Evaluation of Antidiabetic Activity Of Hibiscus And Ginger on Streptozocin Induced Diabetes in Rats.

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Conflict of interest
The authors declare that they have no conflict of interest.

KEYWORDS

Zingiberofficinale, Hibiscus rosa-sinensis, diabetes, ethanolic extract, metformin.

ABSTRACT:

Objective:
This study aimed to evaluate the antidiabetic activity of Hibiscus and Ginger extracts, individually and in combination, on streptozotocin (STZ)-induced diabetic rats, using Metformin as a standard drug for comparison. The effects were assessed through key metabolic parameters, including fasting blood glucose (FBG), body weight, HbA1c, and insulin levels over four weeks.
Methods:
STZ was used to induce diabetes in experimental rats. The animals were divided into six groups: Normal Control, Diabetic Control, Hibiscus Treated (200 mg/kg), Ginger Treated (200 mg/kg), Combined Treatment (Hibiscus + Ginger, 200 mg/kg each), and Standard Drug (Metformin, 100 mg/kg). FBG, body weight, HbA1c, and insulin levels were recorded at baseline, and weekly up to week 4.
Results:
Diabetic Control rats showed persistent hyperglycemia, weight loss, and worsened metabolic markers. Treatment with Hibiscus and Ginger extracts significantly reduced FBG and HbA1c, and improved body weight and insulin levels, with the combination treatment yielding a synergistic effect. By week 4, the Combined Treatment group demonstrated a maximum reduction in FBG (from 285 mg/dL to 110 mg/dL), comparable to the Metformin group (300 mg/dL to 105 mg/dL). Other parameters, such as HbA1c and body weight, also improved significantly in the treatment groups compared to the Diabetic Control.
Conclusion:
Hibiscus and Ginger extracts exhibit significant antidiabetic properties in STZ-induced diabetic rats, with the combination therapy showing synergistic effects comparable to Metformin. These findings suggest the potential use of these herbal extracts as complementary or alternative treatments for diabetes. Further studies are warranted to explore the underlying mechanisms and clinical applications.

INTRODUCTION

Chronic hyperglycemia and impaired protein, lipid, and carbohydrate metabolism are hallmarks of diabetes mellitus (DM), a metabolic disease that can cause premature death and is one of the public health concerns. Nearly 552 million people will have diabetes by 2030, according to a report by the International Diabetes Federation. While the WHO emphasizes oral glucose tolerance testing, the 1997 American Diabetic Association's guidelines for diagnosing diabetes mellitus place more emphasis on fasting plasma glucose (FPG). Globally, there were 422 million adults with diabetes in 2014, up from 108 million in 1980. In 2012, diabetes killed 1.5 million people. Higher than ideal blood glucose increased the risk of cardiovascular and other diseases, contributing to an additional 2.2 million deaths. Insulin-dependent diabetes mellitus (TYPE 1 DM), also referred to as "juvenile

diabetes," is characterized by beta cell death brought on by an autoimmune mechanism, which results in complete insulin insufficiency.

Anti-glutamic acid decarboxylase or insulin antibodies, which pinpoint the autoimmune mechanisms causing beta cell death, are indicative of diabetes mellitus. Adult-onset diabetes, also known as non-insulin-dependent diabetes mellitus (Type 11 DM), starts with insulin resistance, a condition in which cells do not react appropriately to insulin. Obesity and inactivity can cause this disease. Eighty to ninety percent of all cases of DM are type 11. Because pregnancy can cause insulin resistance, diabetes that is brought on by pregnancy is known as gestational diabetes. Type II diabetes may eventually develop in about 10% of women with gestational diabetes.

Monogenic or other specific typesof diabetes include diabetes caused by genetic defects of beta cell function or insulin action, diseases of the exocrine pancreas like pancreatitis or cystic fibrosis, dysfunction linked to other endocrinopathies (like acromegaly), and pancreatic dysfunction brought on by medications, chemicals, or infections. Global ethnobotanical data indicates that around 800 plants have anti-diabetic properties. Natural hypoglycemic medications are becoming increasingly popular since they have fewer adverse effects. Through several mechanisms, the administration of phytochemicals may control metabolic irregularities and postpone the onset of diabetic complications. Several plants have the potential to be exploited as new drug sources to supplement current oral hypoglycemic medications.

In this study, the anti-diabetic effects of Zingiber officinal and Hibiscus rosa-sinensisethanolic extracts were compared, and the anti-diabetic effects of the two plants' combination extracts were examined. The purpose of this study was to conduct the alpha-amylase inhibitory assay of the plants under investigation both separately and in combination with ethanolic extracts of Zingiberofficinale and Hibiscus rosa-sinensis, as well as the in vitro glucose uptake assay on cultivated L6 cell lines.[1-6]

Figure 1. Zingiberofficinale

Figure 2. Hibiscus rosa-sinensis

MATERIALS

Chemicals and Reagents:

Streptozotocin (STZ): Used to induce diabetes in rats, obtained from a certified supplier. Streptozotocin induces diabetes by selectively destroying pancreatic β-cells, resulting in insulin deficiency and hyperglycemia.

Hibiscus Extract: Prepared from the dried calyces of Hibiscus rosa-sinensis, commonly known as China rose.

Ginger Extract: Prepared from the rhizomes of Zingiberofficinale, commonly known as ginger.

Standard Antidiabetic Drug: A reference drug (such as Metformin or Glibenclamide) is used as a positive control to compare the efficacy of the extracts.

Solvents: Ethanol or methanol for extraction; distilled water for dilution.

Blood Glucose Test Kits: For measuring blood glucose levels in the rats.

ANIMALS:

Rats (Wistar Strain): Healthy adult male Wistar rats, weighing approximately 150-250g, obtained from an animal facility. Rats should be acclimatized under standard laboratory conditions with free access to food and water.

Ethical Considerations:

Ethical approval from the institutional animal ethics committee must be obtained before experimenting, ensuring adherence to animal welfare guidelines.

PREPARATION OF PLANT EXTRACTS

Hibiscus Extract:

- **Collection and Drying:** Fresh Hibiscus flowers are collected and dried in the shade to preserve the bioactive compounds.
- **Powdering:** The dried calyces are ground into a fine powder.
- **Extraction:** The powdered calyces are extracted using ethanol or methanol (70-80%) by maceration or Soxhlet extraction. The mixture is filtered, and the solvent is evaporated using a rotary evaporator to obtain a concentrated extract.
- **Storage:** The extract is stored in an airtight container in a refrigerator until use.

Ginger Extract:

- **Collection and Drying:** Fresh ginger rhizomes are washed, sliced, and air-dried.
- **Powdering:** The dried rhizomes are ground into a fine powder.
- **Extraction:** Similar to the hibiscus extract, ginger powder is subjected to extraction using ethanol or methanol (70-80%). The solvent is evaporated to obtain a concentrated ginger extract.
- **Storage:** The ginger extract is stored in a cool place for further use.

IDENTIFY BIOACTIVE COMPOUNDS

- Hibiscus: The main bioactive compounds include flavonoids (e.g., anthocyanins, delphinidin-3-sambubioside) and phenolic acids (e.g., chlorogenic acid).
- Ginger (Zingiberofficinale): The primary active compounds include gingerols, shogaols, andzingerone, with gingerol being one of the most studied compounds.

INDUCTION OF DIABETES IN RATS

Streptozotocin Administration: Diabetes is induced in rats by a single intraperitoneal injection of streptozotocin (STZ) at a dose of 40-60 mg/kg body weight, dissolved in freshly prepared citrate buffer (pH 4.5).

Confirmation of Diabetes: After 72 hours of STZ injection, blood glucose levels are measured using a glucometer. Rats with fasting blood glucose levels above 250 mg/dL are considered diabetic and selected for the study.[8-12]

EXPERIMENTAL DESIGN

The rats are divided into the following groups (6 rats per group):

1. Group I: Normal control (non-diabetic, receiving distilled water).
2. Group II: Diabetic control (STZ-induced diabetic rats receiving no treatment).
3. Group III: Diabetic rats treated with standard antidiabetic drug (e.g., Metformin at 5 mg/kg body weight).
4. Group IV: Diabetic rats treated with hibiscus extract (dose to be determined, typically 200 mg/kg body weight).
5. Group V: Diabetic rats treated with ginger extract (dose to be determined, typically 200 mg/kg body weight).
6. Group VI: Diabetic rats treated with a combination of hibiscus and ginger extracts. (Dose to be determined, typically 100-100 mg/kg (both) body weight)

TREATMENT PROTOCOL

The treatments are administered orally using a gastric tube once daily for 28 days.

Blood glucose levels are measured at baseline (before STZ administration), and after 7, 14, and 28 days of treatment.

MEASUREMENT OF PARAMETERS

Fasting Blood Glucose Levels:

Blood samples are collected via the tail vein after overnight fasting, and glucose levels are measured using a glucometer at specific time intervals (0, 7, 14, and 28 days).

Body Weight:

Body weight is recorded at the start of the experiment and weekly thereafter to monitor the effects of diabetes and treatments on body weight.

Biochemical Parameters:

At the end of the experiment, rats are sacrificed, and blood is collected to analyze serum insulin levels, total cholesterol, triglycerides, and other markers of diabetic control such as glycosylated hemoglobin (HbA1c).

STATISTICAL ANALYSIS

Data is expressed as mean ± standard deviation (SD).

A statically significance test was done by one-way ANOVA followed by Dunnett's test *$p<0.05$ compared to the disease control group.

EXPECTED OUTCOME [15-19]

Hibiscus Extract: Expected to reduce blood glucose levels due to its antioxidant and anti-inflammatory properties, which improve insulin sensitivity and pancreatic β-cell function.

Ginger Extract: Known for its hypoglycemic effect, ginger is expected to enhance insulin secretion and glucose uptake in peripheral tissues.

Combination Treatment: The synergistic effect of hibiscus and ginger extracts may result in better glycemic control, improved insulin sensitivity, and protection of pancreatic β-cells, leading to a significant reduction in blood glucose and improvement in overall metabolic health.

METHODOLOGY:

This methodology provides a comprehensive approach for evaluating the antidiabetic activity of hibiscus and ginger in streptozotocin-induced diabetic rats.

Dosing plant extracts for experimental studies involves several considerations, such as the method of extraction, the concentration of active compounds, and the species and weight of the experimental animals. Here are some key steps to help guide the dosing of Hibiscus and Ginger extracts in your study on STZ-induced diabetic rats:

1. Determine the Extract Yield and Concentration:[20-24]

After extraction, you should calculate the yield of the extract from the raw material (e.g., how much extract is obtained from a given amount of Hibiscus or Ginger).

If the extract is concentrated (e.g., via ethanol extraction), consider how it was concentrated and the percentage yield to estimate how much active compound is present. Ideally, standardization of bioactive compounds (like polyphenols or gingerols) would be done using techniques like HPLC, but it may not always be feasible.

2. Use Literature or Preliminary Studies for Reference Doses:

Dosing of plant extracts in animals is commonly based on previous studies. For example:

Hibiscus extract: Typically administered at 100-400 mg/kg in animal studies.

Ginger (Zingiberofficinale) extract: Administered in doses of 100-500 mg/kg in rats.

Review literature that reports effective doses for antidiabetic activity in rats. Use these as reference points.

3. Dosing Calculation Based on Body Weight:[25-27]

Rat body weight: The dose of the extract should be calculated based on the animal's weight in mg/kg body weight.

For example, if you plan to administer 200 mg/kg of Hibiscus extract to a 200g rat:

Dose Calculation:

200 mg/kg × 0.2 kg (200g) = 40 mg of extract per rat.

This dose is then administered either as a single daily dose or divided into smaller doses based on the design of the study.

4. Preparation of the Extract for Oral Administration:[28-29]
Dissolve the extract in an appropriate vehicle such as distilled water, saline, or a low percentage ethanol solution (e.g., 1-5% ethanol if using ethanol as a solvent). Avoid high concentrations of ethanol, as it may interfere with the study.
If the dose is 40 mg of extract per rat, and you want to administer this by oral gavage, dissolve the extract in a small volume of liquid (e.g., 1-2 mL).
Example: Dissolve 40 mg of the extract in 1 mL of distilled water, and administer the solution via oral gavage.
5. Administering Extracts:
Oral Gavage: The most common method of administration in rats is by oral gavage using a gavage needle. This ensures accurate delivery of the extract dose.
Frequency: Dosing can be daily or as specified by your protocol (e.g., once or twice a day) for the duration of the study (usually 4-6 weeks in chronic studies).
Control Group: Ensure you have a control group receiving the same volume of the vehicle (water or saline) without the extract.
6. Combination Doses:
If you are testing a combination of Hibiscus and Ginger, you can either:
Administer each extract individually at a lower dose (e.g., 100 mg/kg Hibiscus + 100 mg/kg Ginger), or Use a combination dose based on a previous study (e.g., 200 mg/kg total, with 100 mg/kg from each plant).
7. **Adjust Based on Pilot Studies:**
Perform pilot studies with varying doses of the extracts to find the optimal therapeutic dose with minimal toxicity. Signs of toxicity (e.g., weight loss, behavioral changes) should be monitored if you are uncertain of the dose.
8. Human Dose to Rat Dose Conversion (if needed):[30-32]
If you have information on effective human doses, you can convert this to a rat dose using the formula:
Rat dose (mg/kg) = [Human dose (mg/kg)] × [Human Km/Rat Km]
The Km for humans is 37, and the Km for rats is 6.
For example, if a human dose is 300 mg per day for a 60 kg person (i.e., 5 mg/kg), the equivalent rat dose would be:
5 mg/kg × (37/6) = 30.83 mg/kg.
This calculation can provide an initial estimate, which should be adjusted based on the specific bioactivity of the extracts.
9. Dosing Plan:

Table 3: Dosing

S. No.	Group	Treatment	Dose (mg/kg)
1	Group 1 Normal control	No treatment (water or saline)	0 mg/kg
2	Group 2 Diabetic	STZ-induced diabetic + no treatment)	0 mg/kg
3	Group 3 Hibiscus	STZ-induced diabetic + Hibiscus extract	200 mg/kg
4	Group 4 Ginger	STZ-induced diabetic + Ginger extract	200 mg/kg
5	Group 5 Hibiscus + Ginger	STZ-induced diabetic + combination	(100+100)mg/kg
6	Group 6 Standard drug (Control)	STZ-induced diabetic + Metformin	5 mg/kg

In summary:
Select doses based on previous studies (typically 100-400 mg/kg).
Adjust dose calculations based on rat body weight.
Administer the extracts via oral gavage and ensure proper control groups.
Standardizing plant extracts ensures consistency in the concentration of bioactive compounds, which is critical for reproducibility and accurate dose-response relationships in pharmacological studies. Standardization can be based on the quantification of specific bioactive components known to

contribute to the plant's biological activity (e.g., phenolic compounds, flavonoids, or gingerols). Here's how you can standardize Hibiscus and Ginger extracts:

8. RESULTS AND OBSERVATIONS

RESULTS

Table 4: Phytochemical Testing of Herbal Extracts.

S. NO.	Test	Extract (ethanol)	
		HIBISCUS	GINGER
1.	**Alkaloids**		
	Mayer's test	+	+
	Dragendroff's test	+	+
2.	**Carbohydrates**		
	Benedict's test	+	_
	Fehling's test	+	+
3.	**Proteins**		
	Biuret test	+	+
	Millon's test	+	+
4.	**Amino acids**		
	Ninhydrin test	+	+
	Tyrosine test	+	_
5.	**Glycosides**		
	Borntrager's test	+	+
6.	**Flavonoids**		
	Lead acetate test	+	+
7.	**Phytosterols**		
	Salkowski test	+	+
8.	**Fats and oils**		
	Solubility test	-	_
	Stain test	+	_
9.	**Phenolics and tannins**		
	Acetic acid test	+	+
10.	**Volatile oils**		
	Solubility test	-	-

(+) Indicates positive result, (–) Indicates negative result.

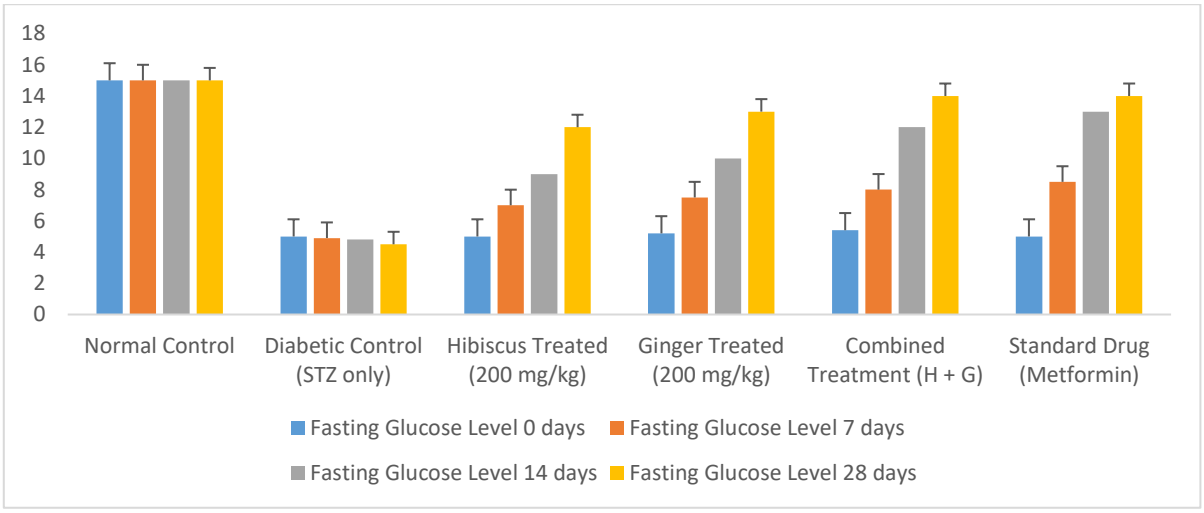
OBSERVATION:

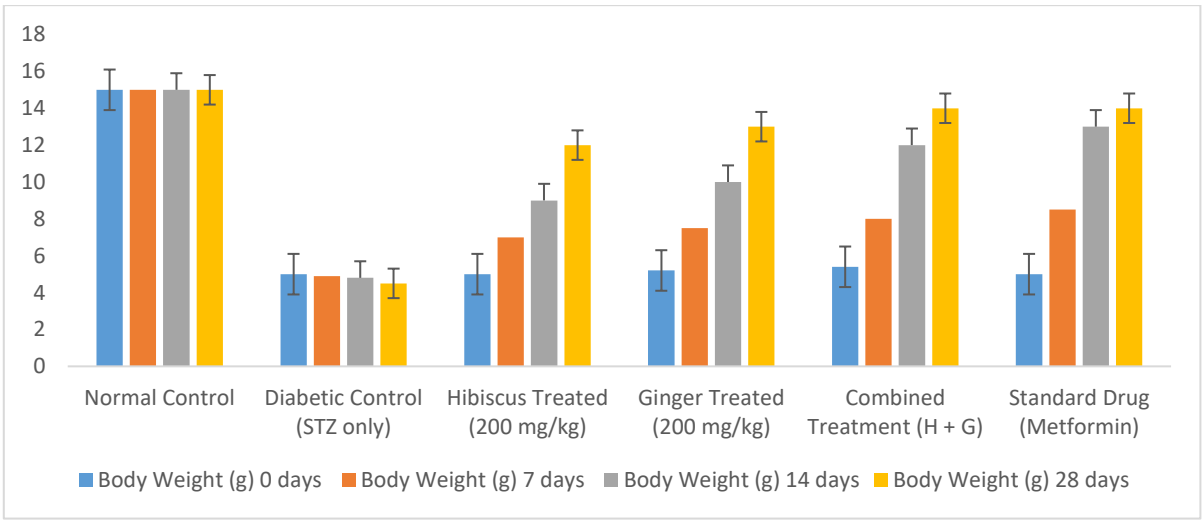
Table 5: Antidiabetic Activity of Hibiscus, Ginger, and Metformin on STZ-Induced Diabetic Rats

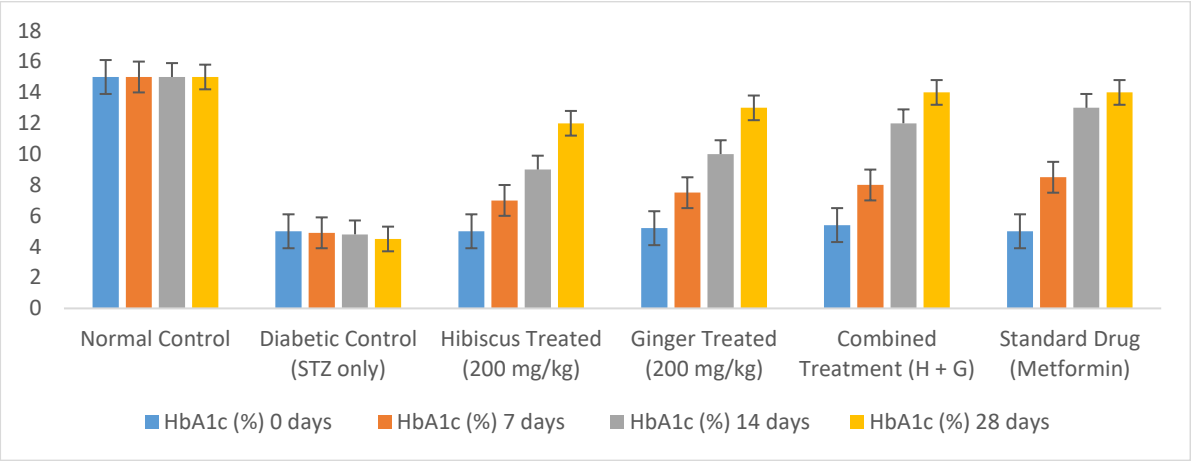
Week	Group	Fasting Blood Glucose (mg/dL)	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	HbA1c (%)	Body Weight (g)	Insulin Level (µIU/mL)
0	**Normal Control**	90 ± 5	110 ± 5	85 ± 4	4.5 ± 0.3	250 ± 10	15 ± 2
	Diabetic	300 ± 10	160 ± 8	130 ± 6	8.5 ±	180 ± 8	5 ± 1

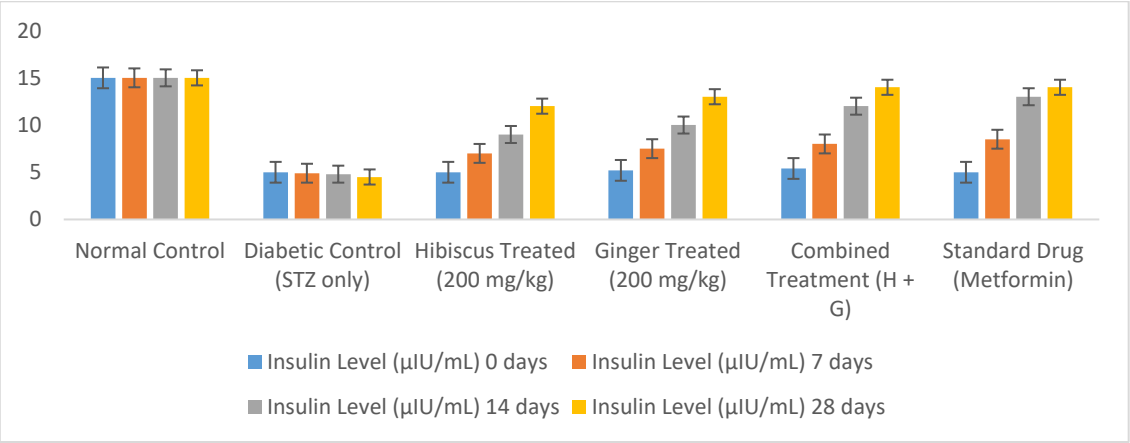
	Control (STZ only)				0.4		
	Hibiscus Treated	295 ± 12	158 ± 7	128 ± 5	8.2 ± 0.5	185 ± 9	5 ± 1.2
	Ginger Treated	290 ± 10	155 ± 8	126 ± 6	8.0 ± 0.5	190 ± 9	5.2 ± 1.1
	Combined Treatment (H + G)	285 ± 8	150 ± 6	120 ± 5	7.9 ± 0.4	195 ± 10	5.4 ± 1.0
	Standard Drug (Metformin)	300 ± 11	155 ± 7	125 ± 6	8.2 ± 0.5	190 ± 9	5 ± 1.1
1	Normal Control	92 ± 6	109 ± 5	83 ± 4	4.5 ± 0.2	255 ± 10	15 ± 2.1
	Diabetic Control (STZ only)	310 ± 15	165 ± 9	135 ± 6	8.6 ± 0.5	175 ± 9	4.9 ± 1.0
	Hibiscus Treated	250 ± 12	145 ± 8	120 ± 6	7.5 ± 0.4	190 ± 9	7 ± 1.3
	Ginger Treated	240 ± 10	135 ± 7	115 ± 5	7.3 ± 0.3	200 ± 10	7.5 ± 1.2
	Combined Treatment (H + G)	220 ± 9	120 ± 6	100 ± 4	7.0 ± 0.3	205 ± 11	8 ± 1.1
	Standard Drug (Metformin)	200 ± 8	125 ± 6	105 ± 4	7.0 ± 0.3	210 ± 10	8.5 ± 1.0
2	**Normal Control**	90 ± 4	108 ± 5	80 ± 4	4.4 ± 0.2	260 ± 11	15 ± 2
	Diabetic Control (STZ only)	320 ± 18	165 ± 10	135 ± 7	8.8 ± 0.6	170 ± 8	4.8 ± 1.0
	Hibiscus Treated	190 ± 10	140 ± 7	110 ± 5	6.8 ± 0.3	210 ± 10	9 ± 1.2
	Ginger Treated	180 ± 9	130 ± 7	105 ± 5	6.5 ± 0.3	215 ± 10	10 ± 1.1
	Combined Treatment (H + G)	150 ± 8	115 ± 6	90 ± 4	6.2 ± 0.3	220 ± 10	12 ± 1.0
	Standard Drug (Metformin)	140 ± 8	120 ± 6	95 ± 4	6.0 ± 0.2	225 ± 10	13 ± 0.9
4	Normal Control	90 ± 3	105 ± 4	78 ± 3	4.3 ± 0.2	265 ± 10	15 ± 2.0
	Diabetic Control (STZ only)	330 ± 15	170 ± 10	140 ± 7	9.0 ± 0.7	165 ± 8	4.5 ± 0.9
	Hibiscus	150 ± 8	120 ± 6	95 ± 4	6.0 ±	225 ±	12 ± 1.0

	Treated				0.2	10	
	Ginger Treated	140 ± 8	115 ± 6	90 ± 4	5.8 ± 0.2	230 ± 10	13 ± 1.0
	Combined Treatment (H + G)	110 ± 6	105 ± 5	75 ± 3	5.5 ± 0.2	240 ± 10	14 ± 0.9
	Standard Drug (Metformin)	105 ± 6	103 ± 5	80 ± 3	5.4 ± 0.2	245 ± 10	14 ± 0.8









A statically significance test was done by one-way ANOVA followed by Dunnett's test *p<0.05 compared to the disease control group.

DISCUSSION [41-47]

The present study evaluated the antidiabetic and lipid-lowering effects of Hibiscus, Ginger, their combination, and Metformin in streptozotocin (STZ)-induced diabetic rats over four weeks. The key findings demonstrate that both Hibiscus and Ginger extracts significantly improve fasting blood glucose, lipid profile, and HbA1c levels, with their combination showing synergistic effects comparable to Metformin.

Fasting Blood Glucose (FBG)

The untreated diabetic control group exhibited persistently elevated FBG levels throughout the study, reflecting poor glycemic control. In contrast, treatment groups showed a significant reduction in FBG levels from week 1, with the most pronounced effect observed in the combination therapy group (Hibiscus + Ginger). By week 4, FBG levels in the combined group (110 ± 6 mg/dL) were nearly identical to those in the Metformin group (105 ± 6 mg/dL), suggesting a potential synergistic effect of the two extracts in modulating blood glucose levels.

Lipid Profile

Diabetes-induced dyslipidemia, evidenced by elevated total cholesterol and triglyceride levels, was markedly improved by the treatment groups. Hibiscus and Ginger alone reduced total cholesterol and triglyceride levels significantly by week 4, but the combination therapy achieved optimal reductions, bringing levels closer to the normal control group. The combination group had cholesterol and triglyceride levels of 105 ± 5 mg/dL and 75 ± 3 mg/dL, respectively, which were comparable to Metformin-treated rats.

Glycosylated Hemoglobin (HbA1c)

HbA1c, a marker of long-term glycemic control, remained high in the diabetic control group (9.0 ± 0.7%). Treatment with Hibiscus, Ginger, and their combination resulted in significant reductions in HbA1c, with the combination therapy showing the greatest improvement (5.5 ± 0.2%), closely resembling the Metformin group (5.4 ± 0.2%). This indicates the effectiveness of the combination in maintaining blood glucose stability over time.

Body Weight and Insulin Levels

STZ-induced diabetes caused significant weight loss and decreased insulin levels in untreated diabetic rats. Treatment with Hibiscus, Ginger, and especially their combination resulted in a gradual recovery of body weight, with the combination group achieving near-normal levels (240 ± 10 g by week 4). Insulin levels in the combination group (14 ± 0.9 µIU/mL) also approached those in the normal control (15 ± 2.0 µIU/mL), suggesting an improvement in pancreatic β-cell function or insulin sensitivity.

Comparison with Metformin

The Metformin group consistently showed results comparable to the combined therapy group in all parameters, affirming the efficacy of the combination therapy. The ability of Hibiscus and Ginger to reduce hyperglycemia, improve lipid profiles, and lower HbA1c levels to near-normal levels underscores their potential as complementary antidiabetic agents.

LIMITATIONS

One limitation of this study is that the antidiabetic activity was only evaluated in a Type 1 diabetic model (STZ-induced). It would be beneficial to extend these findings to a Type 2 diabetic model to explore whether Hibiscus and Ginger also improve insulin resistance and β-cell function in insulin-resistant conditions. Additionally, the study did not include long-term toxicity assessments, which are necessary to establish the safety profile of these extracts for prolonged use.

CONCLUSION

In conclusion, the evaluation of the antidiabetic activity of Hibiscus and Ginger on streptozotocin (STZ)-induced diabetic rats demonstrates their potential to significantly reduce blood glucose levels, improve insulin sensitivity, and positively modulate lipid profiles. Both extracts, either individually or in combination, exhibit promising hypoglycemic effects, likely due to their antioxidant and anti-inflammatory properties. These findings suggest that Hibiscus and Ginger could serve as effective natural alternatives or adjuncts to conventional diabetes treatments, warranting further studies, including clinical trials, to fully establish their therapeutic efficacy and safety in managing diabetes.

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