Effect of Chickpea-Wheat-based Functional Cookies Enriched with Myo-inositol and D-Chiro-inositol on Glycemic Regulation in Streptozotocin-induced Diabetic Rats

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ABSTRACT

Background: Diabetes mellitus is a metabolic disorder characterized by persistent hyperglycemia, which is often accompanied by increased oxidative stress and inflammatory responses. The inclusion of functional foods enriched with bioactive compounds may offer additional therapeutic benefits in managing the condition. This study aimed to investigate the effect of chickpea- and wheat-based cookies, enriched with Myo-inositol (MI) and D-Chiro-inositol (DCI), on parameters such as glycaemic regulation, oxidative stress and inflammatory biomarkers in rats with streptozotocin-induced diabetes.

Methods: Diabetic Wistar rats (n=6/group) were divided into six groups: Control, disease control, wheat, wheat+MI+DCI and chickpea-wheat+MI+DCI. Blood sugar levels, including fasting blood glucose (FBG), glycated hemoglobin (HbA1c), insulin and results from the oral glucose tolerance test (OGTT), were evaluated. Oxidative stress markers [malondialdehyde (MDA), superoxide dismutase (SOD)], inflammatory cytokines (IL-6, TNF-α), Food consumption and body mass were also evaluated.

Result: Diabetic controls showed elevated FBG, HbA1c, oxidative stress and inflammatory cytokines ($p<0.05$). Wheat-based cookies produced negligible effects. Chickpea-wheat cookies moderately improved glycemic indices, while inositol-enriched wheat cookies enhanced insulin sensitivity. The combined formulation (chickpea-wheat+MI+DCI) yielded the greatest benefits, with near-normal FBG (105.1 mg/dL), reduced HbA1c (7.93%), improved OGTT, elevated SOD, reduced MDA and significantly lower IL-6 and TNF-α ($p<0.01$). This group also demonstrated improved weight recovery and reduced polyphagia. Cookies incorporating chickpea flour, wheat and inositols demonstrate synergistic effects in mitigating diabetes, oxidative stress and inflammation in STZ-induced rat models. These findings indicate their possible application as functional dietary components for diabetes management.

Key words: Chickpea, Diabetes mellitus, Functional foods, Glycemic control, Inositol, Oxidative stress.

INTRODUCTION

Diabetes mellitus is a persistent and progressively worsening metabolic disorder characterized by chronic hyperglycemia. This condition mainly arises due to insulin resistance, reduced insulin production, or a combination of these factors (American Diabetes Association, 2023). Considering its lifelong progression and the limitations of pharmacological approaches in fully addressing its complex etiology, there is increasing focus on sustainable dietary strategies that can serve as adjuncts to standard medical therapies (Nurkolis *et al.*, 2025; Devi *et al.*, 2023).

Functional foods-those providing health advantages beyond essential nutrition-have gained recognition as a strategic intervention for glycemic regulation and metabolic homeostasis (Ninomiya *et al.*, 2017; Rathore, Prakash, and Bala, 2021). Among these, *Cicer arietinum* L., commonly known as chickpea is notable for its minimal impact on blood sugar levels, high dietary fibre content and superior protein quality, which collectively render it appropriate for inclusion in diabetic dietary management (Antony *et al.*, 2017; Nam *et al.,* 2023). Additionally, both myo-inositol (MI) and D-Chiro-inositol (DCI), naturally synthesized isomers exhibiting insulin-sensitizing effects, have demonstrated beneficial impacts on glucose assimilation and pancreatic function in various clinical and experimental studies (Larner and Larner, 2003).

In our earlier investigation, a variety of snack cookies incorporating different ratios of chickpea and wheat flour were developed and subjected to sensory evaluation. These formulations were enriched with myo-inositol and D-chiroinositol (DCI). The primary objective was to achieve an optimal balance between consumer acceptability and potential health benefits. The design aimed to preserve

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the traditional flavor and texture characteristic of conventional cookies while integrating bioactive constituents that may enhance glycemic control (Chauhan *et al.*, 2025).

To advance beyond mere sensory and compositional validation, this study examines the biological efficacy of these functional cookies utilizing a streptozotocin (STZ)-induced diabetic rat model. Wistar rats were selected due to their extensive use in diabetes research and their physiological resemblance to human pancreatic beta-cell functionality. The investigation involves administering the cookies orally daily and monitoring variables such as fasting blood glucose levels, serum insulin concentrations, body weight and pancreatic histopathology over a 60-day duration. This in vivo approach enables an assessment of whether these functional cookies can be feasibly incorporated as a dietary intervention for managing diabetes.

MATERIALS AND METHODS

Cookie formulation

Four experimental cookie formulations were prepared and standardised in the Food Technology Laboratory:

- F1 (WF): Wheat flour cookie.
- F2 (WF+MI+DCI): Wheat flour cookie enriched with Myo-inositol and D-chiro-inositol.
- F3 (CF): Functional cookie prepared with chickpea flour (70%) + wheat flour (30%).
- F4 (CF+MI+DCI): Functional chickpea-wheat cookie enriched with Myo-inositol and D-chiro-inositol.

The inositol compounds were incorporated at standardised nutraceutical doses known to support insulin signalling and glucose metabolism (Arefhosseini *et al.*, 2023). All cookies were baked and stored in airtight containers under hygienic conditions until use.

Experimental animals and ethical approval

Male Wistar albino rats weighing between 200 and 250 grams and aged 6 to 8 weeks were sourced from the Institutional Animal Facility. The animals were kept in standard laboratory conditions, which included a temperature of 22±2°C, relative humidity ranging from 55% to 65% and a 12-hour light/dark cycle. They had unrestricted access to a standard pellet diet and water.

The research plan received approval from the Institutional Animal Ethics Committee (IAEC) under Protocol No. GDCUIAEC/2024/49 and all related procedures adhered to the CPCSEA guidelines.

Diabetes induction

After fasting overnight, diabetes was experimentally induced in rats through a single intraperitoneal injection of streptozotocin (STZ) at a dose of 50 mg/kg, dissolved fresh in 0.1 M cold citrate buffer at pH 4.5. Three days later, rats exhibiting fasting blood glucose levels of 200 mg/dL or higher were classified as diabetic and incorporated into the study (Ghazal *et al.*, 2024).

Experimental design and grouping

Animals were assigned to six separate groups through a random process (n = 6 per group) and studied for 60 days:
Group 1 (Control): Standard diet with no cookie consumption.
Group 2 (Disease Control): STZ 50 mg/kg body weight
Group 3 (Wheat): Wheat flour-based cookie.
Group 4 (Functional): Chickpea flour (70%) +Wheat flour (30%) cookie.
Group 5 (Wheat+MI+DCI): Wheat flour cookie enriched with Myo-inositol.
Group 6 (Functional+MI+DCI): Chickpea+Wheat flour cookie enriched with Myo-inositol.

Feeding protocol

Following confirmation of diabetes, a 7-day acclimatization period was allowed before intervention. Cookies were administered once daily for 60 days at the same time to minimize circadian variability. Cookies were administered as a replacement for the standard diet. All animals were observed during feeding and uneaten portions were recorded and weighed to assess feed intake (UKY DLAR, 2025).

Biochemical and histological analyses

At the conclusion of the experimental phase, the animals underwent an overnight fasting, following which blood samples were obtained through the retro-orbital plexus while the animals were lightly anesthetized. The following parameters were analyzed: Fasting blood glucose (FBG): Measured using a glucometer. Oral Glucose Tolerance Test (OGTT, Day 60): Blood glucose levels were recorded at 0, 30, 60, 90 and 120 min following oral glucose load (2 g/kg); area under the curve (AUC) was calculated. Glycated Hemoglobin (HbA1c): Determined using commercial assay kits. Serum Insulin: Measured using ELISA. Oxidative Stress Markers: Malondialdehyde (MDA) and superoxide dismutase (SOD) levels measured by spectrophotometric methods. Inflammatory Markers: Serum IL-6 and TNF-α quantified using ELISA kits. Histopathology: Pancreatic tissues were excised, fixed in 10% neutral-buffered formalin, paraffin-embedded, sectioned and stained with hematoxylin-eosin (H and E) for microscopic examination of islet morphology (Maske *et al.*, 2023).

Statistical analysis

Preliminary assessments of data distribution were conducted using the Shapiro-Wilk test. Most variables did not conform to a normal distribution ($p<0.05$), non-parametric statistical methods were subsequently applied.

The Kruskal-Wallis test, a non-parametric statistical method was employed to evaluate differences among groups and determine if these differences are statistically significant. For instances where the test showed notable findings, pairwise analyses were performed employing Dunn's post hoc method, incorporating the Bonferroni-Holm adjustment to account for multiple testing.

The results are expressed as mean±standard deviation (SD) for descriptive purposes; however, all inferential

statistics were conducted utilizing non-parametric methods. Effect sizes for pairwise comparisons are reported as rank-biserial correlation coefficients.

A p-value less than 0.05 was regarded as indicative of statistical significance. All statistical analyses were conducted utilizing SPSS software.

RESULTS AND DISCUSSION

Glycemic control

The administration of streptozotocin (STZ) led to a considerable increase in fasting blood glucose (FBG) and HbA1c levels in the disease control group (Group 2) when compared to the healthy control group (Group 1) (Ghazal *et al.*, 2024). These results confirm the effective development of a diabetic model. Cookie supplementation demonstrated variable effects across different types (Table 1). The wheat-based Group 3's cookies had little effect on blood sugar regulation, whereas Group 4's chickpea cookies notably reduced fasting blood glucose levels (FBG) and HbA1c levels, likely attributable to their elevated protein and resistant starch content. Additionally, wheat cookies fortified with Myo- and D-Chiro-inositol (Group 5) produced moderate improvements in glycemic parameters, suggesting an insulin-sensitizing effect of these inositols.

Group 6, which received the combination of chickpea, wheat, MI and DCI cookie, exhibited the most notable improvement, with fasting blood glucose (FBG) and glycated hemoglobin (HbA1c) levels approaching normal ranges. Additionally, insulin levels showed significant normalization, suggestive of improved insulin sensitivity. These findings consistent with earlier studies reporting similar benefits of inositol-enriched functional foods (Antony *et al.*, 2017). The oral glucose tolerance test (OGTT) results corroborated these findings, indicating that subjects in this group regained baseline glucose levels more efficiently than those in other groups (Table 2).

Food intake and body weight

Diabetic rats in Group 2 showed symptoms of polyphagia and weight loss, as presented in Table 3. Cookie supplementation alleviated these symptoms, with Group 6 exhibiting the most significant improvement in appetite regulation and body weight recovery. Notably, the final body weight data (Table 3) indicate that functional Groups 4, 5, and 6 achieved higher mean final weights compared to the healthy control group 1 (*e.g.*, G4: 311.83 g vs. G1: 248.17 g). Similar improvements in metabolic and weight parameters have been reported following the supplementation of Myo-inositol and D-Chiro-inositol in diabetic animal models (DiNicolantonio and O'Keefe, 2022). These findings suggest a potential restoration of metabolic homeostasis.

Oxidative stress and antioxidant status

In the control group, rats demonstrated increased levels of malondialdehyde (MDA) and decreased activity of superoxide dismutase (SOD). Both chickpea and inositol enriched cookies independently contributed to a reduction in oxidative stress; however, the group receiving combined supplementation (Group 6) showed the most significant decrease in MDA levels and the highest SOD activity (Table 4). These findings suggest a potential synergistic antioxidant effect of the combined treatment. These observations align with previous findings on the antioxidative role of legume-based and inositol-supplemented diets in diabetes management (Croze *et al.*, 2015; Aljutaily *et al.*, 2022).

Table 1: Effect of cookie supplementation on fasting blood glucose (FBG), HbA1c and Insulin in STZ-induced diabetic rats.

Groups	Fasting blood glucose (mg/dL)	HBA1C (%)	Insulin (µU/mL)
Group 1 (Control)	91.7	5.445	21.93
Group 2 (Disease Control)	178.14	11.785	69.18
Group 3 (Wheat)	173.42	11.66167	68.03
Group 4 (Functional)	127.82	9.22	54.98
Group 5 (Wheat+MI+DCI)	126.99	9.123333	53.2
Group 6 (Functional+MI+DCI)	105.1	7.933333	46.35

(Mean ± SEM, n = 6 rats per group).

Table 2: Oral glucose tolerance test (OGTT) results across groups.

	Blood glucose level (mg/dL) for OGTT					
	Mean					
Groups	0 min	15 min	30 min	60 min	90 min	120 min
Group 1	91.38	131.38	161.38	131.38	111.38	91.38
Group 2	177.57	217.57	247.57	217.57	197.57	177.57
Group 3	172.63	212.63	242.63	212.63	192.63	172.63
Group 4	128.15	168.15	198.15	168.15	148.15	128.15
Group 5	126.56	166.56	196.56	166.56	146.56	126.56
Group 6	106.44	146.44	176.44	146.44	126.44	106.44

Inflammatory cytokines

In diabetic rats (Group 2), levels of interleukin-6 (IL-6) and alpha tumor necrosis factor (TNF-α) were markedly increased. The administration of cookies enriched with chickpeas and inositol individually caused a decrease in these inflammatory cytokines; however, the most significant anti-inflammatory effect was observed in Group 6 (Table 5). This aligns with reports of inositols' anti-inflammatory potential in diabetic and metabolic disorders (DiNicolantonio and O'Keefe, 2022).

Histopathological evaluation of pancreatic tissue

Histological examination supported the biochemical results. Control rats (Group 1) displayed normal pancreatic islets and acinar architecture (Ghazal *et al.*, 2024). Diabetic rats (Group 2) showed shrunken islets acinar degeneration and significant fibrosis. Rats fed with wheat cookies (Group 3) continued to exhibit fibrosis and smaller islets, indicating limited therapeutic benefit. Groups 4 and 5 had partially preserved islets and reorganized acinar tissue. Most notably, Group 6 showed restored islet structure, decreased fibrosis and reorganized acinar tissue, providing strong morphological evidence of pancreatic recovery (Fig 1). Similar pancreatic protection and regeneration effects have been reported with chickpea-and inositol-based interventions in diabetic rats (Antony *et al.*, 2017).

Summary of findings

- Rats subjected to streptozotocin-induced diabetes (designated as Group 2) demonstrated characteristic features of the diabetic condition, such as sustained hyperglycemia, increased oxidative stress, systemic inflammatory responses and morphological changes in pancreatic islets, including atrophy and fibrosis.
- Wheat-based cookies (Group 3) did not result in statistically significant improvements, as animals continued to exhibit islet degeneration and fibrotic alterations.
- Cookies formulated with chickpeas and wheat (Group 4) exhibited moderate glycemic advantages, potentially owing to their low glycemic index, elevated protein content and resistant starch levels. Microscopic examination showed that these rats maintained partially preserved islet architecture with fibrosis being reduced yet still discernible.
- Inositol-enhanced wheat cookies (Group 5) demonstrated a significant improvement in insulin sensitivity and contributed to the partial regeneration of pancreatic islets, accompanied by reorganization of acinar tissue.
- The composite cookie containing chickpeas and wheat, enriched with MI and DCI (Group 6), exhibited the most notable improvements across all evaluated parameters. Specifically, this group demonstrated near-normal fasting blood glucose (FBG) and HbA1c levels, enhanced insulin secretion, increased antioxidant capacity, decreased levels of IL-6 and TNF-α and histological evidence of restored islet architecture characterized by reorganized acinar tissue and minimal fibrosis.

Together, these findings imply that cookies formulated with chickpea and wheat flour, enriched with inositols, may have a multifaceted therapeutic potential. Specifically, these cookies appear to enhance glycemic control, mitigate oxidative and inflammatory stress and maintain the structural integrity of pancreatic tissue.

Future directions

While these findings are promising, more research is necessary to establish translational relevance:

1. Long-term efficacy should be assessed through extended feeding trials to confirm sustained glycemic and histological improvements.
2. Optimization of dosage of Myo-inositol and D-Chiro-inositol is required to determine the most effective therapeutic ratio.
3. Clinical translation through human intervention studies is essential to evaluate safety, acceptability, adherence and therapeutic outcomes in diabetic populations.

Table 3: Food intake and body weight changes across experimental groups.

Groups	Body weight (g)	Food intake (g/day)
Group 1 (Control)	248.17	22.47
Group 2 (Disease Control)	224.17	40.5
Group 3 (Wheat)	235.5	36.5
Group 4 (Functional)	311.83	33.17
Group 5 (Wheat+MI+DCI)	314.67	33.75
Group 6 (Functional+MI+DCI)	298.33	30.25

Table 4: Oxidative stress markers in different groups.

Groups	MDA (umol/l)	SOD (u/mL)
Group 1 (Control)	2.1	146.67
Group 2 (Disease Control)	7.31667	52
Group 3 (Wheat)	6.43333	58.5
Group 4 (Functional)	4.86667	85
Group 5 (Wheat+MI+DCI)	5.05	82.5
Group 6 (Functional+MI+DCI)	3.78333	108

Table 5: Inflammatory cytokines markers in different groups.

Groups	IL-6	TNF-Alpha
Group 1 (Control)	31.74	15.96
Group 2 (Disease Control)	142.11	91.92
Group 3 (Wheat)	119.72	79.12
Group 4 (Functional)	90.73	60.42
Group 5 (Wheat+MI+DCI)	92.45	61.9
Group 6 (Functional+MI+DCI)	62.83	37.94

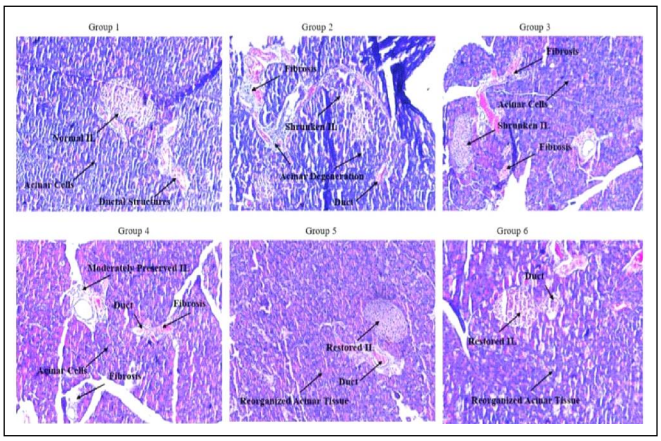


Fig 1: Histopathological examination of pancreatic tissues using hematoxylin and eosin (H and E) staining across all experimental groups.

CONCLUSION

This research article presents findings indicating that cookies formulated with chickpea and wheat flours, enriched with Myo-inositol and D-Chiro-inositol, significantly enhance various health parameters in rats with streptozotocin-induced diabetes. Specifically, these cookies improved blood sugar regulation, antioxidant capacity, inflammatory response and pancreatic tissue structure. Notably, cookies made solely with wheat showed minimal effects, whereas those based on chickpea provided moderate benefits. The addition of inositols further improved insulin responsiveness. The most effective formulation was a combination of chickpea and wheat flours enriched with both Myo-inositol and D-Chiro-inositol, which restored blood glucose and HbA1c levels, decreased oxidative and inflammatory markers and preserved pancreatic islet architecture. These findings suggest the potential utility of incorporating functional ingredients like chickpea flour and inositols into dietary products aimed at managing diabetes.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Ethical approval/Informed consent

All animal experiments were conducted following the guidelines and approval of the Animal Committee of GD Goenka university. All procedures adhered to institutional and national standards for the care and use of laboratory animals.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis and decision to publish or preparation of the manuscript.

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