***Pak. J. Bot.*, 58(10): October 2026.**

ARTICLE **DOI: http://dx.doi.org/10.30848/PJB2026-10(17)**

DEVELOPMENT OF MULTIGRAIN FORMULAS AND *IN VIVO* EVALUATION OF ANTI-DIABETIC POTENTIAL

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Abstract

Diabetes mellitus is a major global health concern, particularly in developing countries. In this study, six multigrain formulations (F_1-F_6) comprising barley (*Hordeum vulgare* L.), buckwheat (*Fagopyrum esculentum* Moench*)*, faba bean (*Vicia faba* L.) and mulberry (*Morus alba* L.) were developed and evaluated for antidiabetic potential using diabetic induced albino rats. Prior to biological evaluation, proximate composition analysis was conducted for all formulations, revealing significant variations among nutritional parameters. Ash content ranged from 2.44 to 2.84%, with the highest value in F_4. Moisture content varied between 6.72% and 9.16%. Protein levels increased across formulations, reaching a maximum of 11.15% in F_6. Fiber content ranged from 9.66 to 11.15%, while fat remained low in all samples (1.16–2.24%). Mineral analysis revealed the highest iron content in F_6 (120.67 mg/100 g) and maximum zinc in F_1 (28.38 mg/100 g). Carbohydrates ranged from 66.83 to 72.72%. *In vivo* evaluation of six-multigrain formulations against diabetic induced albino rats demonstrated promising antidiabetic effects across all treatments. Initial diabetic levels (IDL) were comparable among groups, indicating uniform induction of diabetes. A progressive reduction in blood glucose levels was observed over 14 days for all formulations. Among them, F_4 exhibited the most pronounced hypoglycemic effect, showing the lowest glucose level at day one to day 14 (402 to 107 mg/dL), followed by F_2 (400 to 112.67 mg/dL) and F_6 (415 to 124.67 mg/dL). Based on the low glycemic levels, F_4 emerged as the most effective formulation, suggesting its superior antidiabetic potential among the tested multigrain diets. Based on these findings, it is strongly suggested that diabetic-affected communities adopt the regular consumption of multigrain-based foods, as they offer a natural, effective and sustainable dietary approach to improve glycemic control and combat diabetes.

Key words: Multigrain flour; Proximate composition; Antidiabetic activity; Albino rats; Composite flour

Introduction

Diabetes Mellitus also known as diabetes is a metabolic disorder characterized by high sugar level in blood (hyperglycemia) over a prolonged time period (Mechchate *et al*., 2021). The glucose level of a diabetic patient is rising exponentially beyond the usual range after meals. It is a fact that the blood glucose level decreases quickly when the body struggle to retain the extra blood glucose for future use. Diabetes is classified as type 1 and 2. Type 1 insulin dependent diabetes in which patients are unable to produce sufficient insulin (Zou *et al*., 2018). However, type 2 diabetes (T2D) usually occurs in adults when the body fails to deliver enough insulin or having insulin resistance (Nolan *et al*., 2019). About 90% of the people with diabetes are patients of T2D around the globe which usually is result of being overweight or physically inactive (Shen *et al*., 2018).

The development of T2D and obesity is promoted by the consumptions of energy dense foods containing high fat and sugar content (Martinez-Saez *et al*., 2017). In contrary, the foods containing high fiber content and low sugar level are beneficial in improving satiety, body weight gain and controlling blood glucose level (Van *et al*., 2012). Due to the given evidences, food industry has intensified efforts to produce high fiber, low-fat foods such as biscuit and porridges to address the public health concern (Cukelj *et al*., 2017).

Owing to modernization and globalization, there is an increased preference for fast foods as compared to traditional ones. Fast foods bring ease for consumers being as light meals and eaten anywhere and, anytime during office works and other assignments. Frequent consumption of fast foods disturbed the balance approach of diets, proven to various diseases resulting in health complications. The growing health concerns thereof have brought a shift in consumer demand for healthier and nutritious foods. Consumption of balance diet containing all required constituents can help in preventing diseases and promoting a healthy lifestyle. To maintain a balanced diet consumption, multigrain products offer better choice for consumers. Including a variety of grains in to flours and making different products ensures considerable increase in nutritional profile and thus provide enormous health benefits (Jagdale *et al.*, 2020).

Cereals and legumes are attributed to lower the chance of developing diabetes, cancer and cardiovascular disease. These foods are extensively grown and represent a staple diet in most of the developing nations (Mohanraj, 2020). Buckwheat, barley and faba bean contains several important primary substances, including phytosterols, fagopyrins, fagopyritols flavonoids and polyphenols (i.e. rutin and catechin). The primary function of fagocyritols in the human body is to reduce cholesterol, which helps in lowering the risk of coronary heart disease and cancer (Baniwal *et al*., 2021).

Received: 12-11-2025 Revised: 22-03-2026 Accepted: 28-03-2026 Online: 30-05-2026

Barley (*Hordeum vulgare*) is considered as a functional food having rich content of soluble fiber and β-glucan. These constituents are attributed to maintain a low-glycemic index (GI). Several clinical trials have shown β-glucan-containing oat and barley meals to reduce blood glucose levels in the upper and lower gastrointestinal tract (GIT) via various distinct processes (Annes *et al*., 2015). Buckwheat (F*agopyrum esculentum* Monech) is a pseudocereals grain belongs to non-grass family. It helps to reduce the risk of DM and associated complications. It reduces fasting sugar levels, increase insulin production, lowers glycosylated serum protein, glycosylated haemoglobin, and reduce blood sugar levels (Lee *et al*., 2017). Faba bean peptides have demonstrated anti-inflammatory, antihypertensive, antioxidant and cholesterol-lowering properties (Martineau-Cote *et al.*, 2022). According to recent studies, faba beans and their derivatives may be an excellent source to manage hypertension, diabetes and prevent cardiovascular disease (Turco *et al*., 2016; Farag *et al*., 2022). Cereal and legume-based multigrain products can meet consumers' requirements and exhibit antidiabetic activity. Thus in this study, multigrain products from barley, buckwheat, faba bean and mulberry were developed considering their functional and nutraceutical properties and assessed for their impact on diabetics.

Materials and Methods

Sample collection: Buckwheat, barley, faba bean and mulberry samples were collected from different locations of Gilgit-Baltistan i.e., Astore, Haramosh and Shiger Skardu regions. These samples were transported to the Food Science Laboratory Karakoram International University Gilgit, where the samples were graded and cleaned. The grains were divided into two lots. One was kept as raw while the other portion was soaked in water and kept in a store till its sprouting. The germinated part was first dried and then both portions were roasted. The grains were then milled into flour using a multipurpose integrator.

Development of the formula: The multigrain flour, including buckwheat, faba bean, barley, and mulberry was mixed in different concentrations. There were two ways of processing i.e., germinated and non-germinated grains flours. The grains were roasted and mixed in different ratios and milled to get a combined flour (Table 1).

The study of antidiabetic properties was conducted at the National Institute of Health (NIH) Islamabad, Pakistan.

Ethical approval: Ethical approval was obtained from the ethical committee of the National Institute of Health (NIH) Islamabad to conduct biological studies on diabetes induced rates (No.F.1-5/ERC/202 & Riphah/IRC/23/3118).

Biological studies: Adult female wistar albino rats of weight 230-350g were selected for the study. The animals were divided into six groups containing 3 animals each. They were housed in polypropylene cages lined with husk and maintained in a semi-natural light/dark cycle (12 hours of light, 12 hours of darkness). The animals were kept in conventional laboratory hygienic settings. Temperature was maintained at 25°C and a relative humidity of 55%. The animals were having access to water and food throughout the trial period.

Diabetes generation and collection of samples: The animals were fasted overnight and their body weight and blood glucose levels were recorded prior to the induction of diabetes. The animals were grouped into six categories induced by intraperitoneal injection of alloxan monohydrate dissolved in normal saline and served a single dose of 120 to 180mg/kg. 10% glucose solution was provided for 24 hours to prevent initial hypoglycaemia. The blood glucose levels were determined after three days using a glucometer strip to confirm the induction of diabetes. Rats with blood glucose levels of more than 200 mg/dl were considered to have diabetes. Fasting blood sugar (FBS) was measured and the rats having FBS> 200 mg/dl were considered as diabetic. To reduce the risk of mortality; the rats with extreme hyperglycaemia (>400) were excluded (Tigstu *et al*., 2025).

Feeding of multigrain food formula: The multigrain food formula doses were fed to albino rats with overnight fasting in the form of pills (Table 1). After 2 hours of feeding the formula to diabetic-induced albino rats the blood glucose levels were recorded by using a glucometer.

Statistical analysis: All measurements were carried out in triplicates, and it was analyzed with the help of statistics 8.1 (Tallahassee FL 32,317, USA). One- way analysis of variance (ANOVA) was applied and the means were compared using Least Significant Difference (LSD) at $p \leq 0.05$. Graph were prepared by using MS Excel software 2007 (Sarian *et al.*, 2017).

Results

Proximate and mineral composition: The proximate and mineral composition of 6-multigrain formulas (F_1-F_6) is given in Table 2. A substantial variation in proximate and mineral compositions was recorded. The ash content representing total minerals was found in the range of 2.84 to 2.44%. The highest value was recorded in F_4 (2.84%) F_5 had the lowest values (2.44%). Moisture content ranged from 9.16 to 6.72% representing maximum amounts in F_3 (9.16) and minimum in F_5 (6.72). Protein percentage was recorded in the range of 11.15 to 9.66%, where F_6 showing significantly higher contents (11.15%) and F_1 having the lowest (9.66%). Fat contents ranged from 2.24 to 1.16% respectively. Highest amounts were noted in F_6 (2.24) and the lowest in F_3 (1.16). Dietary fibre varied significantly ranging from 7.64 to 3.87%. F_5 displayed the peak value (7.64%) and F_1 the lowest (3.87%). Carbohydrate content ranged from 72.7 to 66.83% showing maximum in F_4 while minimum in F_1. Iron concentration ranged from 120.67 mg/100g to 49.35mg/100g DW with higher amounts in F_6 and lower in F_3. Zinc levels were highest in F_1 (28.38 and lowest in F_4 (16.06mg/100g DW)

Antidiabetic activity of multigrain food formula: Antidiabetic activity after feeding food formula to diabetis induced rats revealed a significant effect on blood glucose levels (Table 3). The initial blood glucose levels (IDL) were uniformly higher across all groups, ranging from 400.00 to 415.00 mg/dL. These levels dropped to 376.00 to 297.33 in the first 7 days and 170.33 to 124.67mg/dl on the fourteenth day showing a significant reduction ($p \leq 0.05$). The decreasing trends over the study period were statistically significant at $p \leq 0.05$. The most significant glucose-lowering effects were observed by the end of the 14-day period. All formulations produced a noticeable decline from initial values. The lowest final glucose level was recorded in F_4 (107.00 mg/dL) followed by F_2 (112.67 mg/dL), respectively. The results of remaining formulas (F_3, F_5 and F_6) did not show much variation. In conclusion, all tested multigrain formulas confirmed efficacy in lowering blood glucose level over 2 weeks, while F_2 and F_4 demonstrated the most substantial and significant reductions in BGL (Table 3 and Fig. 1). Figure 2 depicted the days and formula wise comparison. Results showed that there was positive correlation between blood glucose level and number of days among all formulas. Highest blood level reduction was recorded on fourteenth day in F_4 treatment, F_3, F_5 and F_6 were found with an intermediate efficacy, whereas F_2 was found having least glucose reduction among the tested formulations.

Table 1. Formulas with different concentration of ingredients g/100g.

S. No.	Formulas	Buckwheat	Barley	Faba Bean	Mulberry
1.	F_1 Germinated	25.00	30.00	25.00	20.00
2.	F_2 Un-germinated	25.00	30.00	25.00	20.00
3.	F_3 Germinated	26.60	26.60	26.60	20.20
4.	F_4 Un-Germinated	26.60	26.60	26.60	20.20
5.	F_5 Germinated	40.00	30.00	30.00	00.00
6.	F_6 Un-germinated	40.00	30.00	30.00	00.00

Table 2. Proximate and mineral Composition of multigrain feed formulas*.

Formulas	Ash (%)	MC (%)	Protein (%)	Fat (%)	Fiber (%)	Carbs (%)	Iron (mg/100gDW)	Zinc (mg/100g DW)
F_1	2.75±0.02^{b}	8.51±0.17^{a}	9.66±0.21^{d}	1.41±0.07^{cd}	3.87±0.07^{e}	66.83±0.37^{d}	87.94±0.05^{d}	28.38±0.07^{a}
F_2	2.58±0.06^{c}	8.66±0.04^{c}	10.51±0.01^{c}	1.8533±0.02^{b}	6.15±0.12^{c}	72.51±0.17^{a}	106.40±0.20^{b}	23.50±0.20^{d}
F_3	2.73±0.02^{b}	6.72±0.04^{f}	10.64±0.03^{bc}	1.16±0.020^{d}	4.64±0.03^{d}	72.63±0.08^{a}	49.35±0.04^{f}	24.45±0.19^{c}
F_4	2.84±0.02^{a}	7.84±0.02^{e}	10.66±0.02^{bc}	1.35±0.01^{cd}	6.24±0.02^{c}	72.72±0.10^{a}	85.57±0.32^{e}	16.06±0.66^{e}
F_5	2.44±0.03^{d}	9.16±0.03^{b}	10.76±0.01^{b}	1.64±0.53^{bc}	7.64±0.03^{a}	68.33±0.53^{c}	103.08±0.03^{c}	23.50±0.20^{d}
F_6	2.74±0.04^{b}	8.15±0.02^{a}	11.15±0.02^{a}	2.24±0.03^{a}	6.82±0.04^{b}	69.46±0.04^{b}	120.67±0.21^{a}	25.43±0.02^{b}
Standard Error	0.031	0.058	0.086	0.176	0.051	0.215	0.138	0.243
LSD	0.070	0.130	0.193	0.392	0.115	0.480	0.307	0.542

Multigrain food formulas denoted as F_1 to F_6 representing different combinations of Ingredient; The values were average of three replications ± SD (standard deviation); Means with different letters were statistically significant at $p \leq 0.05$; Note: Carbs (carbohydrates); MC (Moisture content); DW (dry weight)

Table 3. Blood Glucose levels after fetching multigrain food formula.

Formulas	Weight (g)	IDL	7 Days	14 Days
F_1	300 ± 52.20^{ab}	404.00 ± 6.92^{a}	350.00 ± 3.78^{a}	170.33 ± 25.23^{a}
F_2	346.67 ± 30.55^{a}	400.00 ± 0.00^{a}	364.67 ± 112.01^{ab}	112.67 ± 2.52^{b}
F_3	255.00 ± 13.22^{b}	405 ± 0.00^{a}	356.00 ± 84.67^{ab}	154.67 ± 41.23^{ab}
F_4	248.33 ± 36.85^{b}	402 ± 0.00^{a}	376.00 ± 22.67^{b}	107.00 ± 29.87^{ab}
F_5	286.67 ± 47.25^{ab}	400 ± 0.00^{a}	343.670 ± 19.97^{ab}	133.00 ± 9.84^{ab}
F_6	301.67 ± 12.58^{ab}	415 ± 0.00^{a}	297.33 ± 19.97^{a}	124.67 ± 10.693^{ab}
CVC	29.02	2.30	34.524	19.47
LSD	63.24	5.03	15.495	42.42
CV	15.54	0.564	2.228	18.96

The values are average of three replications ± SD (standard deviation); Means with different letters were statistically significant at $p \leq 0.05$
Note; CVC; Critical Value Constant, LSD (least significant difference test; CV (Coefficient of variance)

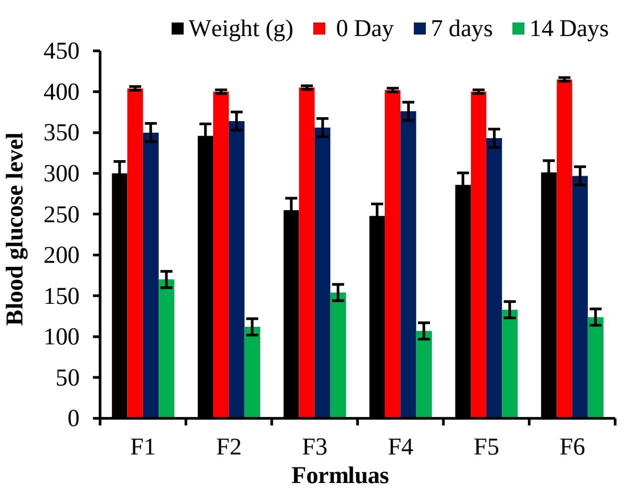

Fig. 1. Effect of developed formulas on blood glucose level in albino rats.

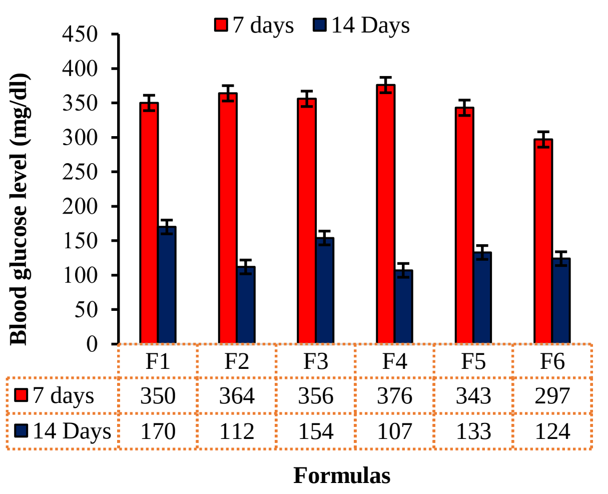

Fig. 2. Days and formula wise blood glucose level reduction.

Discussion

The current investigation clearly exhibits the anti-diabetic potential of multigrain formulations comprising barley, buckwheat, faba bean and mulberry. The cereals and legumes are important part in human diet. The in-vivo blood glucose level of the food formulas after feeding to albino rats exhibit their efficacy against DM. These formulations contain a potential profile of iron, zinc and protein (Table 2). Studies have shown that iron, zinc and protein contents are capable of lowering the concentration of blood sugar level (Famakin *et al*., 2016:Ijarotimi *et al*., 2015 (Lopez-Ridaura *et al*., 2004) : (Barbagallo & Dominguez, 2007) The finding of the current study suggests that remediation of DM by local sources presents a unique opportunity for their utilization and improving, nutrition, health and socio-economic status. In general, the deficiency or lack of micronutrients in the body may have a significant impact on diabetes and insulin resistance and some micronutrients have been known to be related to the pathogenesis of diabetes (Xia *et al*., 2023). Cereal β-glucans display all the physiological properties that have been attributed to dietary fiber like water holding capacity, swelling, diffusion suppressing ability (viscous, gel formation) and binding properties. Therefore, grains with high levels of soluble β-glucans, such as oat and barley are generally more effective in controlling blood glucose levels, improving insulin responses and reducing serum cholesterol levels than wheat, which contains predominantly insoluble dietary fiber. Diets containing foods enriched with oat and barley β-glucans have reduced glycemic index in clinical trials (Biliaderis and Marta 2007). Studies also suggest that the inhibition of glucose diffusion in the small intestine is due to the adsorption or inclusion of the smaller sugar molecules within the structure of the fiber particles (Nishimune *et al.* , 1991; Lopez *et al*., 1996).

Protein, an essential nutrient for the functions of human body, also features prominently in the formulas, with contents ranging from 9.66 to 11.15%. This aligns with the World Health Organization's protein intake recommendations, which suggest that adults weighing 50 and 70 kg should consume approximately 42 to 58 g of protein daily to meet their nutritional needs (Anon., 2007). Given the burgeoning global diabetes epidemic, these findings emphasize the importance of integrating natural dietary interventions into diabetes management and prevention strategies. The antidiabetic, antioxidative, and anti-inflammatory properties of these formulas suggest a holistic approach to tackling not only the symptoms of diabetes but also its underlying causes, such as insulin resistance and metabolic syndrome. In summary, these formulas showcase a potential therapeutic approach to managing diabetes through natural dietary interventions and contribute to addressing prevalent micronutrient deficiencies, demonstrating a multifaceted approach to improving public health in regions like Gilgit-Baltistan.

Role of sprouting in nutrient bioavailability: The utilization of germinated grains in the food formula (F_1, F_2, F_3) is an approach strategically significant to increase the nutritional quality of composite flour. It triggers the enzymatic activity results in the reduction of anti-nutritional factors like phytic acid, which otherwise chelates essential minerals such as iron and zinc (Lian *et al*., 2024). According to emerging evidence sprouted grains have favorable regulatory effects, which helps in maintaining glucose homeostasis while reshaping the gut microbiota (Wang *et al*., 2026). The increase bioavailability of these microelements germinated multigrain diet may provide better support for β-cells of pancreatic function along with insulin signaling than traditional un-germinated cereal diets (Wang *et al*., 2026).

Synergistic effect of bioactive compounds: The incorporation of faba bean and buckwheat into the cereal based product create a significant effect to address the high glycemic nature of refined flour. There are various components presents in faba bean and buckwheat including flavonoids, polysaccharides and triterpenoids demonstrating significant effect on blood glucose regulations. (Lian *et al*., 2024; Zhang *et al*., 2025). This flour combination ensures that the formulation provide postprandial glucose control on immediate basis along with long-term metabolic benefits through the This combination ensures that the formulations provide both immediate postprandial glucose control and long-term metabolic benefits through the attenuation of oxidative stress (Song *et al*., 2026).

Socio-economic impact and sustainability: These formulations offer a sustainable food and nutritional security solution beyond its clinical efficacy. Utilizations of locally adopted crops like barley, buckwheat and faba bean reduces reliance on expensive imported supplements. The proposed multigrain formulation has an alignment on modern strategy of food as medicine, providing an affordable shelf-stable and culturally acceptable dietary intervention (Saleh *et al*., 2019). Such interventions have a vital significance for low income communities facing burden of metabolic disorder in high level, as they utilize natural products that offer a low incidence of adverse effects compared to synthetic drugs (Saleh *et al*., 2019).

Conclusions

The *In vivo* evaluation using albino rats provided convincing evidence of the hypoglycemic efficacy of these multigrain formulas. The reduction in blood glucose levels from 402±0.00 mg/dl to 107.00±29.87 mg/dl within fourteen days highlights their strong therapeutic potential. The tested formulations demonstrated a significant blood glucose lowering effect confirming that the grains have a good potential to be used as anti-glycemic agents. Thus, the functional properties of the multigrain synergistically contribute to diabetes management. Beyond glycemic control, the use of locally available grains and mulberry enhances the sustainability, affordability and cultural acceptability of these formulations. Therefore, the multigrain composite flours represent a practical, safe, and nutritionally rich dietary strategy for managing diabetes while simultaneously improving food and micronutrient security. Besides, this study also paves way for socio-economic development of the region by promoting local products of high consumer demand through small businesses and entrepreneurs.

Acknowledgments

The authors acknowledge the funding support provided by Higher Education Commission (HEC), of Pakistan under HEC Indigenous Scholarship II Phase VI to complete this study. We further acknowledge the technical and logistic support provided by NIH, Islamabad to complete the study.

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