ACCSCIENCE PUBLISHING

INNOSC Theranostics and Pharmacological Sciences

ORIGINAL RESEARCH ARTICLE

Effects of *Napoleonae imperialis* leaf extract and fractions on metabolic signaling and glycemic control in streptozotocin-induced diabetic rats

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Citation: Mba OJ, Anoh RE, Obila MU, Ogbonna BO. Effects of *Napoleonae imperialis* leaf extract and fractions on metabolic signaling and glycemic control in streptozotocin-induced diabetic rats. *INNOSC Theranostics and Pharmacological Sciences*. 2026;9(3):026050006. doi: 10.36922/ITPS026050006

Received: January 30, 2026

Revised: June 2, 2026

Accepted: June 11, 2026

Published online: July 24, 2026

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Abstract

Napoleonaea imperialis has been investigated for its potential antidiabetic effects due to its reported bioactive phytochemicals, although the underlying molecular mechanisms remain incompletely understood. The current study evaluated the effects of crude extract and fractions from *N. imperialis* leaves on G-protein-coupled receptors (GPCRs), AMP-activated protein kinase (AMPK), cyclic AMP (cAMP), insulin, glucagon, and glycated hemoglobin (HbA1c) in streptozotocin-induced diabetic rats. In phase one, 42 male albino rats of mean weight 140 g were divided into seven groups (n = 6/group): Group 1 (normal control), Group 2 (diabetic control), Group 3 (drug control), Groups 4, 5, and 6 were the test groups administered 250, 500, and 1,000 mg/kg of the crude extract, respectively, and Group 7 was normal rats receiving 500 mg/kg of the extract only. There was a significant ($p < 0.05$) decrease in cAMP, glucagon, blood glucose, and HbA1c, as well as a significant ($p < 0.05$) increase in GPCR, insulin, and AMPK, in Groups 3–7 compared with Group 2. In phase two, 54 animals (mean weight, 140 g) were divided into nine groups (n = 6/group): Group 1 (normal control), Group 2 (diabetic control), Group 3 (drug control), and Groups 4–9 received Fractions 1–6, respectively, for 28 days. The results showed that Fractions 2, 5, and 6 (Groups 5, 8, and 9) significantly reduced cAMP, glucagon, blood glucose, and HbA1c, and increased GPCR, insulin, and AMPK, compared with Group 2. These findings suggest that the crude extract and fractions of *N. imperialis* ameliorated the studied indicators of diabetes and its complications in streptozotocin-induced diabetic rats. The possible synergistic action of the bioactive agents present in the leaves can account for these effects.

Keywords: G-protein coupled receptors; AMP-activated protein kinase; Cyclic AMP; Insulin, Glucagon; *Napoleonae imperialis*

1. Introduction

Diabetes mellitus is a protracted ailment as ancient as humans and is responsible for significant risk globally in the present era.[1] It is the most common endocrine disorder, due to an imbalance in carbohydrate, fat, and protein metabolism. The distinctive feature of diabetes mellitus is hyperglycemia with or without glycosuria.[2] Prolonged hyperglycemia is associated with protracted damage to the kidneys, eyes, heart, and blood vessels, resulting in several complications.[3] Diabetes is a prevalent disease in society, and over the past two decades, there has been an increase in its occurrence.[4] Data from the World Health Organization indicate that about 177 million individuals had diabetes in 2000; by 2030, this number is expected to reach 360 million.[5] Hyperglycemia is also associated with a decline in high-density lipoprotein cholesterol and an increase in low-density lipoprotein cholesterol, thereby increasing the risk of coronary heart disease.[6] Therefore, it is important to monitor both glucose and lipid levels.[7] Diabetes is associated with reduced concentrations of G-protein-coupled receptors (GPCRs), insulin, and AMP-activated protein kinase (AMPK), and results in increased blood glucose concentration, glycated hemoglobin (HbA1c), glucagon, and cyclic AMP (cAMP). Presently, diabetes is managed by adjusting lifestyle, exercise, food, antidiabetic drugs, and insulin medications.[8] Due to the severe side effects of the antidiabetic drugs (gastrointestinal side effects by metformin and alpha-glucosidase inhibitors; hypoglycemia/weight gain by sulfonylureas; and the expense and risk of hypoglycemia associated with meglitinides), it is difficult to manage diabetes.[9] Despite impressive advances in recent years, there are insufficient antidiabetic medications. Consequently, phytomedicines have become relevant, and the quest for novel lead agents from plant origin has persisted. Researchers are currently focusing on safe, inexpensive plant-derived hypoglycemic medications.[10] Of interest in this study is *Napoleonae imperialis*, a Nigerian folk medicinal plant with antibacterial and wound-healing effects in albino rats.[11]

Earlier research in our laboratory has revealed the antidiabetic, hepatoprotective, and antioxidant effects of *N. imperialis* in streptozotocin (STZ)-induced diabetic rats.[12,13] However, the effects of the crude extract and fractions from *N. imperialis* leaves on metabolic signaling and glycemic control in STZ-induced diabetic rats have not yet been studied. Therefore, the objective of this study is to evaluate the effects of crude extract and fractions from *N. imperialis* leaves on GPCRs, AMPK, cAMP, insulin, glucagon, and HbA1c in STZ-induced diabetic rats.

2. Materials and methods

2.1. Collection and authentication of plant material

Napoleonae imperialis was obtained from a native farm in the Umuariaga community, Umudike, Abia State, Nigeria, and was authenticated by a plant taxonomist in the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike. The plant materials were carefully rinsed under running water, dried at room temperature (25 °C), milled, and weighed before extraction.

2.2. Extraction

Two hundred grams of the milled plant materials were soaked in 4 L of methanol for 48 h. The extract was filtered through a Whatman No. 1 filter paper, and the filtrate was air-dried at 40 °C and stored in the refrigerator for further use as the crude extract. During the experiment, the crude extract was freshly diluted with distilled water (20 g of crude extract dissolved in 200 mL of water to obtain a stock solution of 100 mg/mL) before use. The fractions were obtained by fractionating the crude extract with increasing-polarity organic solvents (hexane, chloroform, ethyl acetate, and methanol). Fractions were collected, and thin-layer chromatography (TLC) analysis was performed. Fractions having similar retention factors (R_f) were pooled. Fractions with biological activities were subjected to biochemical analysis.

2.3. Experimental animals

Albino rats were purchased from the Breeding Unit, Department of Zoology and Environmental Science, University of Nigeria, Nsukka, and kept in clean iron cages under conditions of 24 ± 1 °C and 45%–50% relative humidity, with a 12 h dark–light cycle. They were allowed to acclimatize for two weeks. Commercial pellet diet (Vital Growers mash by Grand Cereals and Oil Mills, Nigeria) and water were given to the animals *ad libitum*.

2.4. Induction of diabetes

The animals were fasted overnight. Diabetes was induced in the animals by intraperitoneal injection of STZ dissolved in 0.1 M sodium citrate buffer of pH 4.5 at a dose of 65 mg/kg body weight. After the injection, they had free access to food and water. The animals were allowed to drink 5% glucose solution overnight to prevent hypoglycemic shock. The development of diabetes was established after one week of STZ injection. Albino rats with fasting blood glucose levels greater than 200 mg/dL were considered diabetic and used in the experiment.

2.5. Experimental design

The experiment was conducted in two phases. In phase one, 42 male albino rats (mean weight 140 g) were used in this research. The animals were divided into seven groups ($n = 6$/group): Group 1 (normal [non-diabetic] control) received food and water only, Group 2 (diabetic untreated group) was diabetic rats without treatment, Group 3 (diabetic + drug control) was diabetic rats treated with 5 mg/kg body weight of the standard drug (glibenclamide), Groups 4–6 (diabetic + extract) were diabetic rats orally administered *N. imperialis* leaves extract at 250, 500, and 1,000 mg/kg body weight, respectively, and Group 7 (normal [non-diabetic] + extract) was normal rats receiving 500 mg/kg extract. The freshly prepared solutions were orally administered daily for 28 days, after which the animals were anesthetized with mild ether. Blood samples were collected by heart puncture and stored in plain sample bottles. Serum was separated immediately by centrifugation at 3,000×g for 15 min. The supernatant (serum) was obtained and used for biochemical analyses. The animals used in this research were carefully and gently cared for in accordance with the National Institutes of Health's guidelines for the care and use of animals in research.

In phase two, 54 animals were divided into nine groups (n = 6/group): Groups 1–3 were identical to those in phase one, and Groups 4–9 (diabetic + fraction) were diabetic rats orally administered 500 mg/kg body weight of the bioactive Fractions 1–6, respectively, from the crude extract for 28 days. Serum was collected in plain sample bottles for biochemical analysis of bioactive fractions with antidiabetic effects.

2.6. Biochemical analysis

Fasting blood glucose was evaluated at various time intervals (weekly) to assess blood glucose levels using the ACCU-CHEK Active Glucometer (MODEL GB, Roche Diagnostics, Germany) with the glucose oxidase-peroxidase method. HbA1c was assessed using the method described in a previous study.[14] GPCRs (GPR120), AMPK (E-EL-H2268), cAMP (E-EL-0056), insulin (E-EL-H2665), and glucagon (E-EL-H2379) were assessed using enzyme-linked immunosorbent assay (ELISA) according to the manufacturers' instructions (Elabscience Biotechnology Co. Ltd., China).

2.7. Statistical analysis

The data were analyzed using analysis of variance to determine statistical significance at the 95% confidence interval ($p \leq 0.05$), and a Duncan multiple-range post hoc test was performed to compare groups. All statistical calculations were performed using SPSS 22.0 (IBM, United States).

3. Results

3.1. Effects of the crude extract from *Napoleonae imperialis* leaves on blood glucose level in streptozotocin-induced diabetic rats

In week four, there was a significant decrease ($p < 0.05$) in blood glucose levels in the treated groups that were orally given 250, 500, or 1,000 mg/kg of the crude extract compared with the diabetic untreated group, indicating that the crude extract can lower blood glucose levels (Figure 1).

3.2. Effects of fractions from *Napoleonae imperialis* leaves on blood glucose level in streptozotocin-induced diabetic rats

In week four, there was a significant decrease ($p < 0.05$) in blood glucose levels in the groups that were orally given Fractions 1–6 compared with the diabetic control (Figure 2). This indicates that these fractions have antidiabetic effects.

3.3. Effects of crude extract from *Napoleonae imperialis* leaves on HbAIc, AMPK, insulin, glucagon, cAMP, and GPCRs in streptozotocin-induced diabetic rats

Table 1 shows that, in phase one, the AMPK, insulin, and GPCR levels of the diabetic control group (Group 2) were significantly lower ($p < 0.05$) than those of the normal control (Group 1), the drug control (Group 3), the test groups that orally received 250 (Group 4), 500 (Group 5), and 1,000 mg/kg (Group 6) of the crude extract, and the normal rat group that received 500 mg/kg extract (Group 7). In contrast, the HbAIc, glucagon, and cAMP levels of Group 2 were significantly higher ($p < 0.05$) than those of Groups 1, 3–7.

3.4. Effects of fractions from *Napoleonae imperialis* leaves on HbAIc, AMPK, insulin, glucagon, cAMP, and GPCRs in streptozotocin-induced diabetic rats

Table 2 shows that, in phase two, the AMPK, insulin, and GPCR levels of the diabetic control group (Group 2) were significantly lower ($p < 0.05$) than those of the normal control (Group 1), the drug control (Group 3), and the groups that received Fractions 2 (Group 5), 5 (Group 8), and 6 (Group 9). In contrast, the HbAIc, glucagon, and cAMP levels of Group 2 were significantly higher ($p < 0.05$) than those of Groups 1, 3, 5, 8, and 9.

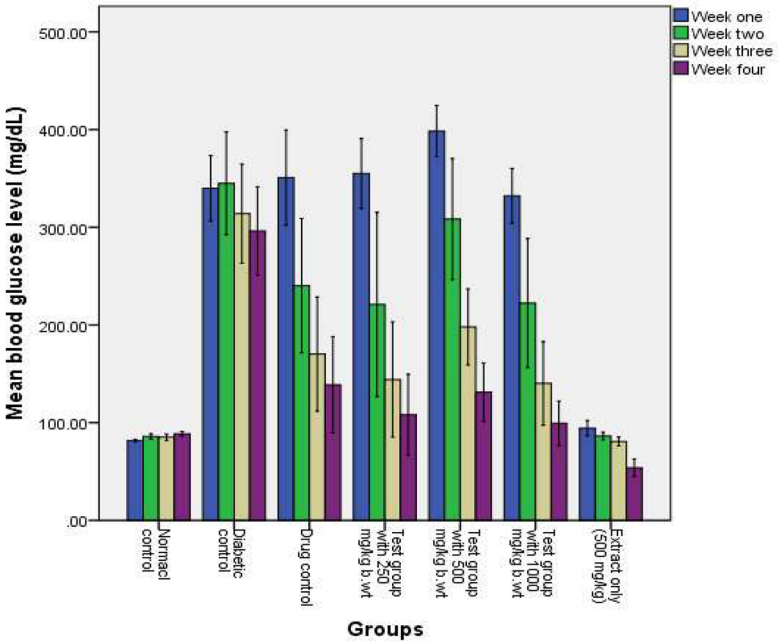

Figure 1. Fasting blood glucose concentration in streptozotocin-induced diabetic rats treated with crude extract from *Napoleonae imperialis* leaves. Values are presented as mean ± standard deviation ($n = 6$).

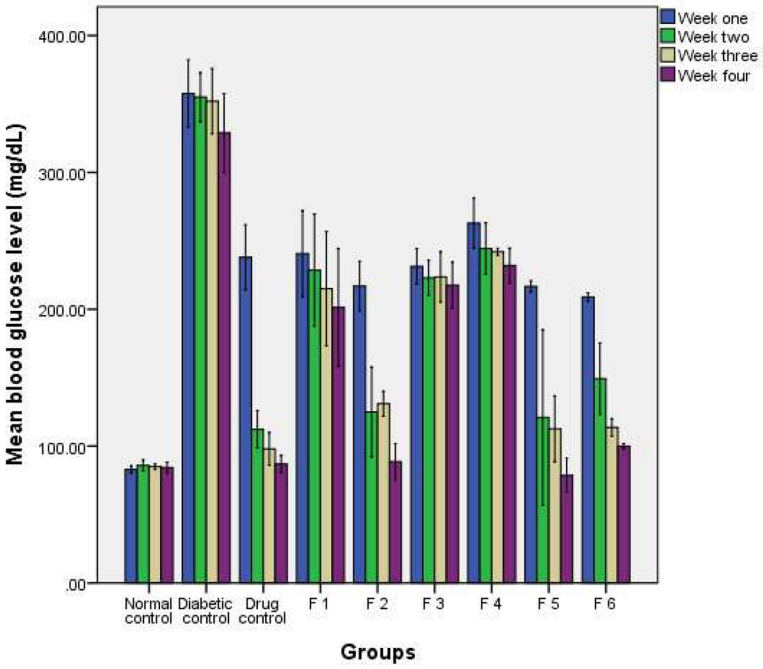

Figure 2. Fasting blood glucose concentration in streptozotocin-induced diabetic rats treated with fractions from *Napoleonae imperialis* leaves. Values are presented as mean ± standard deviation ($n = 6$).

Table 1. Serum HbAIc, AMPK, insulin, glucagon, cAMP, and GPCR levels of streptozotocin-induced diabetic rats administered with the crude extract from *Napoleonae imperialis* leaves

Groups	HbA1c (%)	AMPK (ng/mL)	Insulin (ng/mL)	Glucagon (pg/mL)	cAMP (pg/mL)	GPCR (pmol/L)
1	8.08 ± 0.18[d]	19.26 ± 0.45[a]	10.91 ± 0.03[a]	86.00 ± 1.22[bc]	83.80 ± 0.86[c]	10.90 ± 0.02[b]
2	11.01 ± 0.02[a]	11.53 ± 0.14[e]	8.38 ± 0.07[c]	114.80 ± 1.85[a]	120.60 ± 1.08[a]	8.54 ± 0.05[d]
3	8.97 ± 0.03[b]	16.34 ± 0.28[b]	10.73 ± 0.10[a]	89.20 ± 1.28[b]	86.00 ± 0.70[bc]	10.75 ± 0.06[c]
4	8.51 ± 0.17[c]	13.01 ± 0.47[ab]	10.33 ± 0.21[b]	89.20 ± 0.86[b]	87.40 ± 0.93[b]	10.50 ± 0.04[c]
5	8.87 ± 0.06[bc]	14.37 ± 0.34[c]	10.21 ± 0.07[b]	86.40 ± 1.12[bc]	85.60 ± 0.51[bc]	10.65 ± 0.04[c]
6	8.47 ± 0.14[c]	16.68 ± 0.44[b]	10.32 ± 0.20[b]	85.80 ± 0.86[bc]	84.00 ± 0.70[c]	10.79 ± 0.03[c]
7	7.27 ± 0.20[e]	19.82 ± 0.25[a]	10.12 ± 0.07[b]	84.00 ± 1.14[c]	81.40 ± 0.51[d]	11.01 ± 0.08[a]

Notes: Values are presented as mean ± standard deviation. Different lowercase letters indicate significant differences between treatments ($p < 0.05$).
Group 1: Normal control; Group 2: Diabetic control (disease control); Group 3: Drug control (5 mg/kg glibenclamide); Group 4: Diabetic rats + 250 mg/kg crude extract; Group 5: Diabetic rats + 500 mg/kg crude extract; Group 6: Diabetic rats + 1,000 mg/kg crude extract; Group 7: Normal rats + 500 mg/kg crude extract.
Abbreviations: AMPK: AMP-activated protein kinase; cAMP: Cyclic AMP; GPCR: G-protein-coupled receptor; HbA1c: Glycated hemoglobin.

Table 2. Effects of fractions from *Napoleonae imperialis* leaves on HbAIc, AMPK, insulin, glucagon, cAMP, and GPCRs in streptozotocin-induced diabetic rats

Groups	HbA1c (%)	AMPK (ng/mL)	Insulin (ng/mL)	Glucagon (pg/mL)	cAMP (pg/mL)	GPCR (pmol/L)
1	7.89 ± 0.05[b]	17.83 ± 0.09[b]	10.07 ± 0.05[c]	82.00 ± 2.00[cd]	83.00 ± 1.15[b]	10.86 ± 0.03[b]
2	10.58 ± 0.02[a]	11.80 ± 0.06[d]	8.37 ± 0.04[d]	112.67 ± 3.06[a]	117.00 ± 2.00[a]	8.82 ± 0.02[a]
3	7.49 ± 0.07[c]	16.84 ± 0.05[c]	10.75 ± 0.04[a]	87.00 ± 2.00[b]	86.00 ± 1.00[b]	10.69 ± 0.04[b]
5	7.61 ± 0.03[c]	17.98 ± 0.07[b]	10.40 ± 0.02[b]	85.00 ± 1.00[bc]	85.33 ± 1.53[b]	10.48 ± 0.02[b]
8	7.34 ± 0.05[c]	22.06 ± 0.06[a]	10.65 ± 0.03[a]	83.00 ± 0.58[cd]	83.67 ± 0.88[b]	10.82 ± 0.04[b]
9	7.10 ± 0.03[d]	22.29 ± 0.05[a]	10.86 ± 0.05[a]	81.00 ± 1.00[d]	83.00 ± 1.15[b]	10.94 ± 0.04[b]

Notes: Values are mean ± standard deviation. Different lowercase letters indicate significant differences between treatments ($p < 0.05$). This experiment focused only on the fractions (Fractions 2, 5, and 6) with the most antidiabetic effects. Therefore, further biochemical assay was carried out only on these fractions. Group 1: Normal control; Group 2: Diabetic control (disease control); Group 3: Drug control (5 mg/kg glibenclamide); Group 5: Diabetic rats + Fraction 2; Group 8: Diabetic rats + Fraction 5; Group 9: Diabetic rats + Fraction 6.
Abbreviations: AMPK: AMP-activated protein kinase; cAMP: Cyclic AMP; GPCR: G-protein-coupled receptor; HbA1c: Glycated hemoglobin.

4. Discussion

Streptozotocin is a broad-spectrum antibiotic used to treat diabetes. In STZ-diabetic animals, β-cells of the islets of Langerhans are damaged, leading to a decline in insulin synthesis and secretion. Insufficient insulin secretion by β-cells leads to hyperglycemia.[15,16]

A significant elevation in fasting blood glucose concentration was observed in STZ-induced diabetic rats compared with normal rats, suggesting pancreatic β-cell destruction (Figure 1). Administration of the crude extract of *N. imperialis* leaf at different concentrations and bioactive Fractions 2, 5, and 6 resulted in a significant reduction in blood glucose compared with the diabetic untreated control at the end of the 28-day study (Figures 1 and 2). These results may be attributed to the presence of bioactive compounds, such as flavonoids, which have been shown to enhance insulin production, and saponins, which increase hepatic glucokinase activity in an insulin-like manner.[17] Mba *et al.*[18] revealed that *N. imperialis* leaves contain phytochemicals, such as flavonoids, steroids, terpenoids, phenols, tannins, glycosides, alkaloids, and saponins, which may enhance insulin production, protect functional pancreatic β-cells from further damage, or promote the regeneration of STZ-damaged β-cells.

Elevated non-enzymatic glycosylation is one of the potential mechanisms associated with hyperglycemia and vascular complications of diabetes. Excess glucose in the blood binds to the amino-terminal valine of the β-chain of hemoglobin, forming HbA1c.[19] HbA1c concentration is an important marker of sustained glycemic control in individuals with diabetes mellitus.[20] The result showed a significant increase in HbA1c in diabetic control rats compared with all other experimental groups, indicating poor glycemic control. We showed that crude extracts and Fractions 2, 5, and 6 from *N. imperialis* leaves significantly reduced HbA1c. Decreased HbA1c levels in the treated groups could result from enhanced insulin secretion by regenerated pancreatic β-cells in diabetic rats, thereby improving glycemic regulation.[21,22] This study is consistent with several reports on the therapeutic effects of diverse antidiabetic plants on HbA1c levels in diabetic animals in short-term studies.[23-25]

AMP-activated protein kinase functions as an important metabolic regulator in both insulin-responsive tissues and tissues that respond to metabolic trauma by inhibiting the secretion of fatty acids and cholesterol, two energy-consuming pathways.[26] AMPK is regarded as an ameliorative pathway for the management of diabetes and dyslipidemia[27], and stimulation of AMPK enhances lipid and glucose breakdown.[28] AMPK is also a crucial component of cellular metabolism and has been implicated in numerous metabolic diseases, including type 2 diabetes.[29] Our study revealed that crude extracts and Fractions 2, 5, and 6 from *N. imperialis* leaves enhanced AMPK activity in diabetic animals, demonstrating the relationship between the insulin-dependent AMPK pathway and diabetes mellitus. The hypoglycemic effect of crude extracts and fractions from *N. imperialis* leaves might be related to the restoration of AMPK activity, a non-insulin-dependent pathway, and an insulin-stimulated transport protein. Enhanced glucose uptake by skeletal muscle and reduced hepatic glucose production contribute to a reduction in blood glucose concentration. Our result is consistent with previous studies reporting an increase in AMPK activity in STZ-induced diabetic mice after treatment with a triterpenoid compound[30] or curcumin.[31]

Insulin concentrations were lower in diabetic rats than in non-diabetic rats. This finding is consistent with other research using STZ to induce type 2 diabetes, which showed that STZ reduces insulin secretion by pancreatic β-cells.[19] Supplementation with crude extracts and Fractions 2, 5, and 6 from *N. imperialis* leaves increased plasma insulin concentration compared with the diabetic untreated animals, leading to increased glucose utilization. The increased insulin secretion may be attributed to insulinotropic compounds in *N. imperialis* leaves, which enhance insulin release, thereby reducing blood glucose levels and improving lipid metabolism. These compounds may also protect pancreatic β-cells from further damage and potentially promote the regeneration and restoration of the islets of Langerhans.[32]

Glucagon is a peptide hormone secreted by alpha cells of the pancreas. It increases glucose and fatty acid levels in the bloodstream and is considered the major catabolic hormone in the body.[33] Its action is the opposite of insulin's, which reduces extracellular glucose.[34] Glucagon usually increases serum glucose levels by stimulating gluconeogenesis and glycogenolysis.[33] In our study, glucagon levels were higher in diabetic rats than in non-diabetic rats. Administration of the crude extract or Fractions 2, 5, and 6 of *N. imperialis* leaves significantly reduced glucagon levels compared with those in the diabetic animals. This reduction may reflect suppressed glucagon secretion, leading to decreased hepatic glycogenolysis and improved glycemic control. The observed effects may be attributed to insulinotropic compounds present in *N. imperialis*.[35]

Cyclic AMP is an intracellular second messenger that is generally stimulated by the binding of hormones and neurotransmitters to GPCRs. cAMP elicits several of its biological actions by stimulating cAMP-dependent protein kinase A (PKA), which also phosphorylates and regulates

downstream protein targets, ion channels, enzymes, and transcription factors.[36] cAMP/PKA signaling controls glucose homeostasis at various stages, including insulin and glucagon production, glucose uptake, glycogen secretion and catabolism, gluconeogenesis, and neural regulation of glucose homeostasis. In our study, cAMP concentrations were higher in diabetic rats than in non-diabetic rats. Administration of the crude extract and Fractions 2, 5, and 6 of *N. imperialis* leaves reduced cAMP concentrations compared with those in diabetic animals. This may decrease glycogen catabolism. Meanwhile, cAMP secretion may be affected by the insulinotropic compounds present in *N. imperialis* leaves.[37]

Glucose homeostasis is a strictly controlled metabolic process that involves numerous physiologic mechanisms and multiple tissues and organs (e.g., the endocrine pancreas, liver, gut, muscles, and adipose tissue). Hormones and other extracellular signaling compounds contribute to the control and crosstalk among these processes, thereby maintaining balanced glycemic regulation. GPCRs contribute to several of these mechanisms, including stimulating insulin secretion. In our study, GPCR concentrations were lower in diabetic rats than in non-diabetic rats. This observation is consistent with previous findings using STZ to induce type 2 diabetes, which reported that STZ reduces GPCR concentrations.[38]

Supplementation with the crude extract and bioactive Fractions 2, 5, and 6 of *N. imperialis* leaves increased plasma GPCR concentration compared with diabetic animals, potentially increasing glucose utilization. The increased GPCR secretion may be attributed to the insulinotropic compounds present in *N. imperialis* leaves.[32]

5. Conclusion

Our study showed that subacute administration of the crude extract and Fractions 2, 5, and 6 of *N. imperialis* leaves exerted antidiabetic effects by reducing blood glucose concentrations in STZ-induced diabetic rats. Treatment with the crude extract and the active fractions significantly increased insulin levels and AMPK and GPCR activities while significantly decreasing glucagon and cAMP levels, thereby improving glucose homeostasis. These effects may enhance insulin activity, stimulate AMPK and GPCR signaling, and promote glucose uptake. The observed changes in AMPK, GPCR, insulin, glucagon, and cAMP levels are consistent with modulation of metabolic pathways associated with glucose homeostasis; however, direct mechanistic studies and chemical characterization of the bioactive constituents are required to confirm these relationships.

Acknowledgments

We sincerely appreciate the Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, for providing the facility for this study.

Funding

None.

Conflict of interest

The authors declare they have no competing interests.

Author contributions

Conceptualization: Obinna Joseph Mba
Investigation: All authors
Methodology: All authors
Writing–original draft: Obinna Joseph Mba
Writing–review & editing: Obinna Joseph Mba, Brian Onyebuchi Ogbonna

Ethics approval and consent to participate

The authors hereby declare that "Principles of laboratory animal care" (NIH Publication No. 85- 23, revised 1985) were followed in this study, as well as specific national laws, where applicable. All experiments were examined and approved by the appropriate ethical committee.

Consent for publication

Not applicable.

Availability of data

Data are available from the corresponding author on reasonable request.

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