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Effect of Rhamnogalacturonan II (RG-II) in Okro against Diabetes Induced-rats Management

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Abstract: Okra (Abelmoschus esculentus L.) is widely known for its nutritional and medicinal benefits, particularly its potential role in managing diabetes. This study explores the therapeutic efficacy of Rhamnogalacturonan II (RG-II), a functional component of okra mucilage, in regulating blood glucose levels in diabetic rats. Okra samples were cultivated, processed, and analysed to quantify mucilage and pectin yields, highlighting their bioactive properties. Fourier-transform infrared spectroscopy (FTIR) was employed to characterise the functional compounds present in the extracts. An in vivo study was conducted using streptozotocin (STZ)-induced diabetic rats over 28 days, evaluating the effects of varying concentrations of RG-II on blood glucose modulation. Experimental groups were administered 100%, 80%, and 60% RG-II extracts, alongside control groups receiving water or the commercial antidiabetic drug glibenclamide. The findings revealed a significant reduction in blood glucose levels in rats administered RG-II with The initial blood glucose are STZ Glibenclamide, (354.01±0.02) STZ 100% RGII,(333.0.00±0.00) STZ 80% RG-II,(341.00±0.03) STZ 60% RG-II, (360.01±0.01) and the final STZ Glibenclamide, (105.01±0.01), STZ 100% RG-II (107.01±0.00), STZ 80% RG-II (129.02±0.10), STZ 60% RG-II (147.00±0.01) demonstrating its potential as an effective natural alternative for diabetes management. The study confirms that the polysaccharide-rich composition of okra mucilage and pectin supports glycemic control through mechanisms such as delayed glucose absorption, enzyme inhibition, and gut microbiota interaction. These findings underscore RG-II's promise as a functional food ingredient for diabetes management.
Further research is recommended to optimize dosing and understand the mechanistic pathways underlying its efficacy.

Keywords: Diabetes, Pectin, Mucilage, Rhamnogalacturonan II (RG-II) Okro,

1. Introduction

Okra (*Abelmoschus esculentus L.*) is a member of the Malvaceae family that is derived from the tropical, subtropical, and warm temperate areas of the world (Pillai *et al.,* 2024). Okra has a prominent position among vegetable fruits due to its high nutritional and therapeutic value, ease of cultivation, wider adaptation to varied weather conditions, year-round cultivation, high yield, resistance to numerous diseases and pests, and export potential (Chawla *et al.,* 2025). Okra contains a lot of mucilage, is low in calories but rich in nutrients, and is a very good source of fiber. It also contains bioactive compounds such as phenolic, vitamin C, carotenoids, thiamin, folic acid, riboflavin, oxalic acid, niacin, and amino acids Fatima *et al.,* 2024). Moreover, okra is a good source of minerals (K, Ca, P, Mg) and is very low in cholesterol and saturated fat. In addition to direct consumption, okra fruit is also exploited by the pharmaceutical industry due to its high content of functional compounds such as polysaccharides and flavonoids (Pillai *et al.,* 2024).
Okra (*Abelmoschus esculentus*) is therapeutic and pertinent. In folk medicine, okra has a long history of being used to treat various human diseases, including stomachic, stimulant, demulcent, antispasmodic, and diuretic properties (Basnet *et al.,* 2023). Its bioactive components are effective in treating human diseases and exhibit various pharmacological actions, including anticancer, immunomodulatory, antimicrobial, antidiabetic, anti-obesity, antihyperlipidemic, anti-atherosclerotic, anti-inflammatory, antioxidant, myocardial protective, and neuroprotective properties (Nechchadi *et al.,* 2024). Okra has a vital role in modulating blood glucose (Ahmed et al., 2025). In 2019, region-wide diabetes mellitus accounts for 284,049 deaths, 139,651 deaths in men, and 144,398 deaths in women (PAHO,2021). Despite significant advances in clinical management, the International Diabetes Federation projects

that 783 million people will be living with diabetes by 2045 (Ciming et al.,2025). It is important to find an effective way of consuming okra to control blood sugar. In this regard, okra-based beverages can be a potential option. The justification of the efficacy of the beverage in controlling blood sugar is essential, using animal models such as the rat model. This research aims to develop an effective diabetes remedial okra plant extract functional beverage and to determine the efficacy of the developed okra-based beverage using a rat model.

2.0 Methodology

2.1 Material

Fresh Okro fruits were purchased and were macerated, and the mucilage was freeze-dried and then ground to pass through a 60-mesh sieve. Subsequently, the samples were stored at –20°C for further experimental work and analysis of the remaining indicators (content of mucilage, bioactive compounds, using FT-IR and animal model experiment for anti-diabetes potential).

2.2 Mucilage content

Mucilage content was determined by the extraction method with ethanol (de Alvarenga Pinto Cotrim *et al.*, 2016). The sample (5 g) was added to 100 ml of distilled water and kept for 24 h. The mixture was filtered through a muslin cloth. The filtrate was treated with 50 ml of ethanol and stirred slowly until the mucilage precipitated. A preweighted Whatman No. 4 paper was used to filter the mixture. The filter paper containing residue was dried at 105°C to constant weight, from which the mucilage content (%) was calculated.

2.3 Pectin content

Pectin content was determined by the pectate calcium method (Girma and Worku, 2016). The sample (1 g) was filled to 100 ml with distilled water and filtered through a piece of Whatman No. 4 paper. The filtrate (20 ml) was mixed with 100 ml of 0.1 N NaOH solution. After 7 hours, the mixture was added to 50 ml of 0.1 N CH_3COOH, left for 5 min, and then added to 50 ml of 1 N $CaCl_2$. After 1 hour, the sample was boiled for 5 minutes and then filtered through filter paper. The filter paper containing the precipitate was dried at 105°C to a constant weight. The pectin content was calculated by Equation 2, where P was the precipitate weight (g), 0.92 was the conversion coefficient from calcium pectate to pectin, and m was the sample weight (g).

$$\text{Pectin (\%)} = (P \times 0.92/20) \times (100/m) \times 100$$

2.4 Experimental Design

An in vivo study evaluated the antidiabetic effects of okra extract RG-II on diabetic rats over 28 days. 30 rats were used for the experiment; 24 rats were induced intraperitoneal with STZ (50mg/kg/body weight). While the 6 rats were given a placebo (sterile water). Ethical approval was obtained from the Ministry of Health and the Public Health Department of Osogbo, Osun State. Nigeria No. OSHRC/PRS/569T/240 The experiment spanned 28 days; the grouping and treatment of the diabetic rats are described below.

Table 1: Shows grouping and administration of RG-II

Group A	Group B	Group C	Group D	Group E
6 rats	6 rats	6 rats	6 rats	6 rats
100% H2O	100% Drug	100% RG-II	80% RG-II	60% RG_II

The experimental rats were administered Group A with water, (Negative control group) Group B with glibenclamide (commercial medication) and Groups C, D and E were administered with 100%, 80% and 60% of the RG-II for 28 days' blood sample were collected at 3 days' intervals for the period of experiment to determine the level of blood glucose.

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Figure 1: a. shows Intraperitoneal induction of STZ. (b) Oral administration (c) Point of sample collection

Blood glucose levels were estimated by drawing blood samples on day 0 (pre-treatment) from the tail vein and at 3day intervals, concluding with the study period. The blood glucose levels were estimated using Accu-Check, a monitoring device

3.0 Results

Table 2: Shows the percentage of mucilage yield in the sample.

Sample size (g)	Total yield (g)
5g	1.45g
Total sample (g)	Total yield (g)
5000g	1450g

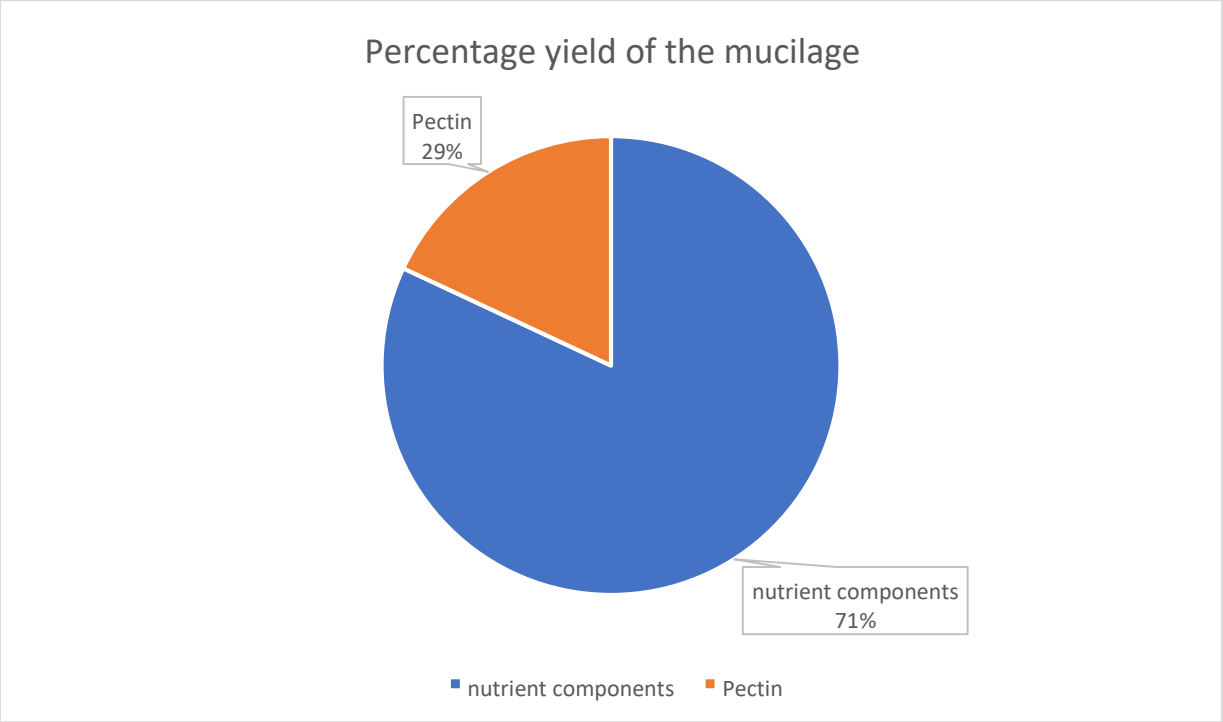

Figure 2: Pie chart representing the percentage of mucilage in the sample

3.1 Percentage Yield of Okro Mucilage

The extraction of 1450g of mucilage from 5000g of okra yields a remarkable 29%, consistent with Ezenwa's study in (2023), which observed yields ranging from 11.65% to 37.885% using conventional and microwave-assisted methods. This high yield indicates efficient extraction parameters. The substantial mucilage percentage implicates a significant presence of rhamnogalacturonan I (RG-I), a polysaccharide known for its potential in diabetes management. RG-I's ability to modulate blood glucose levels presents promising therapeutic benefits, thereby optimising the health impact of okra mucilage within diabetes treatment protocols.

Table 3: Shows the percentage of Pectin yield in the sample.

Sample size (g)	Total yield (g)
5g	1.1g
Total sample (g)	Total yield (g)
5000g	1100g

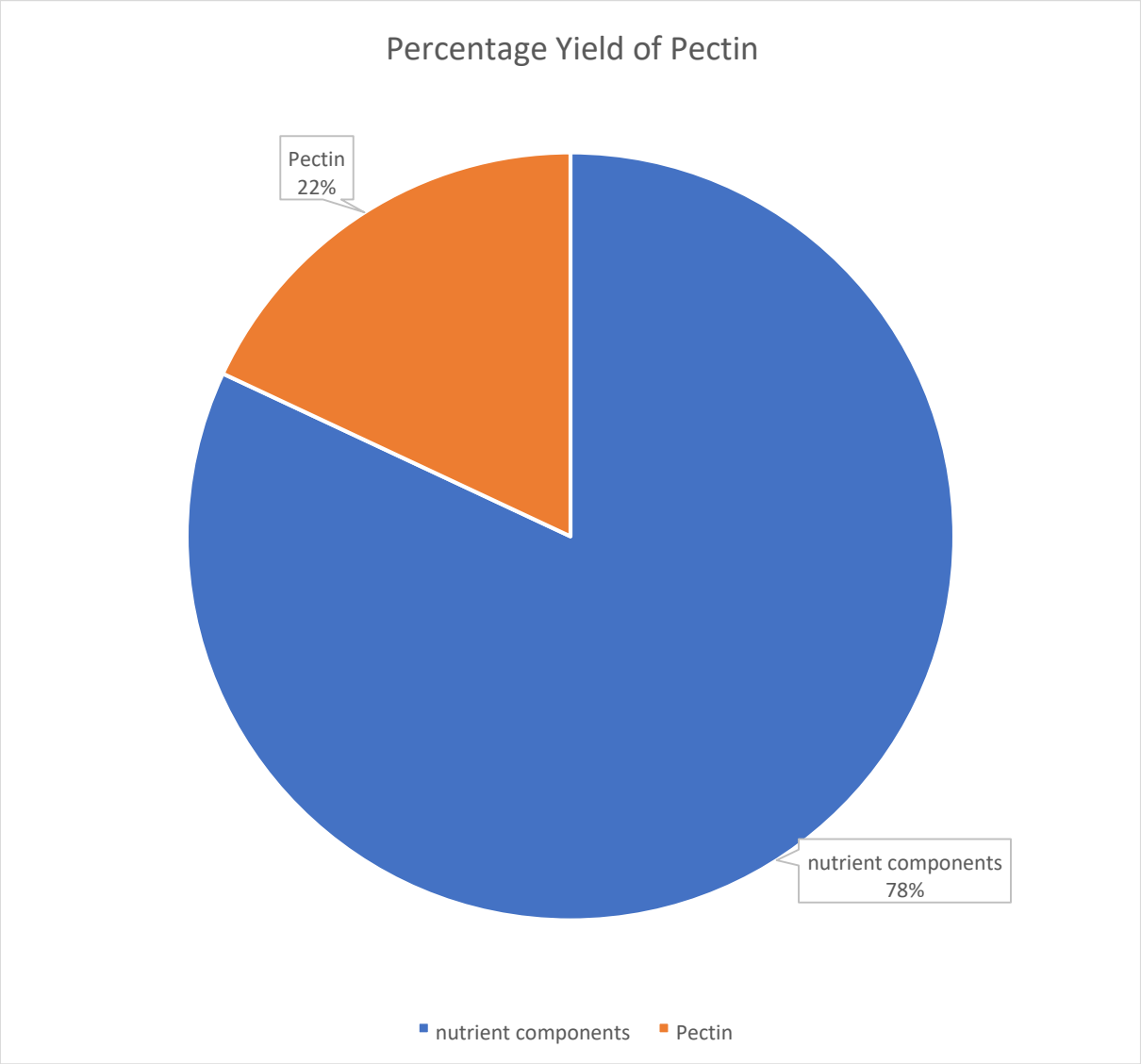

Figure 3: Shows pie chart representing the percentage of pectin in the sample

The extraction of 1100g pectin from 5000g okra represents a 22% yield, aligning with studies reporting 11.65–37.885% yields for okra pectin using methods like citric acid or phosphate buffer extraction. This efficiency reflects optimised parameters such as particle size (0.5–2 mm) and solvent choice. This is tamderm with work done by Afotey *et al.*, 2023)

The result of Okro samples was extracted and analysed using Fourier-transform infrared spectroscopy (**FTIR**) to characterise the active functional compound that can manage blood glucose.

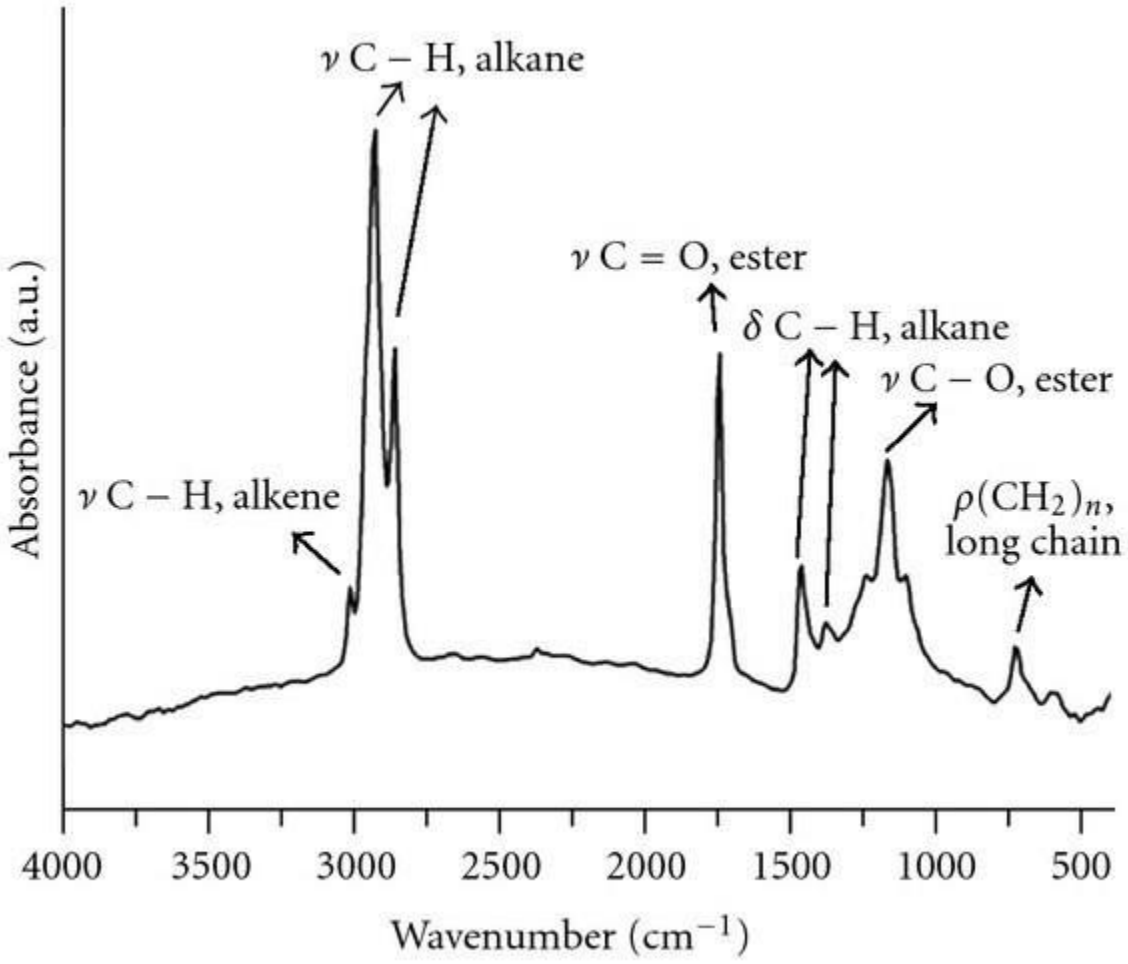

Figure 4. Shows FT-IR spectrum of Okro extracts

The FTIR analysis of okra revealed characteristic functional groups associated with its anti-diabetic properties. The spectrum shows distinct peaks for C-H alkane stretching (around 3000 cm^{-1}), C=O ester stretching (near 1750 cm^{-1}), C-O ester bonds (1200-1100 cm^{-1}), and long-chain $(CH_2)_n$ rocking vibrations. Okra mucilage contains polysaccharides identified in the fingerprint region between 3279-1030 $cm^{-,1}$ the result aligns with the research conducted by Daniel *et al*., 2024, and Elkhalifa *et al.,* 2021.

Table 2: Shows the effect of rhamnogalacturonan RG-II on blood sugar

Parameter (mg/dl)	No STZ +ve control	STZ Not Treated	STZ Glenclamide	STZ 100% RG-II	STZ 80% RG-II	STZ 60% RG-II
Initial fasting	91.03±0.00	343.02±0.04	354.01±0.02	333.0.00±0.00	341.00±0.03	360.01±0.01
Final Fasting	91.01±1.70	351.0.29±1.20	105.01±0.01	107.01±0.00	129.02±0.10	147.00±0.01

Key: Group I: Control (1ml/kg of normal saline) Group II: Diabetic Control (STZ distilled H_2O (0.5ml). Group III: STZ Drug (2ml/kg 0.5ml) Group IV: STZ + RG-II (100% in 0.5ml). Group V: STZ + RG-II (80% + 20% distilled H_2O in 0.5ml). Group VI: Group V: STZ + RG-II (60% + 40% distilled H_2O in 0.5ml)

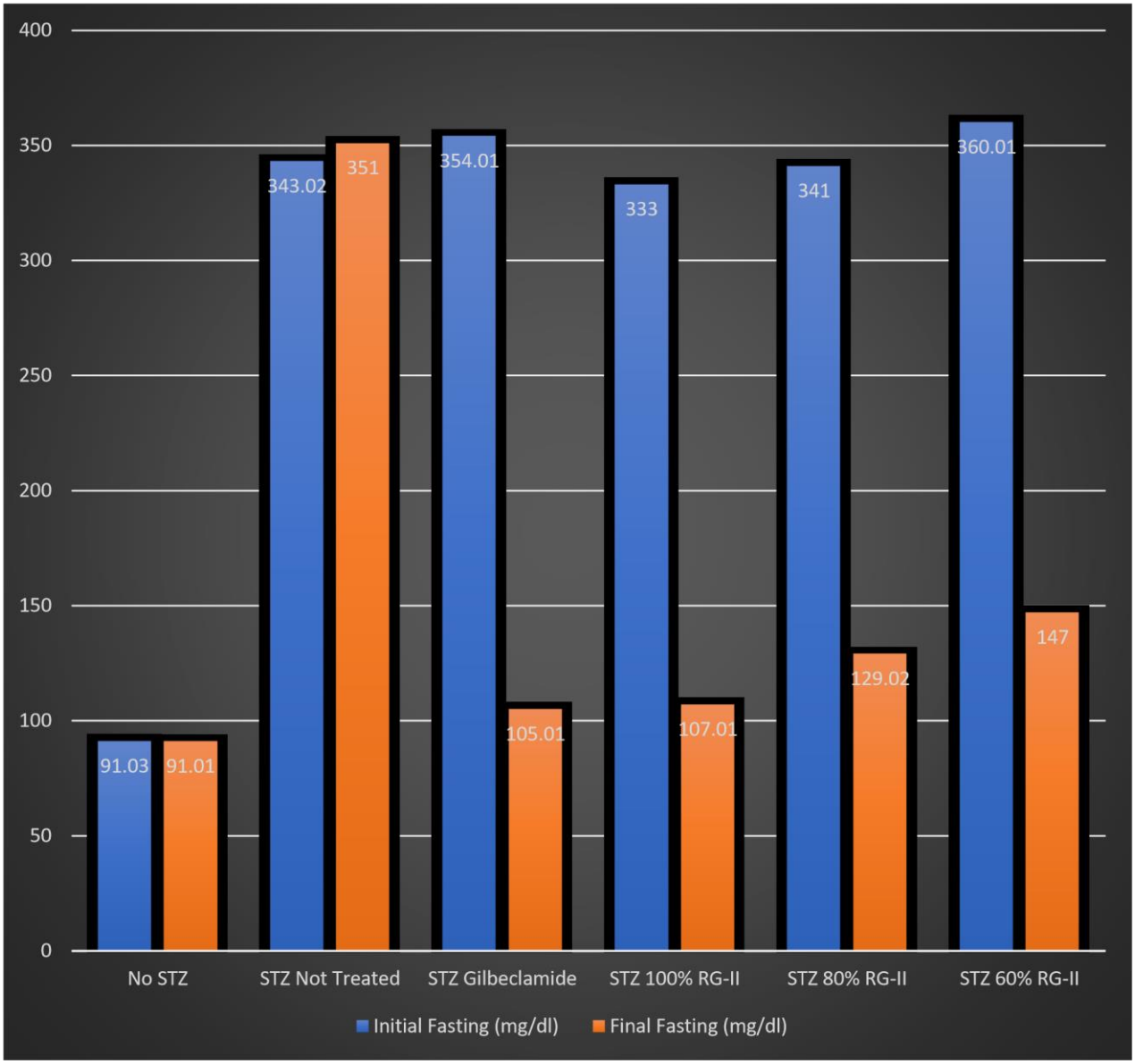

Figure 5, Shows the effect of different levels of RG-II and glibenclamide on blood sugar level

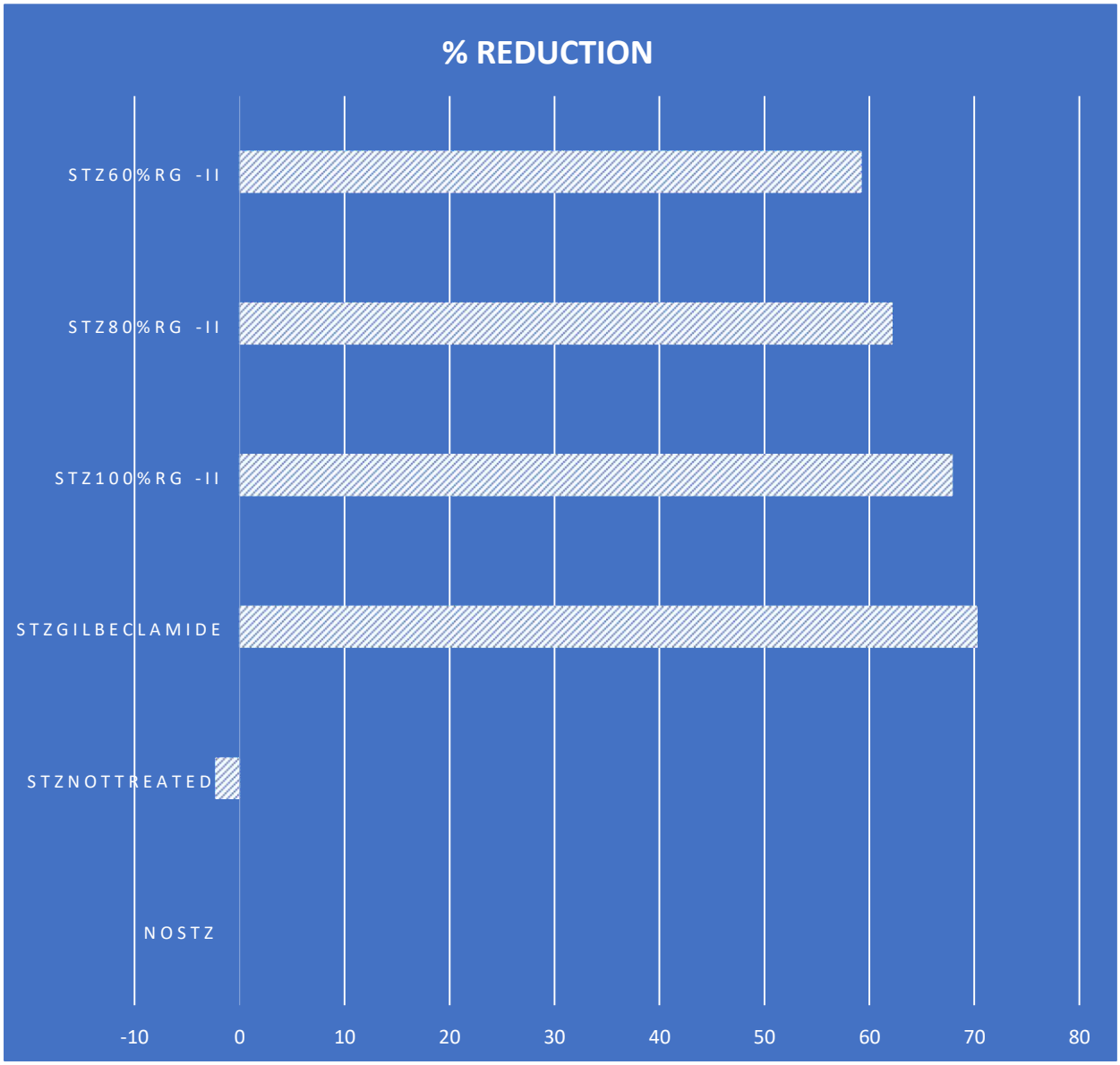

Figure 6 shows percentage reduction of the effect of RG-II on hyperglycemia in diabetic rats Anti-diabetic effect of the RG-II

The data demonstrate that rhamnogalacturonan-II (RG-II) significantly reduces fasting blood glucose levels in streptozotocin (STZ)-induced diabetic rats, with efficacy comparable to the antidiabetic drug glibenclamide. The 100% RG-II treatment lowered final fasting glucose from 333 mg/dl to 107 mg/dl, mirroring glibenclamide's effect (105 mg/dl), while lower RG-II concentrations (80% and 60%) showed reduced potency (129 mg/dl and 147 mg/dl, respectively). The result is in-line with work done by (Akbari *et al.,* 2022; Benalaya *et al.,* 2024). This dosedependent response suggests RG-II's therapeutic potential hinges on adequate dosing.

RG-II's mechanism may involve interactions with gut microbiota or AMPK pathway modulation, as seen in related pectic polysaccharides. The structural conservation of RG-II across plant species supports its biological stability and reproducibility as a treatment candidate. This result aligns with work done by Ghasemi and Jeddi (2023).

Discussion of Results

Mucilage and pectin are powerful soluble dietary fibers (SDF) that offer significant benefits for diabetes management through multiple mechanisms. These plant-derived polysaccharides have emerged as promising natural components for controlling blood glucose levels and improving overall metabolic health in diabetic individuals Mucilage works through several pathways to regulate blood sugar. It increases digestive viscosity, delaying gastric emptying and slowing glucose absorption, which prevents rapid blood sugar spikes after meals. Mucilage also inhibits carbohydrate-metabolising enzymes (α-amylase and α-glucosidase), reducing glucose release into the bloodstream. When fermented by gut bacteria, mucilage produces short-chain fatty acids that enhance insulin sensitivity, improving cellular glucose uptake. Studies in diabetic rats have shown that mucilage can significantly improve lipid profiles by lowering LDL and triglycerides while raising HDL levels, addressing the cardiovascular risks common in diabetes.
Pectin, especially its rhamnogalacturonan II fractions, offers complementary benefits. It strengthens intestinal barrier integrity by modulating tight junction proteins, preventing inflammatory triggers that worsen insulin resistance. Pectin reduces sodium-glucose co-transporter (SGLT-1) expression, limiting intestinal glucose absorption. Like mucilage, pectin's viscous properties delay carbohydrate digestion, moderating post-meal blood sugar increases. Research with STZ-induced diabetic rats demonstrated pectin's effectiveness over 28 days, with RG-II fractions reducing blood sugar levels from 344-365 mmol/dl to 107-147 mmol/dl, comparable to commercial medications.

Conclusion

These natural polysaccharides work through multiple pathways: they create physical barriers to slow glucose absorption, inhibit digestive enzymes, improve insulin sensitivity, enhance gut barrier function, and modify lipid profiles Studies confirm their effectiveness in both animal models and human subjects
For diabetic patients, incorporating mucilage and pectin-rich foods into dietary plans can provide significant benefits for glycemic control and overall metabolic health, supporting conventional diabetes management approaches with natural, food-based interventions

Interest Conflict

The authors declare that there is no conflict of interest whatsoever

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