Research Square

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Binary Combination of Hibiscus sabdariffa and Psidium guajava Improves Glucose Homeostasis, Insulin Sensitivity and Lipid Metabolism in HFD/STZ-Induced Type 2 Diabetic Rats

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Abstract

Background

T2DM is defined by hyperglycaemia, insulin resistance and dyslipidaemia. *Hibiscus sabdariffa* and *Psidium guajava* have been reported to possess antidiabetic properties individually but the combination of these two plants has not been explored for their therapeutic potential.

Objective

To evaluate the antihyperglycaemic, antihyperlipidaemic and insulin-sensitising effects of hydroethanolic extracts of *H. sabdariffa* and *P. guajava* alone or in combination in a high-fat diet/streptozotocin (HFD/STZ)-induced T2DM rat model.

Material and Methods

Male Sprague-Dawley rats with HFD/STZ-induced T2DM were orally treated with *H. sabdariffa* (200 or 400 mg/kg), *P. guajava* (200 or 400 mg/kg), their binary combination (200 + 200 mg/kg) or acarbose (10 mg/kg) for four weeks. Fasting blood glucose (FBG), oral glucose tolerance (OGTT), HOMA-IR, HbA1c, triglycerides (TG), total cholesterol (TC), and LDL-cholesterol were analysed.

Result

Combination therapy significantly improved metabolic parameters compared with diabetic controls (all $p < 0.001$) by reducing FBG (268.3 ± 41.6 to 178.7 ± 33.5 mg/dL), OGTT-AUC (39,700 ± 1,369 to 29,280 ± 2,432.9), HOMA-IR (7.6 ± 0.2 to 3.3 ± 0.2), LDL-cholesterol (96.3 ± 2.1 to 20.5 ± 2.4 mg/dL), TG (199.0 ± 2.0 to 81.9 ± 2.0 mg/dL), and TC (166.3 ± 3.8 to 93.3 ± 3.1 mg/dL). Only the high-dose extract and acarbose groups showed a significant decrease in HbA1c.

Conclusion

The binary combination of *H. sabdariffa* and *P. guajava* was more effective than either extract alone in improving glucose homeostasis, insulin sensitivity and lipid metabolism, supporting its potential as a complementary therapeutic strategy for T2DM. Further long-term preclinical and clinical studies are needed.

Introduction

Type 2 diabetes mellitus (T2DM) is a common metabolic disease characterized by two primary defects, which are the insufficient production of insulin by pancreatic β-cells and the reduced sensitivity of certain tissues to insulin (Motto et al., 2022). These defects are linked to the poor regulation of blood glucose levels, resulting in hyperglycemia. The hallmark of diabetic complications has been shown to be the excessive production of advanced glycation end products (AGEs), which result from non-enzymatic protein glycation(Gutierrez and Baez, 2014).

Presently, diabetes mellitus is being treated with oral antidiabetic drugs such as metformin, sulphonylureas, and thiazolidinediones. Besides, insulin is needed in some conditions. Even though these treatments are effective in controlling blood sugar, they have some disadvantages such as gastrointestinal disorders, hypoglycemia, pancreatic damage, and hepatotoxicity (Adjia Hamadjida et al., 2023; Jugran et al., 2021). Hence, a growing need emerges to explore new, safer, and better approaches in the management of T2DM.

Hibiscus sabdariffa, commonly known as the Roselle plant or Sorrel and belonging to the Malvaceae Family, likely originated in either Asia (India to Malaysia) or Tropical Africa. Today, it is widely cultivated throughout the Tropics and is an important crop in both Tropical and subtropical areas of the world including: Caribbean Islands, Central America, India, Africa, Brazil, Australia, Hawaii and Florida (Mahadevan et al., 2009). The antioxidant content, including anthocyanins (such as flavonoids and polyphenols), offers protection for the cardiovascular system. Flavonoids decrease the oxidation of LDL in a laboratory setting and decrease cholesterol levels in the bloodstream of both mice and rabbits (Lin et al., 2007). Roselle's calyces are high in antioxidants, and thus may reduce the negative impact of free radicals. Many of today's chronic illnesses are the result of exposure to an excessive amount of free radicals, such as kidney damage, diabetes, heart disease, and cancer (Babalola et al., 2001).

Guava (*Psidium guajava* L.) is one of the most recognized fruits that are consumed raw, cooked, or processed due to its high nutritional value, particularly in regard to the phytochemical value, fiber, and essential mineral content of the fruit. In the tropics and subtropics, guava has been used as a food plant and a medicinal plant (Butt et al., 2025; Takeda et al., 2022). In the case of animals that were diabetic, the activity of glycogen phosphorylase showed an increase, but that was not statistically significant (P 0.05). Moreover, the effect of administering the extract to both diabetic as well as normal animals led to a decrement in the activity. Additionally, the *Psidium guajava* extract was able to reduce the expression levels of glycogen phosphorylase in diabetic patients (Tella et al., 2022). The aqueous extract of *P. guajava* leaves is reported to increase secretion of insulin by pancreatic beta-cells and glycogen synthesis in the liver. The extract also inhibits glucose absorption from the upper intestine and improves dyslipidemia to some extent (Rahman et al., 2023). *Psidium guajava* treatment restored the enzyme activity to almost normal levels. Glycogen phosphorylase activity was increased in diabetic animals, although not significantly (P 0.05) and treatment of diabetic and normal animals with the extract lowered activity of the enzyme (Tella et al., 2022).

To develop and evaluate new anti-diabetic drugs, an appropriate experimental animal model that mimics human Type 2 diabetes is required. The High-Fat Diet/STZ (streptozotocin)-induced Type 2 diabetes rat model is one such commonly employed experimental model. This model proposes that feeding rats a high-fat diet (and sometimes a diet rich in sugars) generates 'insulin resistance, hyperinsulinemia, and glucose intolerance, which are exacerbated by low doses of STZ (Skovsø, 2014). Gauvin B, and flavonoids such as quercetin, leucocyanidin, kaempferol are present in guava leaves that exhibited anti-hyperglycemic effects by stimulating various cellular functions such as the activation of PTP1B, GLUT4, AKT, PI3Kp85 and AKT308 (Rahman et al., 2023).

The antidiabetic properties of *Hibiscus sabdariffa* and *Psidium guajava* are well documented, but their possible synergistic action is not well researched, especially in the HFD-STZ-induced diabetic models. Thus, the proposed research had a purpose of assessing the individual and synergistic outcomes of these extracts on glucose homeostasis, insulin resistance and lipid metabolism among diabetic Sprague Dawley rats.

Material and Methods

Chemicals

Thiobarbituric acid (TBA) purchased from Loba chemie (India), malondialdehyde bis(dimethyl acetal), Streptozotocin (99.8%), Acarbose procured from Sigma Chemical Co. (India), casein protein was purchased from Ceres organic Pvt. Ltd. (India). Trichloroacetic acid (TCA) purchased from Sigma Aldrich Soln. (India).

Collection of Plant Material:

Hibiscus sabdariffa L. calyces and *Psidium guajava* L. leaves were collected at the peak season of August-September, 2023 from the natural habitat of Imphal Valley of Manipur, India. The collected plant samples were washed thoroughly with distilled water to remove dirt and other plant pollutants. The calyces and leaves were grouped individually and dried in the sun at room temperature (25 ± 2°C) for about 7–10 days or until crisp. The dry material was then ground to a fine powder using an electric grinder. The obtained powders were maintained at room temperature, in a dry environment, in sealed, light resistant containers until the extraction process. Hydroethanolic extracts were produced by the usual maceration procedure with modification (Hussen and Endalew, 2023).

Extraction:

In brief, 100 g of the finely powdered plant material (*Hibiscus sabdariffa* calyces and Psidium guajava leaves) was soaked in 500 mL of 70% (v/v) ethanol, in a sealed glass container, with a solid-to-solvent ratio of 1: 5 (w/v). The mixtures were macerated for 72 h at room temperature (25 ± 2°C) with periodic agitation to aid the extraction. The extracts were filtered through muslin cloths to remove coarse plant

debris, and then through Whatman No. 1 filter paper after maceration. The leftover plant material was again re-macerated with 200 mL of 70% ethanol for 24 h to ensure the bioactive compounds were extracted fully. The filtrates of both macerations were mixed. The combined ethanolic filtrate was concentrated under reduced pressure at 30°C using a rotary evaporator (Lavfit Pvt. Ltd.). The final dried extracts were maintained at 4°C in sterile sealed containers until use.

Animal procure and feeding:

Male Sprague Dawley rats (180-200g) were obtained from Lovely Professional University Animal house, Jalandhar, Punjab India. The animals were kept at standard cage 6 rats per group with total 8 groups and maintained under controlled temperature and humidity with 12-12h light and dark cycle. On first day of the experiment the body weight of each rat will be recorded. All rats were acclimatized for one week with commercially available normal pellet diet (NPD) for rats. The research proposals were submitted to the CPCSEA, New Delhi after approval of Institutional Animal Ethics Committee (IAEC) No: LPU/IAEC/2025/11.

Research design and induction of type-2 diabetes:

After 1 week of acclimatization, the experiment was started. For three weeks, the control group was given a normal control diet (NCD), whereas the other seven groups were given a high-fat diet (HFD) to induce diabetes shown in Table 1.

Table 1
The nutritional value of high fat diet fed animals (in weight %).

Component	HFD(g/kg)	Percentage (%)
Powdered NCD (Pellet base)	355	35.5
Ghee (Animal fat)	280	28
Casein (80%)	200	20
Starch	104	10.4
Cholesterol	10	1
Vitamin and Minerals mix	50	5
Sodium cholate	1	0.1

Single dose of Streptozotocin (STZ) (i.p. 35mg/kg) was given intraperitonially at the last day of the 2nd week to all animals fed on HFD. The induction of diabetes was analyzed after seven days of STZ injection, non-fasting plasma glucose level (PGL) and the rats with non-fasting PGL greater or equal to 300mg/dl will be considered diabetic (Srinivasan et al., 2005a). Treatment was administered during week 4 following diabetes inductions in week 3. The positive control animals were given 10 mg/kg of

Acarbose (p.o.) at the last week. During this phase, oral administration of 200–400 mg/kg (b.w) hydroethanolic extract of *Hibiscus sabdariffa*(HS) extract, *Psidium guajava* (PG) extract and binary combination extract was provided instead of water to HS, HS-low dose, HS-high dose, PG, PG-low dose, PG-high dose and Combination group (Group IV-VIII) with different doses. Group I animals act as negative control, Group II animals act as diabetic control and Group III animals as positive control shown in below Table 2(Shah et al., 2016).

Table 2
Systematic representation of treatment regime

Group No.	Group Name	Dose and route of drug treatment	No. of animals
Group 1	Vehicle control	Normal control diet (p.o.)+ water	6
Group 2	Diabetic control	35 mg/kg of STZ (single dose, i.p.) + high fat diet	6
Group 3	Positive control-1	35 mg/kg of STZ (single dose, i.p.) + high fat diet + 10 mg/kg of Acarbose (p.o.)	6
Group 4	*Hibiscus sabdariffa* L-1 low dose*	35 mg/kg of STZ (single dose, i.p.) + high fat diet + 200 mg/kg (p.o.)	6
Group 5	*Hibiscus sabdariffa* L 1 high dose*	35 mg/kg of STZ (single dose, i.p.) + High fat diet + 400 mg/kg (p.o.)	6
Group 6	*Psidium guajava*-2 low dose*	35 mg/kg of STZ (single dose, i.p.) + High fat diet + 200 mg/kg (p.o.)	6
Group 7	*Psidium guajava* – 2 high dose*	35 mg/kg of STZ (single dose, i.p.) + High fat diet + 400 mg/kg (p.o.)	6
Group 8	Combination	35 mg/kg of STZ (single dose, i.p.) + High fat diet + 200 mg/kg (p.o.) of *Psidium guajava and Hibiscus sabdariffa*	6
		Total	48
Dose reference: (Srinivasan et al., 2005b),			

All these animals were continued to feed on their respective diet as mentioned in induction phase till the end of experiment. The studies were conduct for total 4 weeks.

Body weight, blood glucose, and insulin measurement:

The animals were weighed on week 0, 3 and 4.A glucometer (Accu-Chek Instant, Roche Diabetes Care India Pvt. Ltd, India) and appropriate blood glucose test strips were used to measure the blood glucose levels in the experimental animals. The blood samples collected on week 0, 1, 3& 4 were withdrawn by tail clipping in overnight-fasted rats to measure the fasting blood glucose levels. The oral glucose

tolerance test (OGTT) was used to evaluate the pancreas ability to generate insulin following a standard treatment. Blood glucose levels were monitored at baseline 0, 30, 60, 90, and 120 minutes after rats in each group were given oral gavages of a glucose solution (2 g/kg b.w.).Overall area under the curve (AUC) of OGTT was determined using the trapezoidal method.

Collection of blood and analytical methods:

Blood samples were collected via retro-orbital puncture under light anesthesia into EDTA-containing tubes. Blood serum was separated by centrifugation at 3000 rpm for 10–15 minutes at 4°C. For biochemical analyses, including HOMA-IR, HbA1c, serum triglycerides (TG), total cholesterol (TC), LDL-cholesterol, and OGTT-AUC, serum samples from three randomly selected animals per group were analysed because of budgetary and laboratory resource limitations. Statistical analyses were performed using these biological replicates (n = 3). Additionally, lipid profile of blood samples, including triglyceride (TG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL), HBA1c and HOMA-IR were analyzed in SPL Synergy Path Lab Pvt. Ltd., India. Insulin resistance was calculated (HOMA-IR) using the following formula: HOMA-IR = [Fasting insulin (μU/mL) × (fasting glucose (mmol/L)] / 22.5 (Haffner et al., 1997).

Statistical analysis:

Data were analyzed using GraphPad Prism (version 8). Results are expressed as mean ± SD. One-way ANOVA followed by Tukey's multiple comparison tests was used. Differences were considered statistically significant at $p < 0.05$.

Result

Effect of Body weight on HFD and total extract:

The changes in body weight of the experimental groups at 0, 3 and 4 weeks of treatment in HFD/STZ caused diabetic rats are represented in Fig. 1 (A–C).

Before treatment beginning, there were no significant changes in body weight across groups ($p > 0.05$; Figure A, Baseline (0 week)), demonstrating the consistency of animals before treatment initiation.

After 3 weeks of therapy (Figure B), the diabetes control group demonstrated a significant rise in body weight (compared with the vehicle control group), reflecting obesity-associated metabolic abnormalities generated by HFD. Administration of extracts of *Hibiscus sabdariffa* and *Psidium guajava* significantly reduced body weight increase, particularly at higher doses, as compared to the diabetes control group. The combination treatment group showed a more significant reduction in body weight indicating a synergistic anti-obesity benefit of the binary mixture.

4 weeks (Figure C), body weight in the diabetes control group was considerably greater than in the vehicle control group. Administration of both the plant extracts continued to reduce weight growth in a

dosage dependent way. Among all therapy groups, the greatest decrease in body weight was observed in the combination group, which was close to normal level. This finding suggested that the concurrent treatment of HS and PG was more effective in controlling diabetes-induced alteration in body weight than the individual therapies.

In conclusion, the results indicated that the hydroethanolic extracts of HS and PG have significant antihyperlipidemic and anti-obesity activity especially when combined in HFD/STZ-induced diabetic rats. The reduction in body weight observed may be related to the improved glucose metabolism, reduction in fat accumulation and modification of energy homeostasis.

Effect of total extract on FBG in HFD-STZ induced diabetic rats:

The levels of fasting blood glucose were similar among the groups at week 0 and the levels were within the normal physiological range, which indicates successful randomization. None of the significant differences were observed at week 1, which means that hyperglycemia had not yet occurred fully after the administration of STZ. By week 3, the level of glucose had increased significantly ($p < 0.001$), which proved the success of diabetes induction. Week 4 showed differences in treatments show in Table 3.The control of the diabetics was hyperglycemic, whereas the positive control of the high-dose of Hibiscus sabdariffa and the combination treatment were very significant ($p < 0.05$).There was no significant change in low-dose groups and *Psidium guajava* treatments. Combination therapy and increased doses were the most responsive to anti-hyperglycemic response under the fasting state, indicating a dose-dependent trend that is worth exploring further.

Table 3
Effect of *Hibiscus sabdariffa*, *Psidium guajava*, and their combination on FBG at 0, 1, 3, and 4 weeks (mean ± SD). One-way ANOVA with Tukey's post hoc test showed significant differences after STZ induction (week 3) and treatment (week 4). ***p < 0.001: **p < 0.01, *p < 0.05, ns vs diabetic control, ns = not significant.

Treatment group (n = 6)	**Fasting glucose level(mg/dl)**			
	0 week	**1 week**	**3 week**	**End of 4 week**
Vehicle control	106.83 ± 2.79	124.67 ± 11.34^{ns}	126.67 ± 10.56	124.2 ± 10.9***
Diabetic control	130.00 ± 12.25***	143.33 ± 43.89^{ns}	243.33 ± 50.24***	268.3 ± 41.6
Positive control-1	121.17 ± 7.63^{ns}	165.17 ± 20.30^{ns}	320.60 ± 32.23***	187.2 ± 44.9*
Hibiscus sabdariffa L-1 low dose	114.83 ± 7.22*	139.17 ± 38.90^{ns}	318.67 ± 24.65***	247.0 ± 32.6^{ns}
Hibiscus sabdariffa L 1 high dose	117.83 ± 3.87^{ns}	159.33 ± 44.26^{ns}	330.40 ± 26.92***	179.4 ± 41.6*
Psidium guajava-2 low dose	126.67 ± 6.12**	177.00 ± 72.66^{ns}	325.25 ± 28.65***	238.5 ± 39.1^{ns}
Psidium guajava – 2 high dose	120.50 ± 11.34^{ns}	163.00 ± 29.57^{ns}	308.50 ± 23.21***	216.8 ± 22.7^{ns}
Combination	117.83 ± 8.5^{ns}	150.00 ± 32.5^{ns}	236.83 ± 46.3***	178.7 ± 33.5*

Effect of total extract on OGTT in HFD-STZ induced diabetic rats:

At the end of the study, oral glucose tolerance test were done at 0, 30, 60, 90 and 120 min to observe the impairment of glucose in the body. The outcomes of OGTT revealed that the glucose levels in the diabetic control were persistently high at all the time points with an AUC which was significantly higher than that of the vehicle control ($p < 0.001$) which validated impaired glucose tolerance (Table 4). The postprandial glucose levels were lowered by all treatment groups, albeit with varying degrees. The positive control caused the greatest decrease in AUC ($p < 0.001$), which is why the experimental model is valid. For test treatment, high-dose *Psidium guajava* and combination therapy attained significant AUC reduction ($p < 0.01$); high-dose *Hibiscus sabdariffa* attained significance at a lower level ($p < 0.05$). Treatments at low dosages did not show significant. The trend in group suggesting, dose-dependent effect and the performance of the combination therapy makes it possible that there is a synergistic action between the two extracts.

Table 4

Effect of *Hibiscus sabdariffa*, *Psidium guajava*, and their combination on oral glucose tolerance expressed as AUC (0–120 min). Values are presented as mean ± SD of three biological replicates (n = 3). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. ***p < 0.001, **p < 0.01, *p < 0.05, ns = not significant compared to diabetic control.

Group	0 min	30 min	60 min	90 min	120 min	AUC (Mean ± SD)
Vehicle control	101.7 ± 10.4	141.7 ± 10.4	148.3 ± 10.4	121.7 ± 10.4	101.7 ± 10.4	15400 ± 955.6***
Diabetic control	236.7 ± 15.3	334.0 ± 25.1	353.3 ± 30.6	351.0 ± 20.4	333.3 ± 25.2	39700 ± 1369.0#
Positive control-1	121.7 ± 20.2	243.3 ± 40.4	276.7 ± 40.4	239.0 ± 35.7	148.3 ± 45.4	26820 ± 3497.0***
Hibiscus sabdariffa L-1 low dose	209.3 ± 34.1	269.0 ± 35.7	300.0 ± 30.0	262.0 ± 40.1	245.0 ± 47.7	31745 ± 4356.7ns
Hibiscus sabdariffa L 1 high dose	173.3 ± 25.2	250.0 ± 30.0	286.7 ± 50.3	264.3 ± 40.7	239.7 ± 47.8	30225 ± 4096.3*
Psidium guajava-2 low dose	201.7 ± 37.5	281.7 ± 30.1	338.3 ± 15.3	262.3 ± 31.5	249.3 ± 35.8	33235 ± 2578.3ns
Psidium guajava – 2 high dose	174.7 ± 35.0	245.7 ± 33.1	296.3 ± 50.6	244.7 ± 39.7	204.3 ± 32.3	29285 ± 1420.4**
Combination	159.3 ± 30	234.7 ± 34.0	293.3 ± 40.4	276.7 ± 14.7	183.3 ± 36.5	29280 ± 2432.9**

Effect of total extract on Serum metabolic index in HFD-STZ induced diabetic rats:

The HOMA-IR, LDL, triglycerides, total cholesterol, and HbA1c were significantly higher in the diabetic control group compared to the vehicle control, which confirms the successful induction of insulin resistance, dyslipidemia, and chronic hyperglycemia (Table 5). In all groups of treatment, the HOMA-IR and lipid parameters significantly decreased ($p < 0.001$).The combination treatment led to the biggest reductions in lipid levels, with the values nearly reaching the normal range. This suggests that *Hibiscus sabdariffa* and *Psidium guajava* work together effectively. The high doses were more effective than the low doses throughout the study, which aligns with a dose-dependent response. The pattern of HbA1c was different Notable decrease occurred only in the positive control and high-dose groups; no significant decrease was observed in low-dose and combination groups. It is interesting to mention this difference in lipid and glycemic results: the interventions were effective in improving insulin sensitivity and lipid metabolism, although their effect on long-term glycemic control was modest within the study period, possibly due to the slow response of HbA1c to treatment instead of it being ineffective.

Table 5

Effect of *Hibiscus sabdariffa*, *Psidium guajava*, and their combination on insulin resistance (HOMA-IR), lipid profile (LDL, triglycerides, cholesterol), and glycemic control (HbA1c). Values are expressed as mean ± SD of three biological replicates (n = 3). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. ***p < 0.001 versus diabetic control; ns = not significant.

Groups	HOMA-IR	LDL(mg/dl)	Triglycerides(mg/dl)	Cholesterol(mg/dl)	HbA1c
Vehicle control	2.2 ± 0.2***	12.3 ± 2.5***	64.0 ± 1.1***	55.3 ± 1.2***	4.4 ± 0.7***
Diabetic control	7.6 ± 0.2	96.3 ± 2.1	199.0 ± 2.0	166.3 ± 3.8	9.5 ± 0.5
Positive control	2.7 ± 0.2***	10.4 ± 0.6***	86.4 ± 1.5***	75.2 ± 1.1***	5.4 ± 0.2***
Hibiscus sabdariffa L– low dose	5.7 ± 0.3***	64.3 ± 2.5***	152.3 ± 2.5***	142.3 ± 2.1***	8.3 ± 0.2ns
Hibiscus sabdariffa L - high dose	5.5 ± 0.3***	57.0 ± 3.6***	154.3 ± 4.0***	137.3 ± 1.5***	6.9 ± 0.9***
Psidium guajava - low dose	6.3 ± 0.3***	64.3 ± 1.5***	165.7 ± 3.1***	152.7 ± 3.1***	8.9 ± 0.5ns
Psidium guajava- high dose	5.4 ± 0.3***	61.3 ± 2.5***	153.7 ± 3.1***	131.7 ± 1.5***	6.5 ± 0.4***
Combination	3.3 ± 0.2***	20.5 ± 2.4***	81.9 ± 2.0***	93.3 ± 3.1***	9.3 ± 0.2ns

Discussion

Diabetes type-2 is increasingly becoming a burden both to the patients and health systems. The growth has been boosted by urbanization and increasing incomes and even though research and investments in public health have resulted in sustained efforts, prevalence continues to increase (Kahn et al., 2014). Plant-based intervention work has also grown in this regard due to cost, in part due to availability and in part since various plant-derived compounds have shown pharmacological effects.

The present study demonstrated that hydroethanolic extracts of *Hibiscus sabdariffa* (HS) and *Psidium guajava* (PG), either alone or in combination, significantly improved glucose homeostasis, insulin resistance and dyslipidaemia in type 2 diabetic rats induced by HFD/STZ. The HFD/STZ model is a well-established accurate simulation of the clinical metabolic abnormalities of type 2 diabetes including insulin resistance, decreased β-cell activity, hyperglycemia and dyslipidaemia (Skovsø, 2014; Srinivasan et al., 2005). Successful induction of experimental diabetes was confirmed by significant increases in fasting blood glucose, HOMA-IR, serum triglycerides, total cholesterol, LDL-cholesterol and HbA1c levels

of the diabetic control rats compared to the vehicle control (all $p < 0.001$). These results are consistent with previous reports of metabolic disturbances following prolonged administration of high-fat diet and low-dose streptozotocin.

Body weight is an important marker of metabolic dysfunction in the onset of diabetes. The diabetic control animals showed a slow increase in body weight compared to the normal control after being fed a high fat diet indicating metabolic problems associated with obesity. Both plant extracts reduced this increase and the combination group showed the greatest reduction after 4 weeks. This decrease corresponds to improved energy metabolism and reduced fat deposition, even though body weight changes are minimal. Flavonoids isolated from Psidium guajava promoted fatty acid oxidation and decreased adipocyte hypertrophy whereas anthocyanins and polyphenols from Hibiscus sabdariffa repressed adipogenesis and enhanced lipid utilisation via activation of AMP-activated protein kinase (AMPK) (Salem et al., 2022; Takeda et al., 2022). Thus, the weight gain might be due to better metabolic efficiency and not appetite suppression. Type 2 diabetes is characterised by chronic fasting hyperglycemia due to increased hepatic glucose production and decreased insulin secretion. Fasting blood glucose in the diabetes control group increased from 130.0 ± 12.3 mg/dL at baseline to 268.3 ± 41.6 mg/dL after 4 weeks while vehicle control animals had normal glucose concentrations (124.2 ± 10.9 mg/dL). High-dose HS treatment significantly decreased fasting glucose to 179.4 ± 41.6 mg/dL ($p < 0.05$), as did the combination group (178.7 ± 33.5 mg/dL, $p < 0.05$). In contrast, benefits of low-dose therapy were small and not statistically significant. These results show the dose-dependent antihyperglycaemic activity and indicate that the combination of both extracts is favourable for glycaemic control. The equivalent glucose lowering effects of H. sabdariffa and P. guajava are mainly attributed to the abundance of anthocyanins, chlorogenic acid, quercetin, gallic acid and other phenolic compounds that enhance insulin sensitivity, inhibit hepatic gluconeogenesis, delay intestinal carbohydrate digestion via α-glucosidase inhibition and promote GLUT4 mediated glucose uptake (McDougall et al., 2005; Rahman et al., 2023; Tella et al., 2022).

The oral glucose tolerance test showed that the treatment further improved glucose utilisation. Glucose area under the curve was significantly higher in diabetic animals (39,700 ± 1,369) compared to vehicle controls (15,400 ± 956, $p < 0.001$) indicating severe impairment of glucose tolerance. High-dose PG (29,285 ± 1,420, $p < 0.01$) and combination group (29,280 ± 2,433, $p < 0.01$) caused a significant reduction of glucose excursions, while high-dose HS caused a moderate but significant reduction (30,225 ± 4,096, $p < 0.05$). The findings suggest that the therapy augments postprandial glucose disposal. In earlier studies, it has been shown that the polyphenols present in hibiscus inhibit digestive enzymes responsible for fast absorption of carbohydrates, and the flavonoids in guava leaves promote glucose uptake in the periphery, increase insulin secretion and improve the functionality of pancreatic β-cells through activation of the PI3K/Akt signaling pathway (Deguchi and Miyazaki, 2010; Rahman et al., 2023). Thus, the increased performance of the combination group may be caused by complementary processes occurring concurrently on glucose uptake, insulin signaling and peripheral glucose disposal.

The defining characteristic of type 2 diabetes is insulin resistance, which is commonly evaluated using HOMA-IR. In the current study, the HOMA-IR score in diabetic rats was 7.6 ± 0.2 compared with 2.2 ± 0.2 in the vehicle control group. Insulin resistance was significantly reduced in all treatment groups ($p < 0.001$), with the greatest reduction observed in the combination group (3.3 ± 0.2) and similar to acarbose (2.7 ± 0.2). Polyphenolic compounds including quercetin, chlorogenic acid and anthocyanins could be implicated in the improvement of insulin sensitivity by improving insulin receptor signaling and GLUT4 translocation. These phytochemicals have been reported to relieve oxidative stress, inhibit inflammatory cytokines such as TNF-α and IL-6 and activate the PI3K/Akt and AMPK pathways, all of which contributed to the restoration of insulin responsiveness (Jugran et al., 2021; Rahman et al., 2023). The higher reduction observed in the combined treatments indicates the bioactive compounds of the two plants acting either additively or synergistically.

Diabetic dyslipidemia is a significant contributor to the cardiovascular complication of type 2 diabetes. The diabetic control group showed a significant increase in triglycerides (199.0 ± 2.0 mg/dL), total cholesterol (166.3 ± 3.8 mg/dL) and LDL cholesterol (96.3 ± 2.1 mg/dL). The greatest reductions in LDL (20.5 ± 2.4 mg/dL), triglycerides (81.9 ± 2.0 mg/dL) and total cholesterol (93.3 ± 3.1 mg/dL) were in the combination group. All treatments improved the lipid profile significantly ($p < 0.001$). The results indicate that the combined extracts have a potent antihyperlipidemic activity. Guava flavonoids have been shown to inhibit hepatic lipogenesis and promote lipid catabolism and the anthocyanins from hibiscus have been shown to inhibit cholesterol synthesis, decrease LDL oxidation and improve endothelial function (Lin et al., 2007; Salem et al., 2022). This remarkable improvement observed in the combination group is consistent with the hypothesis that simultaneous targeting of multiple lipid metabolic pathways may provide enhanced cardio metabolic protection.

Interestingly, the course of HbA1c was not the same. The combination therapy showed improvements in fasting glucose and insulin resistance, albeit not statistically significant, with only the positive control and high dose treatment groups having statistically significant reductions. HbA1c is a measure of average glycaemic control over a period of several weeks and therefore the relatively short treatment period of four weeks may not have been sufficient to demonstrate meaningful reductions. Thus, longer intervention studies are needed to determine whether changes in fasting glucose translate into clinically meaningful reductions in HbA1c.

In conclusion, present results show that binary combination of Hibiscus sabdariffa and Psidium guajava has a better Antihyperglycemic, insulin sensitising and antihyperlipidemic effect than each extract alone. The effects observed are likely mediated by complementary phytochemicals acting on several metabolic targets including oxidative stress, insulin signaling, glucose transport, lipid metabolism, and inflammatory pathways. The multi-target effects suggest the therapeutic potential of this herbal combination as an adjunct in management of type 2 diabetes and associated metabolic problems. However, to confirm synergistic processes and translational relevance of these findings future studies should involve longer treatment periods, histological analysis, molecular pathway analyses and well designed clinical studies.

Conclusion

The current research demonstrate that *Hibiscus sabdariffa* and *Psidium guajava*, as well as a combination of the two, have a significant anti-hyperglycemic, antihyperlipidemic, and insulin-sensitizing effect on the diabetic rats fed on HFD-STZ. Emphasis was made on fasting glucose, OGTT, HOMA-IR, and lipid parameters ($p < 0.001$), and the combination therapy was more effective, which suggests that the effect might be synergistic. The changes on HbA1c were however limited with only high dosages of treatment and the positive control showing significant changes ($p < 0.001$).Primarily, the positive changes were made without steady variations in body weight ($p > 0.05$), which indicates that the effect can be mediated by direct metabolic processes instead of modulation of body weight. These results indicate the therapeutic potential of these plant extracts, especially in combination, in the treatment of insulin resistance and dyslipidemia. Their effect on long-term and mechanistic research is justified, and their role in controlling chronic glycemia is to be evaluating further.

Declarations

We hereby declare that this manuscript entitled **“Binary Combination ofHibiscus sabdariffaandPsidium guajavaImproves Glucose Homeostasis, Insulin Sensitivity and Lipid Metabolism in HFD/STZ-Induced Type 2 Diabetic Rats”** is an original work and has not been published or submitted elsewhere for publication. All authors have read and approved the final manuscript. There are no conflicts of interest to declare.

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Author Contribution

During the preparation of this work "K. S. S., N. K., K. B. S., R. G., R. K. and N. S." conceived the article. "K. S. S." collected literature and drafted the manuscript. "N. K., K. B. S., R. G., R. K., B. T. and N. S." helped with the manuscript revision. All authors read and approved the final manuscript for publication.

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Figures

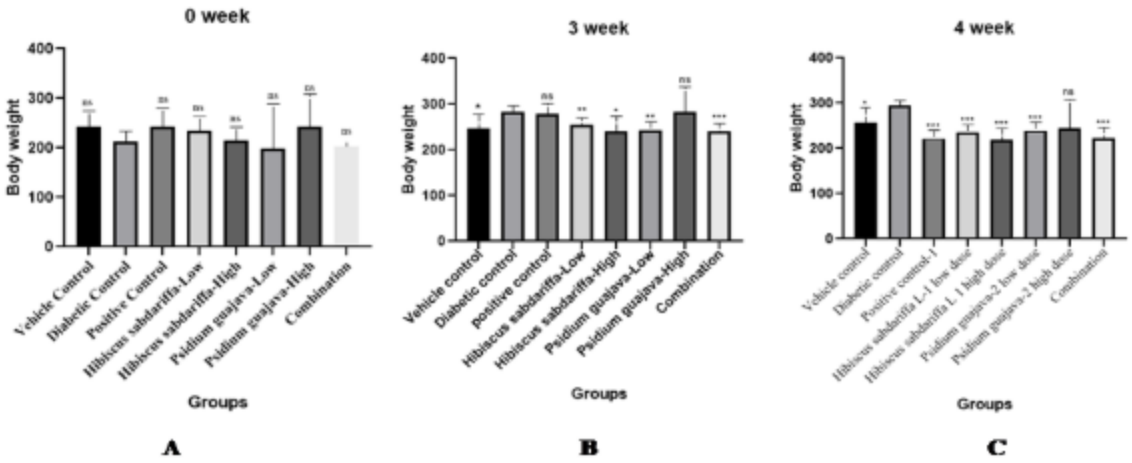


Figure 1

Effect of treatments on body weight at (A) baseline (week 0), (B) after diabetes induction (week 3), and (C) after treatment (week 4). Values are expressed as mean ± SD (n = 6). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. While significant differences were noted at weeks 3 and 4. *p < 0.05, **p < 0.01, ns = not significant.

Supplementary Files

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