent on insulin for their treatment. Sulfonylureas were the first diabetes medications used orally. Due to the adverse effects of most medications, sulfonylureas might result in hypoglycemia, skin rashes or itching, sensitivity to sunlight, stomach upset, and weight gain. Normally, chia is utilized as a therapeutic food. The so-called functional foods have traditionally been consumed as daily staples, depending on their availability. Recent approaches advocate for integrative treatments for diabetes, targeting multiple organ systems rather than just reducing hyperglycemia, with plant-based drugs showing promise in managing chronic diseases like diabetes. These days, it is clear that functional foods have significant nutritional values since they include unique kinds of bioactive chemicals. The presence of active components and the bioactivities of substances that were originally present in the plant are the main sources of advantages for functional meals. Certain observational and experimental studies have demonstrated the potential of chia seeds to mitigate certain risk factors, including inflammation, fatty oils, insulin resistance, and visceral obesity. They may also increase "excellent" HDL cholesterol. The seeds are also a good source of minerals, dietary fiber, proteins, and phytochemicals with lipid-lowering, antioxidant, and cardioprotective qualities such as myricetin, quercetin, kaempferol, and chlorogenic acid. Quercetin, an antioxidant found in chia seeds, has been shown to lower the risk of a number of illnesses, including heart disease and coronary illness (de Silva Marineli *et al*., 2014). Again, antioxidants play a critical role in the prevention of cancer by limiting free radical damage, which has been linked to the growth of cancer cells. An investigation showed the effects of consuming chia seeds (*Salvia hispanica* L.), which are high in fiber and alpha-linolenic acid, on insulin resistance (IR) and dyslipidaemia (Chicco *et al*., 2008). IR was brought on by the consumption of meals high in sucrose (62.5%). In sugar rich diet-fed rats, chia seeds help to lower visceral fat through diet supplementation. Another study that used *Salvia hispanica* to treat Wistar rats on a high-fat, high-fructose diet also revealed reduced blood glucose, triglycerides, and body weight (Batista *et al*., 2023). Ground and whole chia seeds added to bakery products reduced blood glucose levels after fasting, as shown by lower area under the curve (AUC) of glucose tolerance test compared to the control group (Ho *et al*., 2013). Therefore, the present study was designed to evaluate the potentiality of CSE to improve metabolic dysregulation in both normal and diabetic mice.

Materials and methods

Chia seed preparation: The edible chia seeds used in this experiment were purchased from the local market of Mymensingh. The seeds were carefully cleaned and separated from other unwanted items before being sun-dried. Dried seeds were finely pulverized in a grinding machine after being sun-dried. Until ethanol extraction, the powdered seeds were kept in an airtight plastic bag.

Preparation of chia seed extract: With a few minor adjustments, the technique outlined by Horbowicz and Obendort (1994) was used to make the ethanolic extract of *Selvia hispanica*. Five grams of chia seed powder and precisely 100 ml of ethanol were combined in a conical flask, which was then shaken at 120 RPM for a full day. Following filtration through filter paper (Whatman number 1), the residue was extracted again using 100 ml of ethanol. In a vacuum rotatory evaporator, two filtrate sections were combined and evaporated at a temperature not exceeding 45°C. Before being administered, the concentrated slurry was freeze-dried and kept chilled.

Food formulation: Food contains various amounts of wheat, wheat bran, rice polishing, fish meal, oil cake, pulses, milk, soy oil, molasses, salt, and embavit (a vitamin premix) (Table 1).

Table 1. Composition of normal food formulation used in this study (for 100g) (Ulla et al., 2017).

Ingredients of normal lab diet	Percent
Wheat	40.0
Wheat bran	20.0
Rice Polishing	5.5
Fish meal	10.0
Oil cake	6.0
Gram	0.39
Pulses	0.39
Milk	0.38
Soybean oil	1.5
Molasses	0.095
Embavit	0.1

Experimental animals: Male Swiss albino mice, four weeks old, were acquired from the Animal Resources Facility of International Centre for Diarrheal Disease Research, Bangladesh (ICDDR, B). This study protocol was approved by the Institutional Ethical Standard of Research Committee (ESRC No.: BAURES/51/2024) of Bangladesh Agricultural University. To help the experimental mice get used to their new surroundings, they were split up into four groups and allowed to adapt for 10 days. The animals were kept in a room with natural daylight and good ventilation, maintained at 28±2°C and 70-80% relative humidity. Water and regular meals were freely available throughout the feeding trial. Each of the four groups of animals included a minimum of three mice.

Induction of experimental diabetes: Mice underwent an eight-hour fast after receiving a subcutaneous injection of freshly made 2% Alloxan solution. With few modifications, the methodology outlined by Sabu and Kuttan (2002) was implemented. One hundred fifty mgs of Alloxan per kg of body weight was administered to induce diabetes in mice after 10 days of acclimatization to the new environment. To help the mice to recover, 5% dextrose solution was administered intraperitoneally.

Experimental design: Experimental mice were divided into four groups normal control group, normal mice with chia administration, diabetic control group, and diabetic mice with chia administration. The experimental mice were provided with the oral administration of chia seed extract solution at a dose of 50 mg/kg body weight after an acclimatization period of 14 days of inducing alloxan.

Biochemical parameters

Measurement of food intake and body weight of the mice: A 15-day feeding trial was performed and mice were provided with *ad libitum* food and water. The food intake was measured on alternative days (at 8:00 AM) up to 14 days of the experiment. The body weights were measured in three-day intervals until the end of the feeding trial.

Intraperitoneal glucose tolerance test (i.p. GTT): At the conclusion of the treatment, the intraperitoneal glucose tolerance test (i.p. GTT) was performed in accordance with the usual protocol as detailed in a different report (Maejima *et al.,* 2015). Mice were fasted for 4 hours in clean cages with continuous access to water. Blood glucose was measured using a Glucoleader IM Enhance Meter. Following measurement, each mouse received an intraperitoneal injection of glucose (2 g/kg BW). Blood glucose levels were recorded at 0, 15, 30, 60, and 120 minutes post-injection. The area under the curve (AUC) for glucose levels was calculated from these measurements to assess glucose tolerance.

Blood sample collection and preparation of serum: Following an 18-hour fast, blood samples were taken from the Posterior Vena Cava using the previously mentioned technique after end of the feeding experiment (Hoff *et al*., 2000). The blood was drawn into a 1.5 ml Eppendorf tube. The blood-containing tubes were spun for 10

minutes at 4°C at 4000 rpm using a Gyrozen 1580R Multi-Purpose High-Speed Refrigerated Centrifuge machine (Gangnamgu, Seoul, Korea). Serum samples were stored at -20°C until lipid profile assay.

Measurement of organs weight: After collecting the blood samples, the internal organs such as liver, heart, and kidney were harvested and weights were measured using a digital balance (Eki300-2n electronic scale. A&D company Ltd. Korea).

Determination of lipid profile parameters: Parameters such as total cholesterol (ascertained by the CHOD-PAP method; Richmond, 1973); TG level (ascertained by the GPO-PAP method; Cole *et al*., 1997); and HDL cholesterol (ascertained by the CHOD-A method; Henry et al., 1974) were analyzed in lipid profile investigations. Serum LDL cholesterol concentrations were calculated using the Friedewald equation (Friedewald *et al*., 1972) as follows:

LDL cholesterol (mg/dl) = Total cholesterol - HDL cholesterol- (Triglyceride/5)

Statistical analysis: For all statistical analysis, Prism 5 (GraphPad Software, CA) was used. The mean SE was shown for every data set. Tukey's post-hoc test was used after an analysis of variance (ANOVA) to support the significant differences ($p<0.05$) between treatment groups.

Results and Discussion

Effect of chia extract on daily food intake

In a 15-day study, chia seed extract significantly impacted food intake in normal but not diabetic mice. Initially, daily food intake was similar between control and chia groups in normal mice (4.68±0.32g vs. 4.17±0.51g) and between diabetic and diabetic + chia groups (6.0±0.14g vs. 5.83±0.30g). By day 10, chia supplementation reduced food intake significantly in chia supplemented mice (3.58±0.22g), compared to controls (5.18±0.32g) and continued to the end of the experiment. In diabetic mice, chia initially decreased food intake which became significant on day 4 (6.67±0.22g for diabetic vs. 5.00±0.29g for diabetic+chia), but this effect did not sustain rather it showed a trend to maintain a constant food intake. Overall, chia seed extract led to a significant decrease in food intake in normal mice, while it had no lasting effect on diabetic mice.

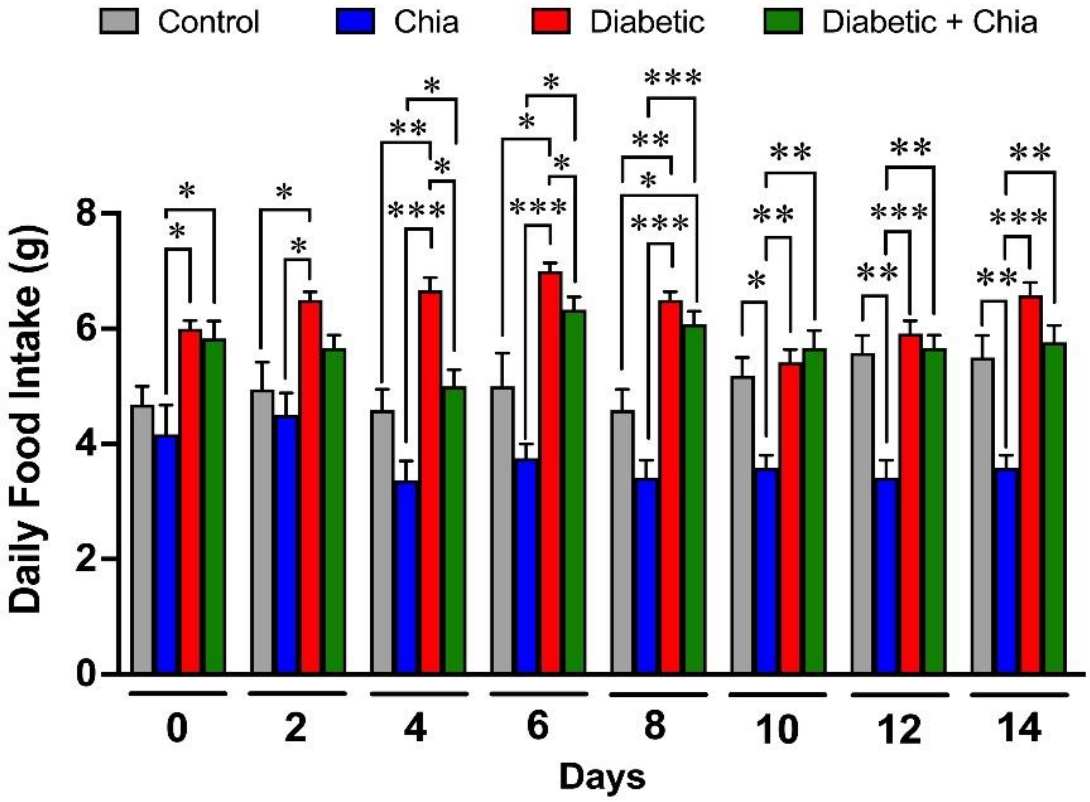


Figure 1: Effect of chia seed extract (CSE) on daily food intake in Swiss Albino male mice. All data were expressed as mean ±SEM (n≥3). Here, "*" indicates $p<0.05$; "**" indicate $p<0.01$ and "***" indicate $p<0.001$.

In this study, we explored the effects of CSE on food intake that is potentially linked to hormonal and neuronal pathways that regulate appetite and satiety (Benton and Young, 2017). A reduction in food intake is associated with decreased energy intake, leading to lower blood glucose levels and fat mass, which may prevent diabetes and obesity. Our findings indicated that CSE administration significantly reduced food intake in normal mice, likely due to increased satiation. This aligns with previous studies where higher long-chain omega-3 PUFA intake correlated with enhanced sensations of fullness (Parra *et al*., 2008). The study came to the conclusion that during weight loss, long chain omega-3 PUFA consumption influences postprandial satiety in overweight and obese volunteers. Increased sensations of satiety may therefore lead to a reduction in subsequent food intake and an improvement in body composition and weight loss (Parra *et al*., 2008). In another study by Ayaz *et al.* (2017), participants reported significantly lower hunger scores, reduced desire for sugary foods, and increased satiety after consuming 7 g and 14 g of chia seeds compared to a control group. Decreased food intake in mice may be due to the omega-3 fatty acids found in chia seeds. However, in diabetic mice, CSE did not significantly alter food intake, suggesting that the mechanisms of appetite regulation may differ in diabetic conditions. The relationship between omega-3 PUFAs, particularly alpha-linolenic acids (ALA) from plant sources and EPA/DHA from seafood, and type 2 diabetes risk remains inconclusive, necessitating further research.

Effect of chia extract on body weight

Body weight was monitored at three-day intervals, and both the control and chia groups showed a natural increase. Initial weights were almost similar in the control (58.33±2.38g) vs chia (56.67±4.71g) and diabetic (51.75±0.94g) vs diabetic+chia (48.33±3.29g) groups. On day 12, chia supplementation tended to decreased body weight (49.00±5.04g) compared to controls (58.67±3.39g), however, the difference was not statistically significant but when we consider the change in body weight (Fig. 2B) the overall change in body weight of chia supplemented group in normal mice was significantly decreased. Diabetic mice lost weight, most likely because of decreased glucose metabolism. Chia supplementation showed no remarkable effect on the weight of diabetic mice. Diabetic mice's body weight declined significantly by day 15 when compared to control group, which was partially alleviated by chia extract (38.25±4.33g in diabetic control vs. 39.67±1.86g in diabetic+chia).

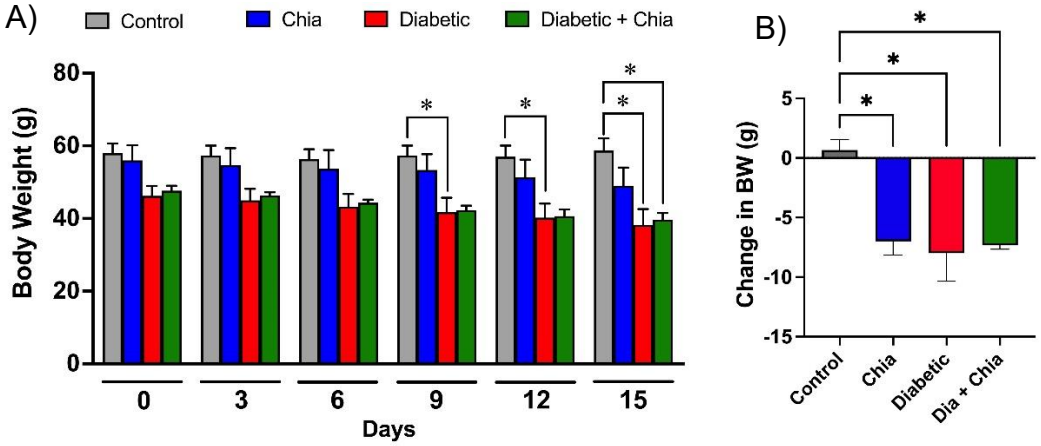


Figure 2: Effects of chia seed extract (CSE) on body weight in Swiss Albino male mice. A) Body weight (g). B) Change in BW (g). All data were expressed as mean ±SEM (n≥3). Here, "*" indicates $p<0.05$.

Diabetic mice experienced a significant decrease in body weight, which was partly alleviated by CSE treatment. Chia seeds extracted with ethanol include omega-3 fatty acids, which may aid in weight maintenance and fat loss via a variety of methods. A 6-month double-blind trial found that overweight or obese type 2 diabetes patients who consumed chia seeds lost more weight and had a greater reduction in waist circumference compared to a control group (Vuksan *et al*., 2017). One mechanism is the increase in postprandial satiety, which leads to decreased food intake and body weight. Dietary fiber is a component of chia seeds that is linked to weight loss. Dietary fiber is well known for helping people to lose weight (Lattimer and Haub, 2010). It does this by

delaying the emptying of the stomach and boosting the release of intestinal hormones that encourage satiety, which in turn reduces calorie intake and aids in weight reduction. Another mechanism includes modifying gene expression associated with fat oxidation and adipogenesis in adipose, hepatic, heart, intestinal, and skeletal muscle tissues. These factors affect gene expression that regulates fat oxidation and reduced fat deposition. Long-chain (LC) omega-3 PUFA-supplemented meals have been proven in studies to result in significantly lower body weights and visceral fat than non-LC omega-3 PUFA diets (Flachs *et al*., 2005).

Effect of chia extract on glucose tolerance

This study looked into the usefulness of CSE in reducing diabetes symptoms. Intraperitoneal glucose tolerance tests (i.p. GTT) were used to assess the impact on blood glucose homeostasis. The chia-supplemented group consistently had lower blood glucose levels than the controls after receiving a 2g/kg BW glucose injection (Fig. 3A). Chia supplementation in diabetic mice resulted in considerably decreased blood glucose levels 30 and 60 minutes after injection. After 15 minutes, the control group's glucose levels increased to 340.83±15.30mg/dL, while the chia group's levels were 279.25±12.50mg/dL. Whereas diabetic mice showed 401.14±35.15mg/dL, while the diabetic+chia group had 341.65±35.23mg/dL after 15 minutes of glucose challenge. AUC analysis revealed considerably lower values in chia-supplemented groups compared to respective control groups (Fig. 3B).

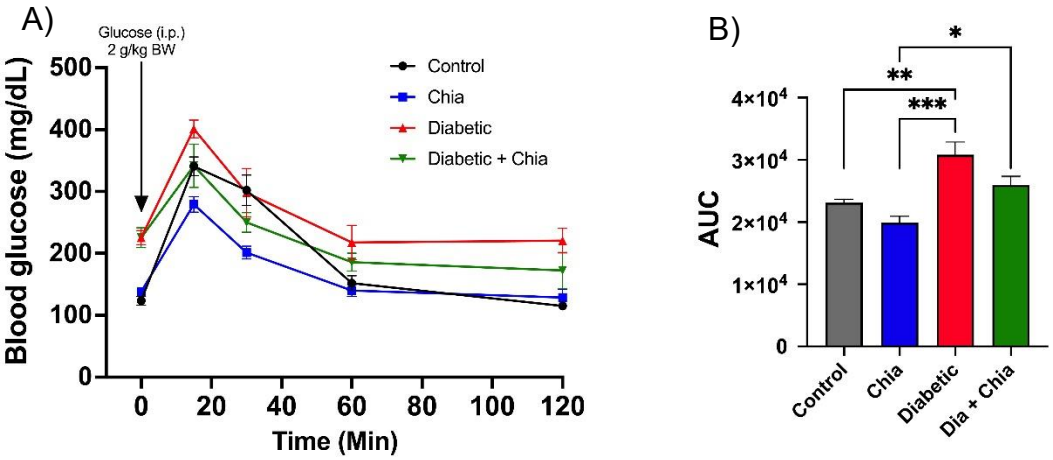


Figure 3: Effects of chia seed extract (CSE) treatment on glucose tolerance in Swiss Albino male mice. A) Change in blood glucose (mg/dL) at 0, 15, 30, 60, 120 minutes after glucose infusion (2 g/kg BW). B) Area under the curve (AUC) during i.p. GTT. All data were expressed as mean ±SEM (n≥3). Here, "*" indicates p<0.05"**" indicate p< 0.01 and "***" indicate p<0.001.

The blood glucose levels in the chia-supplemented groups were considerably lower than in the controls. A study of rats on a high-fat, high-fructose diet found that consuming chia seed (13.3%) and chia oil (4%) enhanced glucose tolerance and insulin resistance (Marineli *et al*., 2015). Chia flour and oil improve glucose metabolism in case of insulin resistance. Rosmarinic acid (RA), the main phenolic ingredient in chia seeds, exhibits hypoglycemic properties (Runtuwene *et al*., 2016). Chia oil improves glucose tolerance by boosting the levels of key proteins and genes involved in insulin signaling and energy metabolism, including AKT1, the insulin receptor, FOXO1, and glycolysis enzymes. Additionally, Chia hydrolyzed phenolic extract (CHPE) reduces the gene expression of enzymes responsible for gluconeogenesis, which is the production of glucose from non-carbohydrate sources (Enes *et al*., 2020). Chia seeds' insoluble dietary fiber gives extra bulk and delays the transit time through the gastrointestinal tract by holding water several times of its weight when hydrated. Greater postprandial blood glucose levels and a longer duration of decreased insulin resistance are directly correlated with longer gastro-intestinal transit times (Munoz *et al*., 2012). Furthermore, omega-3 fatty acids in chia may help to protect kidney function in type 2 diabetic patients with hypertriglyceridemia.

Effect of chia extract on organ weight

Organ weights were determined for each mouse to compare between groups. There were no significant differences in heart weights between the following groups:

control (4.48±0.35mg/g BW), chia (4.47±0.36mg/g BW), diabetic (4.66±0.30mg/g BW), and diabetic+chia (4.56±0.57mg/g BW). Chia supplementation led to a decrease in liver weight: control (54.42±1.17mg/g) vs. chia (47.29±1.86mg/g), and diabetic (53.15±3.97mg/g BW) vs. diabetic+chia (58.41±6.30mg/g) (Fig. 4B). Kidney weights differed somewhat among the groups, but not significantly.

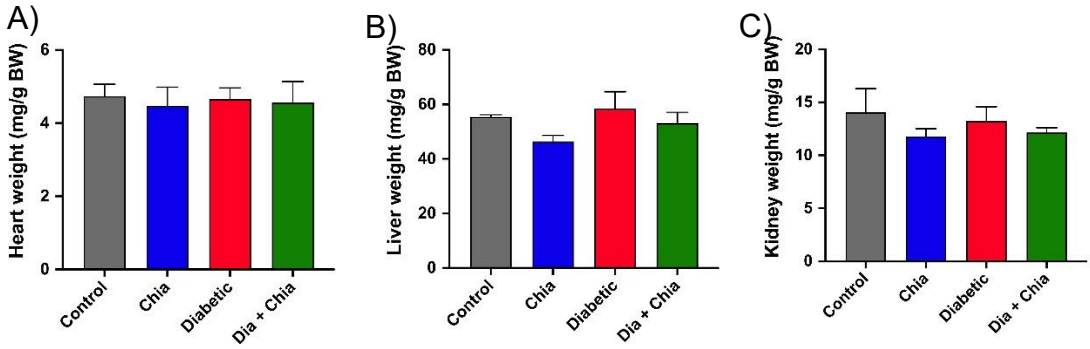
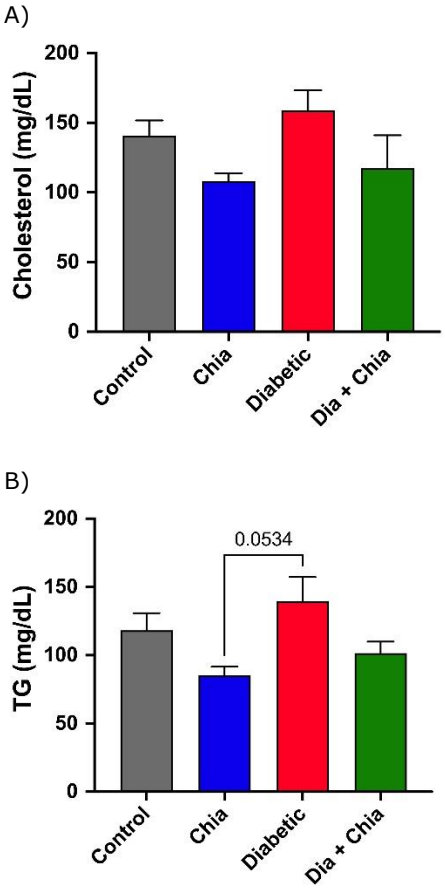


Figure 4: Effects of chia seed extract (CSE) treatment on organ weight in Swiss Albino male mice. A) Heart weight (mg/g BW). B) Liver weight (mg/g BW). C) Kidney weight (mg/g BW). All data were expressed as mean ±SEM (n ≥3). Here, "*" indicates $p<0.05$.

Chia-fed animals had lower liver weights than the control group. The reduction in hepatocyte volume in animals fed various chia processing methods was directly related to a decrease in fat globule content in the liver (da Silva *et al*., 2016). Chia was able to reduce liver fat percentages and weights by lowering lipid buildup in the body, which may have resulted in enhanced fat excretion in the feces.

Effect of chia extract on lipid profile

Serum samples were analyzed for total cholesterol (TC), triglycerides (TG), and high-density lipoprotein (HDL) using specific reagents, and low-density lipoprotein (LDL) calculated from TC, TG, and HDL values. No significant differences were observed in TC, HDL, and LDL among the groups (Fig. 5). After 14 days of chia supplementation, the cholesterol level decreased in the chia group (108.10±5.66mg/dL) compared to the control (140.87±10.82mg/dL) and diabetic (158.77±14.56mg/dL) groups. TG levels tended to decrease in the chia-supplemented groups (85.13±6.42mg/dL) compared to controls (118.23±12.65mg/dL) and diabetics (139.40±17.89mg/dL), though not statistically significant (p=0.0534). LDL level was lower in the chia group compared to control and diabetic groups (Fig. 5D) but without statistical significance.



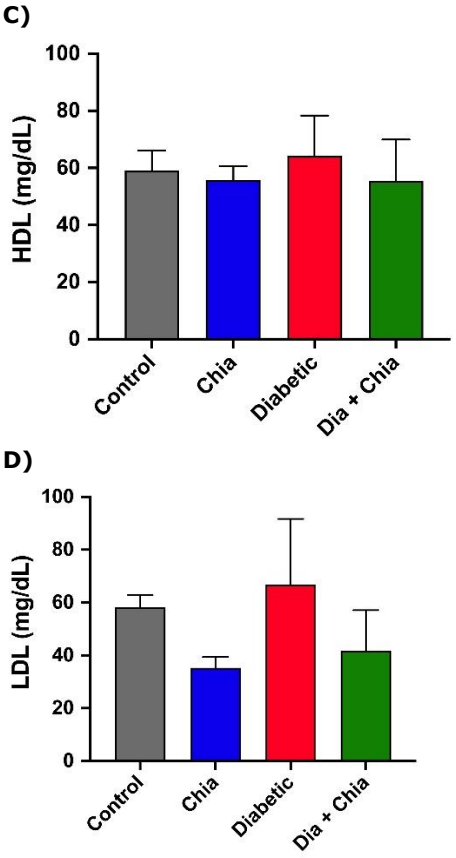


Figure 5: Effects of chia seed extract (CSE) treatment on serum lipid profile in Swiss Albino male mice. A) Cholesterol (mg/dL). B) TG (mg/dL) C) HDL (mg/dL). D) LDL (mg/dL). All data were expressed as mean ±SEM (n ≥3). Here, "*" indicates $p<0.05$.

The chia-supplemented group exhibited lower levels of total cholesterol, LDL, HDL, and triglycerides (TG) compared to the control group. This lipid profile improvement aligns with other studies on dietary fibers in rats and humans (Anderson *et al*., 2000). Chia seed soluble fiber dissolves in water and moves through the digestive tract more slowly than insoluble fiber. Healthy eating combined with soluble fiber may help reduce LDL cholesterol levels without affecting HDL cholesterol (Mohamed *et al*.,2018). Dietary omega-3 fatty acids, abundant in chia seeds, have been shown to lower plasma triacylglycerol levels in rats (Mohamed *et al*., 2002). In Diabetes Mellitus (DM), dyslipidemia is characterized by increased TG and decreased HDL levels (Goodman, 1996). The interaction between omega-3 fatty acids and cholesterol is critical, as cholesterol content and organization significantly affect cell membrane structure and function. Therefore, CSE, rich in omega-3 fatty acids, can improve lipid profiles in both normal and diabetic mice, as observed in this study. The lack of significant results may be due to the short duration of the study. Further research is necessary to elucidate the mechanisms by which CSE reduces blood glucose and improves lipid profiles.

Conclusion

Chia seed extract decreased body weight, liver weight, and LDL cholesterol while enhancing glucose tolerance in normal mice. Chia seed administration led to maintain body weight and reduced blood glucose levels in diabetic mice, improved glucose tolerance compared to diabetic controls, lowered LDL, total cholesterol, and triglyceride levels, and decreased liver weight, indicating better control of hyperlipidemia and metabolic improvements. Incorporation of chia seeds into the regular diet may help to reduce the risk of metabolic disorders, including those linked to excess body weight, glucose intolerance, and hyperlipidemia, may be due to their high content of omega-3 fatty acids and antioxidants, which support metabolic health and reduce inflammation. However, further research is required to elucidate the mechanisms by which chia seeds maintain glucose and lipid homeostasis and protect against the development of diabetes, obesity, and other metabolic conditions.

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Authors contribution

The authors confirm contribution to the paper as follows: study conception and design: Chayon Goswami, Rakhi Chacrabati, Md. Rezwanul Haque; data collection: Romana Jahan Moon, Ohi Alam; analysis and interpretation of results: Romana Jahan Moon, Md. Kamrul Hasan Kazal, Mst. Khadiza Khatun, Chayon Goswami; draft manuscript preparation: Romana Jahan Moon. Md. Kamrul Hasan Kazal, Hafij Al Kader, Chayon Goswami. All authors reviewed the results and approved the final version of the manuscript.

Data availability

The data supporting these research findings will be made available as per the authorization of the authors.

Conflict of interest

The authors declare that they have no conflict of interest regarding the publication of this article.

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