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RESEARCH ARTICLE

Therapeutic Impact of Bitter Gourd Seed-fortified Crackers on Alloxan-induced Diabetic Rats

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ABSTRACT

Diabetes mellitus (DM) is a metabolic condition marked by a decreased body's capacity to produce or react to endocrine hormones to keep blood glucose levels within normal ranges. DM is categorized into type 1 DM (T1DM), type 2 DM (T2DM), and gestational diabetes. T2DM accounts for 90% of DM cases and is largely associated with diet. In this research, bitter gourd seeds (BGS) were incorporated at 10% into crackers to make BGS-fortified crackers (BGS-C), to investigate their therapeutic impact on DM. A 35-day therapeutic trial on 15 male Wister rats was performed. Rats were divided into five groups where G0 (non-diabetic: control diet), G1 (diabetic: Metformin), G2 (diabetic: Metformin + 25% BGS-C), G3 (diabetic: Metformin + 50% BGS-C), and G4 (diabetic: Metformin + 75% BGS-C). The results of this study revealed that the inclusion of BGS-C into the diet of diabetic rats brought much faster, highly significant, and health-promising outcomes. The results of G4 and G3 were quite close to the normal healthy lab values for almost all biomarkers including body weight, diabetic biomarkers, lipid profile, liver and renal function tests. The outcomes of G4 when compared to G1 indicated highly significant improvements in diabetic biomarkers i.e. percentage decline of 11.98% in serum glucose, 33.7% in serum insulin, and 54.8% in fasting blood glucose. These outcomes have strengthened the nutraceutical significance of BGS against DM. Moreover, the treatment of DM only relying on the medicine may prolong the disease but the use of BGS along with the use of medicine may help in speeding up the recovery process from DM. Hence, the regular dietary intake of BG or BGS is highly recommended to lower the negative health outcomes of DM and its associated metabolic disorders.

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INTRODUCTION

Diabetes Mellitus (DM) is one of the chronic and metabolic diseases that impairs the body's capacity to either produce or react to the hormone insulin, which controls blood sugar levels. As a result, there is an irregular metabolism of carbohydrates and increased blood and urine glucose levels (Niaz *et al.*, 2018). Insulin is essential for the movement of blood glucose from the circulatory system into cells where it is converted into energy. Every macronutrient after its consumption in broken down into simple sugar in the blood, which is then transported into cells by the endocrine system for either energy storage or usage (Olisah *et al.*, 2022). Current reports of the World Health Organization (WHO) have shown that 415 million people worldwide are affected with diabetes. People with diabetes are said to increase by 2045, it is predicted that 48% of the world's population will have this disease (Kotwas *et al.*, 2021).

The most common symptoms of DM include polyuria, polydipsia, polyphagia, and weight loss despite having a healthy appetite, increased susceptibility to infections, particularly urinary tract and skin infections (Niaz *et al.*, 2018), while untreated DM can cause severe complications such as cardiovascular disease (CVDs), chronic kidney diseases (CKD), nerve damage, blurred vision, and hearing problems. Common risk factors of DM include obesity, unhealthy eating habits, a sedentary lifestyle, and low levels of physical activity. DM is categorized into 3 different types; type 1 DM (T1DM), type 2 DM (T2DM) and gestational diabetes from which 90% of cases reported are of T2DM (Mikhael *et al.*, 2020).

Uncontrolled glycaemia in T2DM patients has been linked to poor eating habits, inactivity, non-adherence to treatment, and irregular blood glucose monitoring. Free radicals are known to cause oxidative stress, which is linked to an increased risk of diabetes because they damage membranes, deplete antioxidant stores, and interfere with cellular functions. Hence the dietary inclusion of naturally present hypoglycemic and antioxidant substances that have the potential to be useful against or with diabetic medications is highly recommended. The American Diabetes Association (ADA) states that diabetes self-management education is essential to prevent long-term problems and improving the quality of life for T2DM patients (Zanzabil *et al.*, 2023).

Despite the abundance of pharmaceutical medicines with anti-diabetic properties, there is a growing interest in plant-based therapies due to their low cost and few side effects (Anwar *et al.*, 2022). Herbs and nutritional supplements are used in complementary and alternative medicine to treat various illnesses. Since ancient times, traditional medicinal herbs and their compounds have been employed for therapeutic purposes. More than fifty plant species are commercially used for medical reasons WHO, with bitter gourd (BG) being the most often used. (Bhuiyan *et al.*, 2020). BG also known as balsam pear, is a widely consumed summer vegetable. It grows mostly throughout Asia and other parts of the world, especially Pakistan. It is a member of the genus Momordica, scientifically known as *Momordica charantia* L., family Cucubitaceae, order Cucurbitales (Saha and Chatterjee, 2022).

The plant is an annual vine, about 2 to 4 meters high, that is monoecious, thin, and tendril-climbing. The fruits have a thin coating of flesh and a hollow cross-section, making them warty. After 45 to 80 days, the fruit ripens, and the straw-colored, flesh-covered seeds are 8 to 15 mm long. The optimal temperature range for germination is 25 to 28 °C. It is a rich source of iron, β-carotene, calcium, potassium, vitamins B1 to B3, C, and dietary fiber (Jamil, 2016). Triterpenes, protein molecules, steroids, alkaloids, saponins, flavonoids, and acids are among the biologically active plant compounds found in them. Even though other plant parts are also used as food and medicinal, the BG plant's fruit is the most important part from the consumption point of view. According to the maturity stages, BG Seeds (BGS) are a great source of protein (28–30%) and oil (18.1-37.6%). BGS contains a significant amount of fatty acids, including conjugated linoleic acid (CLA) and antioxidants. It has significant lectin that has hypoglycemic effect and thus is believed to possess insulin activity. Moreover it has anti-fungal, anti-bacterial, anti-parasitic, antiviral, anti-fertility, anti-tumorous, hypoglycemic and anti-carcinogenic qualities (Azeez, 2023).

There are multiple ways that BGS might reduce blood sugar. Firstly, the bioactive ingredients in these, such as charantin, polypeptides, and saponins, may have hypoglycemic effects by raising insulin sensitivity, causing the pancreatic beta cells to release insulin, and inhibiting the liver's ability to synthesize glucose. All of these actions would facilitate the uptake and utilization of glucose by cells (Boyar *et al.*, 2023). Secondly, bioactive components in BG may inhibit the intestinal enzymes alpha-amylase and alpha-glucosidase, which oversee breaking down and absorbing carbohydrates. Thus, BGS may aid in controlling postprandial blood sugar levels by decreasing the pace at which glucose is absorbed and carbohydrates are broken down. Furthermore, pancreatic beta cells can be shielded from oxidative stress-triggered damage by the antioxidant qualities of BG, maintaining their functionality and ability to secrete insulin (Yakubu Magaji Yuguda, 2023). Additionally, it has been discovered that extracts from BGS promote the synthesis of nitric oxide, lower lipid peroxidation, and improve vascular health, all of which contribute to improved insulin sensitivity and glucose metabolism in general. Moreover, these extracts have demonstrated noteworthy antibacterial action against *Bacillus cereus*, underscoring their potential for the management of hyperglycemia and related microbial infections that are frequently observed in individuals with diabetes (Gayathry and John, 2022).

For the dietary inclusion of BGS, crackers were selected as a baseline food vehicle. Crackers are among such popular snacks that can have functional ingredients added to them to create higher-value nutritional meals especially to target diseases that restrict the consumption of sugary items. Crackers are described as thin, crispy, dry-baked snacks with minimal levels of sugar, fat, and moisture that are mostly produced with wheat flour (Giannoutsos *et al.*, 2023). In this research, the standard cracker's recipe was fortified with BGS at 10% fortification levels to investigate their therapeutic impact on physical and laboratory biomarkers of diabetic rats.

MATERIALS AND METHODS

Procurement of materials and chemicals: All the raw materials including BGS, and wheat flour were procured from the local merchant market of Faisalabad, Pakistan, likewise, all the required chemicals, equipment, and kits including alloxan, metformin, drug feeding kits, sample collection vials were purchased from local chemist shops and pharmaceutical companies.

Phytochemical analysis of BGS by HPLC-MS: Using an HPLC Acquity H-CLASS system (Waters, Milford, MA, USA) connected to a Xevo TQD triple-quadrupole mass spectrometer outfitted with a Z spray TM electrospray ionization (ESI) source, the phytochemicals in BGS powder were identified and measured. BGS powder was diluted 10 times prior to injections and filtered using a hydrophilic PTFE syringe filter (Analitica, São Paulo, SP, Brazil) with a diameter of 13 mm and pore diameter of 0.45 µm before the chromatographic analysis. A final solution containing 5.0 µL was injected into an Acquity HPLC® BEH C18 column (50 × 2.1 mm, 1.7 µm) (Waters, Milford, MA, USA) maintained at 40 ± 1 °C to perform chromatographic separation of the phenolic compounds. The mobile phases were water-acidified with 0.1% formic acid (solvent A) and methanol (solvent B) at a flow rate of 0.220 mL/min. Gradient elution with the methanol % changing linearly was used as follows: 0–3.0 min, 13% B; 3.0–7.2 min, 65% B; 7.2–8.3 min, 100% B; 8.3–12 min, 87% B, according to the method described by (Lopes *et al.*, 2018).

Preparation of BGS-fortified crackers (BGS-C): BGS were cleaned and ground into fine powder and were

incorporated at a 10% fortification level by replacing 10 g wheat flour with BGS in a total of 100 g wheat flour. Briefly, 100 g of BGS-fortified wheat flour, 2 g of salt, 4 g of sugar, 30 g of ghee and 40 mL of cold water were taken in a bowl mixer and mixed for about 5 minutes to prepare a dough for BGS-C. The dough was sheeted (around 3 mm in thickness) and the crackers were shaped by using a molder. Finally prepared crackers were baked at 180 °C for about 15 minutes. Baked BGS-C were allowed to cool down at room temperature and were stored in properly labeled zip lock bags.

Housing of animals: A total of 15 male adult Wistar rats, 100–170 g weight, 6–8 weeks of age, were adequately housed in standard square ft. cages with three rats per cage at the animal room near the National Institute of Food Science and Nutrition, University of Agriculture, Faisalabad, Pakistan, with the consent of the university's Ethical Review Committee (D#1913/ORIC). The rats were reared in constant environmental conditions of an equal length light-dark cycle, water available ad libitum, and 25±5 ℃ room temperature. Rats were given a commercially available rat pellet diet having 35% carbohydrates, 25% proteins, 7% lipids and 3% vitamins.

Induction of T2DM: Rats were induced with DM with alloxan in sodium citrate buffer. For this purpose, 0.1 mol of citrate buffer was prepared with 2 g of sodium citrate dissolved in 100 ml of distilled water. To make 60 mg of alloxan, 0.6 g of alloxan was dissolved in 1 ml of citrate buffer. An active blood glucose meter was used to measure the animals' fasting blood glucose 72 hours after they were induced, following a single injection of alloxan (140 mg/kg body weight of rats in solution form) via the intraperitoneal vein. Diabetic rats had blood glucose levels higher than 200 mg/dl. The control group rats were not induced with DM following the study plan (Onyibe *et al.*, 2021).

Experimental design, drug and diet allocation: A 35-day therapeutic trial was conducted on 15 male Wister rats divided into five groups of three rats each. The first group, i.e., G0 is considered the control group with no induction of T2DM and was fed a normal diet throughout the trial. In the next four groups, rats were induced DM using alloxan on the 7th day of the trial and were shifted to the treatment plan by giving standard drug metformin [Dose of metformin in mg= 0.01 x 40 x weight of rat (in g)] at day 10 (Mobasher *et al.*, 2020). The diabetic rats of G1 were fed a normal pellet diet and were only treated with metformin. The diabetic rats in G2, G3, and G4 were given 25, 50, and 75% BGS-C replaced with their normal pellet diet in addition to the standard drug metformin.

Physical parameters: The body weight change in terms of increase or decrease in the total body weight was recorded on a weekly basis.

Biochemical parameters: At the end of the trial, the rats were decapitated after being given anesthesia with ether. The blood samples were taken from the retro-orbital venous plexus of rats. The blood samples were placed into the vials and were centrifuged (Eppenfrof AG, Hamburb, Germany) at 3700 rpm for 20 minutes at room temperature. After the serum was separated, 10 µL was used right away to measure blood glucose, and the remaining serum was kept at -20 °C to measure other parameters (Mahmoud *et al.*, 2017). Blood samples from the rats were collected, serum was isolated, and amylase activity was measured by using an amylase activity assay kit as the method proposed by (Gao *et al.*, 2021). Serum glucose level or random blood glucose (RBG), serum fructosamine (s-fructosamine), serum insulin (s-insulin), pancreatic malondialdehyde (p-MDA), and pancreatic reduced glutathione content (p-GSH) (for insulin resistance and β-cell function) were determined as the methods described by Mahmoud *et al.*, (2017). Pancreatic amylase (p-amylase) activity in rats was measured using an amylase activity assay kit. This involved isolating the serum from the rats and then measuring the amylase activity using a colorimetric or fluorometric assay. Lipid profile test including total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL); liver function test (LFT) including alanine transaminase (ALT), and aspartate transaminase (AST), alkaline phosphatase (ALP); and renal function test (RFT) including serum creatinine and urea were performed as the methods described by (Siques *et al.*, 2014;Thammitiyagodage *et al.*, 2020).

Statistical analysis: To get the mean ± S.D. for each analysis, triple runs of the analyses were conducted. Using IBM® SPSS® Modeller 16.0, one-way analysis of variance (ANOVA), independent sample t-test, and Tukey HSD test were applied to examine differences among treatments and means at a 95% ($p<0.05$) confidence interval. The Omni online percentage increase calculator was used to determine the percentage increase or decrease in three BGS group readings as compared to the readings of the Metformin group.

RESULTS

Phytochemical analysis of BGS by HPLC-MS: The phytochemicals detected after HPLC-MS analysis of BGS powder are shown in Table 1 which represents that BGS powder is a rich source of phenolic compounds.

Changes in body weights on BGS consumption: The gain in body weight was noted on a weekly basis and the mean weight recorded at Day 1, 7, 10, 14, 21, 28, and 35 in all five groups have been presented in Fig. 1a and 1b. All the groups showed normal weight gain at the start, but later, the diabetic groups indicated slight reductions in their weight from day 21 onwards, and these reductions are most prominent in higher BGS-fed groups as shown in Fig. 1b. Overall the changes in body weight remain non-significant for all groups.

Changes in diabetic biomarkers on BGS consumption: The results of all blood glucose biomarkers including fasting blood glucose (FBG), s-glucose or random blood glucose (RBG), s-insulin, s-fructosamine, p-amylase, p-GSH, and p-MDA have been listed in Table 2. The FBG level was monitored weekly in rats and has been presented in Fig. 2 and Table 2. As diabetes was induced at day 7, the peak FBG levels in G1 to G4 at day 10 confirm the

Table 1: Phytochemical analysis of BGS powder by HPLC-MS.

Phytochemicals detected	Trans-cinnamic acid	Caffeic acid	Gallic acid	4-Hydroxybenzoic acid	*p*-Coumaric acid	Vanillic acid	Crisin	Total
Composition (mg/g)	55.74±1.32	3.85±0.56	2.78±0.09	33.98±1.76	10.34±0.64	1.49±0.12	15.23±0.31	123.41±0.49

*All the values are taken as triplicates and expressed as mean ± standard deviation after subjecting to statistical analysis, p≤ 0.05.

Table 2: Effect of BGS-C on diabetic biomarkers of diabetic rats

Biomarkers	G0	G1	G2	G3	G4	G1XG2	G1XG3	G1XG4	F-Value
S-Glucose	123.0±2.65a	139.3±2.52b	134.3±2.081cb	127.0±2.00a	122.6±1.53a	3.58%v	8.83%v	11.98%v	33.590***
S-Insulin	16.0±1.00a	24.6±1.53b	22.6±1.53cb	20.0±1.00dc	16.3±1.53ad	8.13%v	18.68%v	33.73%v	24.296***
S-Fructosamine	252.7±2.52a	283.3±2.52b	277.7±2.52cb	267.7±2.52d	255.3±2.52a	1.97%v	5.50%v	9.88%v	85.50***
P-Amylase	79.0±1.52a	58.7±1.52b	66.7±2.08c	71.0±2.00dc	76.7±2.08a	13.62%^	20.95%^	30.66%^	62.15***
P-GSH	8.20±0.264a	6.33±0.208b	6.80±0.20cb	7.10±0.10dc	7.63±0.305ad	7.42%^	12.16%^	20.53%^	30.79***
P-MDA	0.662±0.003a	0.982±0.003b	0.837±0.001c	0.775±0.003d	0.667±0.002a	14.76%v	21.08%v	32.07%v	701.80***
FBG Day-1	81.67±1.15a	78.00±5.29a	73.33±3.21a	74.33±2.08a	72.67±8.32a	5.98%v	4.71%v	6.8%v	1.89NS
FBG Day-21	90.67±1.53a	120.67±5.13a	115.67±3.51cb	98.33±2.00a	91.67±7.67a	4.14%v	18.51%v	24.03%v	28.83***
FBG Day-35	92.66±3.05a	103.00±2.64b	98.33±3.21ab	90.33±2.51ac	84.00±1.00c	4.53%v	12.30%v	18.44%v	23.65***
FBG Change	11.00±3.46a	25.00±6.24a	25.00±6.00a	16.00±4.58a	11.33±7.37a	0%	36%v	54.8%v	4.502*

*Means/%±SD; Means sharing the same letters in a column are not significantly different from each other at *** = Very highly significant at p < 0.001; ** = highly significant at p < 0.01; *= significant at p < 0.05; ns = non-significant at p < 0.05; G1XG2 (Percentage effect among G1 and G2); G1XG3 (Percentage effect among G1 and G3); G1XG4 (Percentage effect among G1 and G4); Percentage effect is calculated as: %^ **(**Percentage increase) and %v (Percentage decrease) = [(D42-D1) ÷ D1] × 100.

Table 3: Effect of BGS-C on lipid profile, LFTs, and RFTSs of diabetic rats

Biomarkers	G0	G1	G2	G3	G4	G1XG2	G1XG3	G1XG4	F-Value
TG	75.0±4.00ad	106.3±4.16b	84.33±4.51a	75.0±2.00ad	69.0±2.00d	20.67%v	29.4%v	35.08%v	52.273***
TC	159.6±4.51a	173.0±3.61b	160.0±4.00a	149.0±3.00c	137.0±2.65d	7.51%v	13.87%v	20.81%v	41.791***
HDL	57.0±2.00a	46.67±2.08b	52.0±1.00c	56.00±1.00ac	61.00±2.00a	11.42%^	19.99%^	30.7%^	30.965***
LDL	87.67±3.21a	105.0±2.64b	91.0±2.64a	77.67±3.21c	61.67±1.53d	13.33%v	26.02%v	41.27%v	105.459***
ALT	21.33±2.08a	30.00±1.00b	27.33±1.53cb	24.33±1.58ac	20.67±1.53a	8.9%v	18.9%v	31.1%v	22.77***
AST	22.33±1.53a	28.00±1.00b	25.67±0.58cb	24.33±0.58ac	18.67±1.53d	8.32%v	13.11%v	33.32%v	29.55***
ALP	279.0±1.00a	298.0±3.00b	291.3±1.53c	285.0±1.00d	276.6±1.53c	2.24%v	4.36%v	7.18%v	74.09***
Urea	21.7±2.52a	34.0±2.00b	28.7±1.53c	24.0±1.00ac	18.6±1.53a	15.58%v	29.41%v	45.29%v	34.18***
Creatinine	0.767±0.057a	1.10±0.100b	1.00±0.100cb	0.867±0.057ac	0.73±0.057a	9.09%v	21.18%v	33.63%v	12.05***
T-Bilirubin	0.500±0.100a	0.900±0.100b	0.733±0.057cb	0.633±0.057ca	0.433±0.057a	18.55%v	29.67%v	51.89%v	17.33***

*Means/%±SD; Means sharing the same letters in a column are not significantly different from each other at *** = Very highly significant at p < 0.001; ** = highly significant at p < 0.01; *= significant at p < 0.05; ns = non-significant at p < 0.05; G1XG2 (Percentage effect among G1 and G2); G1XG3 (Percentage effect among G1 and G3); G1XG4 (Percentage effect among G1 and G4); Percentage effect is calculated as: %^ **(**Percentage increase) and %v (Percentage decrease) = [(D42-D1) ÷ D1] × 100.

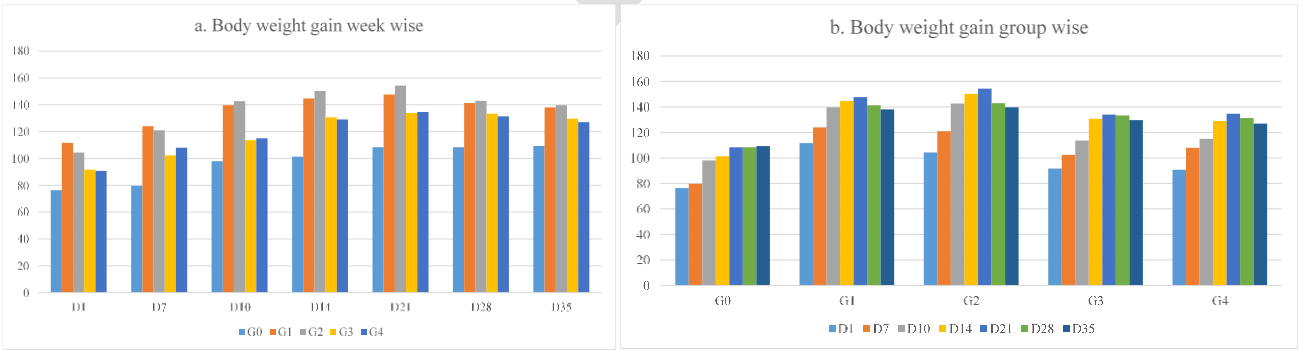

Fig. 1 (a): Effect of BGS-C on body weight gain presented week wise in groups, (b) Effect of BGS-C on body weight gain presented group wise in weeks.

induction of DM in these groups. Later, significant reductions in FBG levels can be noted in all groups, whereas the reductions in FBG levels were maximum in G4 (Metformin+75% BGS-C). The FBG levels marked highly significant changes from day 21 onwards as presented in Table 2.

Changes in lipid profile on BGS consumption: The results of lipid profiles including TG, TC, HDL, and LDL have been displayed in Table 3 and Fig. 3. In diabetic rats, the levels of TG were maximum for the G1 (Metformin) group, which made highly significant decreases of 20.67%, 29.4%, and 35.08% in G2, G3, and G4 along with the increase of BGS in the diet. The final TG levels of G4 were 8% lower than those of non-diabetic normal control rats of G0. Similarly, the levels of TC were at their peak for the G1 (Metformin) group, which reduced significantly in G2, G3, and G4 along with the increase of BGS in the diet. Here again, the TC of the G4 group was 14% lower than G0 rats. The results of HDL level showed a highly significant increase in BGS-fed rats of G2, G3, and G4 when compared to G1, and the final levels of HDL in G4 were notably higher than in G0 rats.

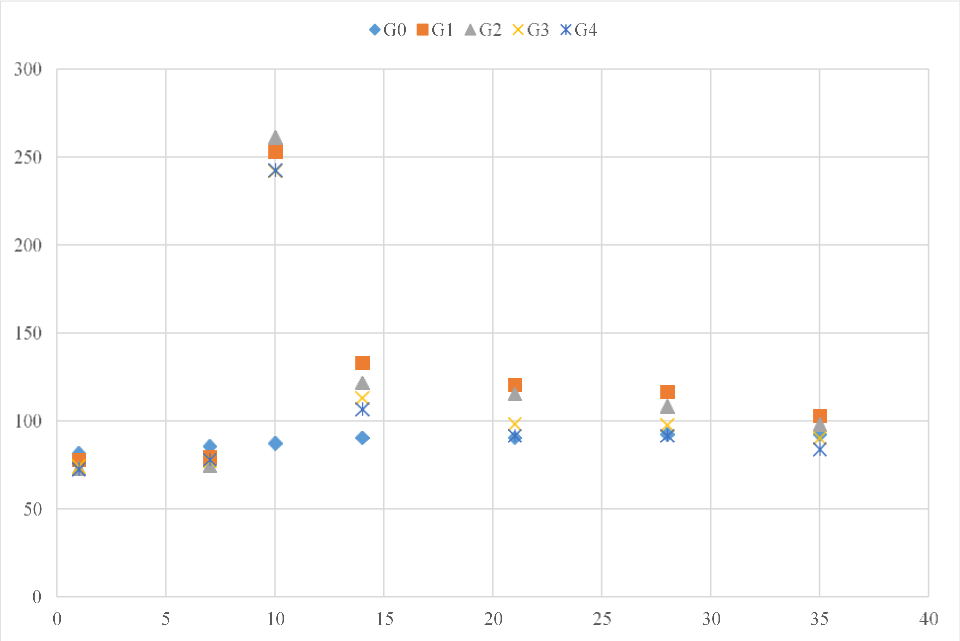

Fig. 2: Effect of BGS-C on weekly fasting blood glucose (FBG) in diabetic rats.

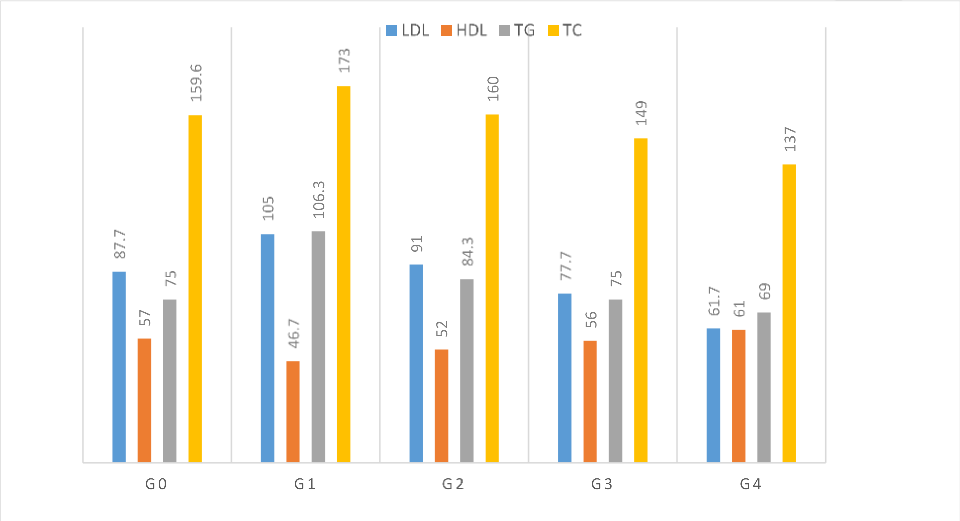

Fig. 3: Effect of BGS-C on lipid profile in diabetic rats.

Changes in LFTs and RFTs on BGS consumption: The results of ALT, AST, and ALP have been documented in Table 3. The induction of diabetes resulted in a prominent and significant elevation of these LFTs in G1 when we compare these from G0. Whereas in the next three groups after the addition of BGS into the diet, these biomarkers have shown a decline towards the normal values of G0. Just like the results of diabetic biomarkers and the lipid profile, again the outcome of G3 and G4 are more promising here to bring down the elevated LFTs to normal.

DISCUSSION

It has been discovered that phenolic compounds found in BGS, such as caffeic acid, gallic acid, vanillic acid, Trans-cinnamic acid, and 4-Hydroxybenzoic acid can regulate diabetes mellitus in several ways. Their antioxidant qualities can aid in lowering oxidative stress, a significant factor in the onset and advancement of DM. They have the ability to inhibit important enzymes that are involved in the metabolism of carbohydrates, like α-amylase and α-glucosidase, which can slow down the absorption of glucose and aid in blood sugar regulation. Better glucose homeostasis can result from their ability to increase insulin sensitivity and secretion from pancreatic β-cells. Insulin resistance can be lessened by them by modulating signaling pathways like the PPAR and AMPK pathways, which are involved in the metabolism of fat and glucose. They have anti-inflammatory properties that may help avert complications from diabetes, such as nephropathy and neuropathy (Gayathry and John, 2022).

While assessing the changes in body weights on BGS consumption, overall G0 the negative control marked lower body weight gain, whereas G1 (Metformin) and G2 (Metformin+25%BGS-C) made almost similar and most weight gains. A study reported similar body weight changes in drug and BG-treated groups (Tripathi and Chandra, 2009). Overall body weight gain remains non-significant at weekly intervals, and there were no significant differences among the groups. The BGS groups (G2, G3, and G4) have indicated notable reductions in body weights when compared to G1. Similarly, body weight reduction on BG consumption has been previously reported by Hussain *et al.* (2022). Another study reported significant body weight reduction

on BG extract administration in diabetic rats (Eweda *et al.*, 2021). Therefore, BG consumption can be used clinically to reduce body weight, especially in diabetic patients if they are overweight or obese and face difficulty in reducing it.

The FBG levels made the peak decline of 54.8% in the G4 (Metformin+75% BGS-C) group when compared to the G1 (Metformin) group. The FBG level has decreased by more than 50 times, which emphasizes the value of nutraceutical meals in the treatment of DM. A previous study reported a similar time-dependent decline in FBG levels of diabetic rats on BG administration (Tripathi and Chandra, 2009). Likewise, similar and significant reductions have been reported in FBG levels of diabetic rats on the feeding of BG juice at day 7, 14, 21, and 28, where the outcomes of BG were found more promising as compared to the insulin treatment (Shachi *et al.*, 2020). The results of our study are also more promising for the BG along with diabetic drugs as compared to the drug treatment alone. Likewise, the RBG recorded from the serum glucose level of rats after decapitation also justifies the hypoglycemic potential of BGS. The results showed highly significant reductions in the serum glucose levels. The RBG of G4 (Metformin+75%BGS-C) was 122.67±1.53mg/dl which was significantly lowered from the rest of the diabetic groups (G1, G2, G3) and is quite comparable to the normal RBG level of non-diabetic group G0 123.0±2.65mg/dl. This blood glucose lowering property of BG was previously reported due to its bioactive components cucurbitane-type triterpenoids, which may aid in inhibiting gluconeogenesis approximately by 80% and reduce glucose production in hepatic cells (Çiçek, 2022).

The s-insulin and s-fructosamine levels of the diabetic groups made highly significant declines along with the increase in the given amount of BGS-C. The s-insulin and s-fructosamine were maximum 24.67±1.53µIU/mL and 283.3±2.52µmol/L in the G1 (Metformin) group, while these made a maximum decline of 33.73% (16.3±1.53µIU/mL) and 9.88% (255.3±2.52µmol/L) in G4 (Metformin+75% BGS-C) respectively. These levels of s-insulin and s-fructosamine of G4 were quite close to non-diabetic control group rats as shown in Table 2. The outcomes are in accordance to a previous study where similar reductions in s-fructosamine have been documented by the administration of BG, whereas, in their results, the s-insulin level was increased (Mahmoud *et al.*, 2017). This difference might be due to the concentrations of bioactive components in BG juice in comparison to BGS-C. However, another study has presented similar ameliorations of increased s-insulin in the drug-treated group as compared to BG-treated groups (Eweda *et al.*, 2021). Results of p-amylase showed that non-diabetic rats of the normal control group (G0:77±1.52U/L) have a higher level as compared to the diabetic groups. The level of p-amylase was lowest for G1 which made a highly significant increase in the BGS-fed group, and the G4 (Metformin+75% BGS-C) indicated p-amylase quite closer to G0. Low amylase content has been associated with pancreatic insufficiency to produce amylase for proper carbohydrate metabolism, and hence results in abnormal blood glucose levels (Nakajima, 2016).

The results of p-GSH indicated a highly significant decline in its value after diabetes induction in rats, which made a significant increase along with the BGS inclusion in diet as elaborated in Table 2. Previously similar increase in GSH content by the oral administration of BG (Tripathi and Chandra, 2009). This gradual and significant increase in p-GSH can justify the improvements in β-cell function. In comparison to p-GSH, the p-MDA levels increased on diabetes induction and were again significantly reduced to normal levels by the inclusion of BGS in the diet of diabetic rats. The GSH levels usually are depleted in diabetic rats due to the heperglycaemia induces oxidative stress, which ultimately leads to an increased lipid peroxidation product p-MDA in body. The anti-oxidative properties of BGS have helped restore the GSH level, which gradually brought the MDA levels back to normal. This property of BG have been supported by (Tripathi and Chandra, 2009).

The results of LDL level indicated a highly significant reduction in LDL levels, which are quite like those of TG and TC. The reduction in LDL of G4 was 29% lower than G0. A similar hypolipidaemic potential of BG was previously reported in diabetic rats (Shachi *et al.*, 2020). Lipid biomarkers improved as a result of BG's previously demonstrated anti-hyperglycemic and anti-oxidative qualities, which were associated with decreased lipid peroxidation in the body (Tripathi and Chandra, 2009). Moreover, a dose-dependent significant amelioration in lipid profile has been justified in a previous study (Yakubu Magaji Yuguda, 2023). Moreover, similar improvements in all biomarkers in the Lipid profile were reported by BG administration in diabetic rats (Eweda *et al.*, 2021). Overall findings of LFTs have revealed highly significant ameliorations in this study. The significant anti-oxidative and anti-glycemic potential of BG may also have worked here for restoring the liver enzymes back to their normal. Similar improvements in liver function and histopathological presentation of liver tissues on administration of BG strengthened its hepatoprotective potential (Shahzadi *et al*., 2024).

The results of RFTs including urea, creatinine, and total bilirubin as documented in Table 2, also reveal highly significant variations among the groups. Just like the LFTs, the RFTs were also raised after the induction of diabetes in G1 when we compared them to G0, but after the inclusion of BGS into the diet of diabetic rats, their values of RFTs made significant improvements. Here again, the valuable addition of BGS into the diet is a true indicator of its nutraceutical significance. Similar improved blood urea and creatinine levels have been reported by the administration of BG to diabetic rats, and this renal protective activity was addressed due to the bioactive polypeptides in BG (Mardani *et al.*, 2014). The kidney's glycoconjugates are crucial to the preservation of the glomerular filtration barrier. Diabetic nephropathy is well known for its glomerular basement membrane thickening. BG supplementation effectively reduced the rise in glucose-associated enzyme activity related to the production and breakdown of glycosaminoglycans associated with diabetes (Kumar *et al.*, 2008). The outcomes of RFTs here in this study are promising for renal biomarkers. Similar improvements a in a previous

study were reported due to the antioxidative property of BG extract that helped to avoid the oxidative damage that diabetic kidney disease (Teoh *et al*., 2010).

Conclusion: DM is a metabolic condition marked by a decreased body's capacity to produce or react to endocrine hormones, which is necessary to keep blood glucose levels within normal ranges. Modern nutrition is targeted towards the dietary management of T2DM that is mostly acquired by inappropriate dietary habits. Despite the abundance of pharmaceutical medicines with anti-diabetic properties, there is a growing interest in plant-based therapies due to their low cost and few side effects. BG is one such medicinal plant therefore, its seeds BGS were incorporated at 10% into crackers to make BGS-C, to investigate their therapeutic impact on DM. The results of this study revealed that the inclusion of BGS-C into the diet of diabetic rats brought much faster and more significant recovery from hyperglycemia-related imbalances. The results indicated that the administration of 50 and 75% BGS-C along with the diabetic drug Metformin in G3 and G4, brought dramatic outcomes towards normalization of the body weight, FBG, RBG, s-insulin, s-fructosamine, p-amylase, p-GSH, and p-MDA, TG, TC, HDL, and LDL, LFTs, and RFTs. These results have indicated the nutraceutical potential of BGS against DM. Moreover, the treatment of DM only relying on the medicine may prolong the disease but the use of BGS along with the use of medicine may help in accelerating the recovery process. Hence, the regular dietary intake of BG or BGS is highly recommended to lower the negative health outcomes of DM and its associated metabolic disorders.

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