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Investigation of insulin resistance and glycemic response to *Dennettia tripetala* essential oil in steptozocin-induced diabetic mice

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Abstract

Background: The growing influence of medicinal plants among individuals living with diabetes in developing nations is a major concern for safety, efficacy, and tolerability. This study investigated the median lethal dose (LD_{50}), antihyperglycemic effects, and potential mechanisms of action of the essential oil (EO) from the dried seeds of *Dennettia tripetala (D. tripetala)* in streptozocin (STZ)-induced diabetic mice.

Methods: The EO was obtained by hydrodistillation of pulverized dried seeds, and its LD50 was determined using Lorke's method, yielding an LD_{50} value of 2150 mg/kg (oral administration). Diabetes was induced in mice using low-dose STZ (40 mg/kg), and after 72 hours, animals with blood glucose levels ≥10 mmol/L were considered diabetic. The diabetic mice were divided into five groups: a negative diabetic control group, a positive control group treated with glibenclamide (5 mg/kg), and three groups treated with 50, 100, and 200 mg/kg EO, respectively, for 20 days. The negative nondiabetic control group received the vehicle. Body weight and blood glucose levels were monitored throughout the treatment, and insulin levels and insulin resistance (as measured by HOMA-IR) were assessed at the study's conclusion. Statistical analysis was performed using ANOVA with Dunnett's post hoc test (p<0.05).

Results: The results revealed that administration of the EO at doses of 50, 100, and 200 mg/kg led to dose-dependent increases in body weight and reductions in blood glucose levels. The most significant improvement in glycaemic control was observed at 100 mg/kg.

Although the insulin resistance index (HOMA-IR) of diabetic mice was higher than that of nondiabetic controls, EO treatment resulted in a significant reduction in insulin resistance across all doses, with the most notable effect at 50 mg/kg. This dose resulted in the lowest mean HOMA-IR value of 5.15 pg/mL, indicating a marked reduction in insulin resistance compared to the negative diabetic control group, though this difference did not reach statistical significance.

Conclusion: The essential oil of dried seeds of *D. tripetala* exhibited mild toxicity and modest antihyperglycemic effects in diabetic mice, primarily through the reduction of insulin resistance, highlighting its potential as an antidiabetic agent.

Keywords: Antihyperglycemic; *Dennetia tripetala;* diabetes; essential oil; insulin resistance.

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Background

Diabetes mellitus (DM) is a chronic metabolic condition caused by deficiencies in insulin synthesis, insulin action, or both [1]. It is characterized by chronic hyperglycemia and abnormalities in carbohydrate, fat, and protein metabolism. It often manifests in individuals with increased thirst, excessive urination and unintended weight loss [1-3]. The acute complications are mostly metabolic derangements including diabetic ketoacidosis, hyperosmolar hyperglycemic state and occasionally treatment-related lactic acidosis and hypoglycemia [1,3,4].

Perhaps, the chronic complications of diabetes are among the most devastating medical conditions and, in most cases, responsible for the huge costs associated with the disease. They include microvascular complications affecting the retinal, nephrons and neurons. The macrovascular complications affect the organ system, namely the brain (cerebrovascular disease), heart (coronary heart disease) and peripheral arterial disease [1,3-5]. The non-vascular complications, although intricately linked with vasculopathy, have other mechanisms implicated and are nonetheless potentially debilitating. They include lower extremity amputations, skin infections and erectile dysfunction among others [2,5,6].

Individuals with diabetes often require multiple pharmacologic agents to achieve optimal glycemic control and forestall the development of complications [7]. In Nigeria, nearly 70% of patients with type 2 diabetes mellitus are on dual therapy and about one-third are on insulin either as basal or pre-mixed [8]. These treatment regimens are often fraught with poor adherence owing to several factors, notably costs of medication, pill burden, socio-cultural and religious beliefs of patients. Given the huge economic burden of diabetes, it must be taken seriously not only by individuals living with, or at high risk of, the condition but also by healthcare professionals and policymakers. It remains a serious and growing challenge to public health and places a considerable burden on individuals affected and their families. It is considered one of the most expensive chronic diseases in the world. It was estimated that global expenditures on diabetes treatment and its complications were at least $966 million in 2021 and projected to be at least $1.054 trillion in 2045 [9]. With the rising cost of medical care occasioned by galloping inflation and economic crises on the heels of the COVID-19 pandemic, individuals living with diabetes continue to grapple with poor access to quality care, especially in developing nations. Healthcare financing in Nigeria remains predominantly out-of-pocket, with disparate access between the rich, middle- and low-income earners. Diabetes and diabetes-related end-organ damage connote a dismal outcome for patients in Nigeria, given that the advanced care is clearly out of reach of average citizens.

Consequently, many patients inevitably resort to medicinal herbs which are ubiquitous, cheap and familiar, notwithstanding concerns about safety, tolerability or efficacy. The growing influence of medicinal plants cannot be over-emphasized in developing nations of the world, given the cultural acceptability and economic challenges in these climes [10].

Dennettia tripetala G. Baker, commonly known as pepper fruit, is a tropical plant in the family Annonaceae, native to West and Central Africa. Traditionally, *D. tripetala* is used as a spice in medicinal preparations and cuisine; its small, reddish-brown fruits are known for their spicy, pungent taste, similar to black pepper [11]. The plant is believed to possess anti-inflammatory, analgesic, and antimicrobial properties [12,13]. Its essential oil has shown insecticidal and fungicidal activities [13,14].

Preclinical studies in our laboratory have also demonstrated multiple biological activities, including anti-inflammatory [14,15] and neuropharmacological properties [16-17] of the essential oil of *D. tripetala*. These findings, together with its recognized ethnomedicinal applications, provide a strong rationale for evaluating its potential antihyperglycaemic properties.

Given the increased use of complementary and alternative medicines among patients with chronic ailments in Nigeria and other resource-limited settings [18], investigating the potential health benefits of *D. tripetala* is critical.

While there has been little reported evidence of the anti-diabetic effect of the extract of *D. tripetala*, its essential oil has been scarcely investigated, and in particular, the mechanisms of the anti-hyperglycemic effect are yet to be fully elucidated.

The study, therefore, was designed to assess the antihyperglycemic effect and the possible mechanisms of the essential oil of the dried seed of *D. tripetala* on streptozocin-induced diabetic mice. Investigating the mechanisms through which the essential oil of *D. tripetala* may exert antihyperglycemic effects is essential for advancing our understanding of its therapeutic potential. Additionally, this study could provoke further exploration of other medicinal plants with similar properties, potentially leading to a broader understanding of plant-based interventions in diabetes management, particularly in settings where conventional medical resources are scarce and expensive [18-20].

Methods

Study location

The study was an experimental comparative study conducted at the Postgraduate Laboratory of the Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

Plant materials

The dried seeds of *D. tripetala* were purchased from a neighborhood market in Ondo city, Ondo West Local Government Area, Ondo State, Southwest Nigeria. Three purchases were made between November 2022 and February 2023. The seeds were reauthenticated by the Herbarium Officer of our institution. The voucher specimen of the dried seed was prepared and deposited at the Herbarium and compared with the previous reference number issued, IFE 15,356, from the Herbarium Unit of our institution.

Experimental animals

Healthy male Swiss mice (17-25 g) bred in the Animal House, Department of Pharmacology, Faculty of Pharmacy, OAU Ile-Ife, were used for both diabetic induction and subsequently for the administration of graded doses of essential oil of *D. tripetala*. The animals were housed in standard plastic cages and allowed access to a high-fat diet (Grand Cereals, United African Company Nigeria Plc) and water *ad libitum* for the initial two weeks to allow them to gain weight and thus promote the induction of diabetes. The procedure for animal care was based on the “Guide for the Care and Use of Laboratory Animals -Eighth Edition” [21] and the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs) guidelines on humane animal

care and use [22]. All experiments were performed according to the "Principles of Laboratory Animal Care" (NIH Publication No. 85; rev. 1985) and World Medical Association (WMA) Statement on Animal Use in Biomedical Research. Ethical approval for the study was obtained from the Health Research Ethics Committee (HREC) of the Institute of Public Health, Obafemi Awolowo University, Ile-Ife, Nigeria, with the approval number: HREC NO: IPH/OAU/12/2398.

Equipment and sundry consumables

Observation cages (25 cm by 25 cm by 30 cm), mortar and pestle, microtitre plate reader (SM 600 Shanghai, Yonchuang Medical instrument Co., Ltd), animal pellet feeds; procured at Livestock feed store, Sabo, Ile-Ife, Osun State, analytical weighing balance (7133781), centrifuge 80-2B (Zenith LAB, deep freezer, MAPADA W-1100D spectrophotometer, Clevenger-type apparatus, Glucometer (Accu-chek® instant by Roche), test strips, lancets, cotton wool, masking tape, indelible markers, hand towel, distilled water, oral cannula, 1ml needle and syringes, plain sample bottles, cotton wool, Gyostyle box and ice packs.

Drugs

Tablet Glibenclamide 5 mg (Daonil, Sanofi®)

Chemicals and reagents

The following chemicals were used: Streptozocin powder (Sigma, St. Louis, MO, USA), 5% Tween 80, distilled water, and 0.1 mL citrate buffer.

Preparation of essential oil of dried seed of D. tripetala

The essential oil of *D. tripetala* was obtained by hydro-distillation using the Clevenger-type apparatus at the Department of Biochemistry, OAU, Ile-Ife. The dried seeds of *D. tripetala* were pulverized into coarse particles using a mortar and pestle. The coarse particles were then stored in a dark bottle and used when required. One thousand five hundred grams of coarse particles were introduced into a 5 L round-bottom flask, and distilled water was added to about 2/3 of the flask and thereafter subjected to hydro-distillation for about 4 h. The oils obtained, measuring about 7.25 g of the characteristic pungent aromatic odor of the essential oil of *D. tripetala,* were stored in a light-proof amber-colored bottle and refrigerated until use. The oil was emulsified with 5% Tween 80 before administration. The density of the oil was determined and found to be 1070 mg/mL. An equivalent volume corresponding to the required weight was taken and emulsified with Tween 80 and then made up to obtain the desired concentration of the oil in mg/ml shortly before administration.

Preparation of experimental animals

Healthy male Swiss mice weighing 17-25 g were obtained from the Animal House of the Faculty of Pharmacy, OAU, Ile-Ife and maintained in well-aerated plastic cages where beddings were replaced each day, at a room temperature of about 25^0C. They were allowed to acclimatize for one (1) week prior to the commencement of experimentation. During this period, they were all provided with commercially available high-fat mice pellets and clean water *ad libitum*. All experiments were performed according to the "Principles of Laboratory Animal Care" (NIH Publication No. 85; rev. 1985).

Ethical approval

Ethical approval for the study was obtained from the Health Research Ethics Committee (HREC), Institute of Public Health, Obafemi Awolowo University, Ile-Ife, which was approved under HREC NO: IPH/OAU/12/2398.

Oral acute toxicity assessment

The oral median lethal dose (LD_{50}) of the essential oil of *D. tripetala* was determined in mice according to the method described by Lorke [23]. The study was done in two phases: In the first phase, nine mice were randomized into three groups of three mice, which were given the essential oil orally at doses of 10, 100 and 1000 mg/kg body weight. The mice were kept under conducive conditions and were observed for signs of acute toxicity, which included but were not limited to stretching, paw-licking, respiratory distress and mortality for 24 h. There was no mortality recorded in the first phase; hence, the test proceeded to the second phase. Three [3] other mice were administered 3 dose levels of 1600, 2900 and 5000 mg/kg, respectively. The animals were also monitored closely for 24 hours after treatment for signs of toxicity and/or mortality. The results obtained in the second phase were used to calculate the LD_{50}.
The LD_{50} was calculated as the geometric means of the maximum dose producing 0% mortality (a) and the minimum dose that produced 100% mortality (b), and mathematically expressed as:

$LD_{50} = \sqrt{ab} = \sqrt{(D_0 \times D_{100})}$ [23]

Induction of diabetes

A commercially available high-fat diet (HFD) (Table 1) was used to feed the mice for two (2) weeks to cause some degree of insulin resistance while allowing animals liberal access to water. On day fifteen (15), they were injected intraperitoneally (*i.p.*) with freshly prepared STZ (dissolved in a 0.1 mL citrate buffer, pH 4.5) at a single dose of 40 mg/kg b.w. The animals were allowed continued liberal access to feed and water.

Confirmation and verification of diabetes

Seventy-two hours after diabetic induction, the blood glucose levels of the mice were determined using an Accu-Check® instant glucometer and the corresponding test strip. A blood sample was obtained by a single prick on the tail tip of each mouse on the test strip by the capillary method, and the values obtained were read off within a few seconds for each mouse. Mice having blood glucose (BG) levels ≥ 10 mmol/L were considered hyperglycaemic.

Grouping of experimental animals

Animals with blood glucose ≥ 10 mmol/L after 72 h following STZ administration were considered diabetic. Animals were thereafter divided into six (6) groups as follows;
Group I (Negative Diabetic Control): administered with 5 mL/kg of 5% Tween 80 daily;
Group II (Positive Diabetic Control): administered with 5 mg/kg of Tab Glibenclamide daily;
Groups III, IV, and V (Diabetic): administered with graded doses of 50, 100 and 200 mg/kg of essential oil of *D. tripetala* daily;

Group VI (Negative Non-Diabetic Control): administered with 5 mL/kg of 5% Tween 80 daily;
The treatment was done for a total of 20 days.

Study procedure

Route of administration

The essential oil of *D. tripetala*, Glibenclamide and 5% Tween 80 were administered orally throughout the study.
Alternate-day body weight change
Animals in all groups were weighed on the day of diabetic induction and thereafter on alternate days during the period of treatment and on the day of sacrifice for changes in body weight and accurately recorded for each mouse.

Blood analysis

On the 20th day of the experiment, all the mice were humanely euthanized by gentle cervical dislocation and blood samples were collected by cardiac puncture. The blood was collected into plain bottles.
The samples were subsequently allowed to clot and centrifuged at 3500 rpm for 15 minutes. The sera were separated, stored at -30˚C and used for evaluation of insulin levels for each mouse.

Serum insulin

A double assay for serum insulin by a quantitative method with microplate enzyme-linked immunosorbent assay (ELISA) insulin kits supplied by Calbiotech Co., Ltd, USA was performed. The sensitivity of this assay is 0.75 µIU/mL, and the test has no cross-reactivity with C-peptide, pro-insulin and glucagon.

Insulin resistance (IR)

The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) was used to estimate the IR as obtained in the original formula described by Matthews *et al.* [24].

The equation is derived as follows:

$$\text{HOMA-IR} = \frac{\text{serum insulin (mIU/mL)}}{\text{X blood glucose (mmol/L)}}{22.5}$$

Serum insulin is the concentration of insulin in blood expressed as mIU/mL, and blood glucose is the concentration in the randomly collected blood specimen in mmol/L. The constant **22.5** is a normalizing factor derived from the product of normal fasting plasma insulin (5 µU/mL) and glucose (4.5 mmol/L) values in an "ideal" individual. This formula is widely used to estimate insulin resistance from a single fasting blood sample [24].

Statistical analysis

All data obtained were expressed as Mean ± SEM, and statistical differences between means were determined by One-way ANOVA. The level of significance was set at $p<0.05$.

Results

Acute toxicity study of EO of D. tripetala

In phase I of the experiment, no mortality was recorded in the 3 dose levels. In phase II, there was no mortality at 1600 mg/kg, but there was mortality at 2900 and 5000 mg/kg within 24 h. Thus, the maximum dose of the essential oil that did not cause mortality was 1600 mg/kg, and the lowest dose that caused mortality within 24 h was 2900 mg/kg ($LD_{50} = \sqrt{(D_0 \times D_{100})}$). The LD_{50} was found to be 2150 mg/kg (*p.o.*), similar to an earlier report [23].

Comparative effect of EO of D. tripetala on body weight of mice

The mean weight of mice in the diabetic group was 25.01±0.22 g, significantly higher ($p < 0.0001$) than the non-diabetic group with a mean weight of 21.53±0.63 g (Table 2). Within the diabetic groups, the mean body weights of animals are as follows: Negative Diabetic Control group, 26.20±0.41 g, Essential Oil 50 mg/kg (25.69±0.50 g, Essential Oil 100 mg/kg (26.78±0.48 g), Essential Oil 200 mg/kg (22.15±0.33 g) and Positive Diabetic Control (24.99±0.44 g). Figure 1 shows the comparative effects of EO on the body weight of mice at incremental doses as stated earlier. At 50 mg/kg, a significant steady increase in mean body weights of mice with increasing days of observation up to Day 20 of the study ($p = 0.034$) was observed. In particular, the initial mean body weight of mice recorded as 22.15±1.22 g on Day 0, increased to 26.17±1.19 g on Day 7 of the observation, accounting for an 18.2% increase ($p = 0.040$). This dose further effected a significant 21.9% weight gain in the mice on Day 15, with the mean body weights of mice at 27.02±1.18 g from the initial Day 0 ($p = 0.016$). Furthermore, at a dose of 100 mg/kg, a significant increase in the mean body weights of mice with increasing days of observation up till Day 7 of the study ($p = 0.001$) was also seen, with Day 9 recording the most significant percentage weight increase of 23.5% ($p = 0.001$). Similarly, a 200 mg/kg dose of EO also produced a significant increase in the mean body weights of mice with increasing days of observation up till Day 20 of the study ($p <0.0001$). In particular, the initial mean body weight of mice recorded on Day 0 was 18.64±0.43 g, showing that the essential oil of *D. tripetala* caused a significant 17.3% gain in body weight of mice (21.86±0.34 g) on Day 7 of the observation ($p = 0.022$). Also, a significant 32.6% gain in the body weight of mice was reported with the administration of the essential oil of *D. tripetala* on Day 20, yielding a mean body weight of 24.71±0.42 g ($p < 0.0001$).

Comparative effect of EO of D. tripetala on the glycaemic profile of mice

There was a notable disparity in the glycaemic profiles of mice with incremental doses of EO of *D. tripetala*. Expectedly, the mean blood glucose of mice in the diabetic groups exhibited a statistically significant higher mean (9.76±0.19 mmol/L) ($p < 0.0001$) compared to the mean blood glucose of mice in the non-diabetic group (7.76±0.22 mmol/L) (Table 3). Within the diabetic groups, the following mean blood glucose levels were recorded: Negative Diabetic Control group (12.31±0.51 mmol/L), Essential Oil 50 mg/kg (8.90±0.33 mmol/L), Essential Oil 100 mg/kg (8.71±0.49 mmol/L), Essential Oil 200 mg/kg (8.79±0.31mmol/L), and Positive Diabetic Control (10.41±0.271 mmol/L) (Tables 4 and 5).
Essential Oil of *D. tripetala* at a dose of 50 mg/kg showed a significant steady reduction in mean blood glucose of mice with

decreasing days of observation up till Day 20 of the study. At this dosage, a significant 31.6% ($p < 0.0001$) reduction in blood glucose was seen on Day 9. Although this pattern was altered on Day 12, it resumed thereafter with a significant 28.4% drop in blood glucose and the pattern continued till Day 20 (Figure 2). Similarly, at 100 mg/kg, a significant reduction in the mean blood glucose of mice was observed with increasing days of observation up till Day 20 of the study ($p < 0.0001$). Although the mean blood glucose (6.69±0.55 mmol/L) on Day 12 was slightly higher than the initial Day 0 (12.65±0.82 mmol/L), the pattern resumed with a significant 47.1% loss in blood glucose on Day 12 and a much more significant reduction of 50.7% by Day 20 (Figure 2). In the same vein, at a 200 mg/kg dosage, a significant reduction in the mean blood glucose of mice with increasing days of observation up to Day 20 of the study ($p < 0.0001$) was noted. The most profound effect was seen on Day 3 with a dip in mean blood glucose (1.60±0.54 mmol/L) amounting to an 86.1% reduction. The pattern resumed steadily on Day 7 (9.37±0.43 mmol/L) with a significant 18.7% reduction ($p = 0.020$) and continued till Day 20 (6.34±0.43 mmol/L) with a significant 45.0% reduction in blood glucose ($p < 0.0001$) (Figure 2).

Comparison of insulin levels in diabetic and non-diabetic mice

There was no statistically significant difference in insulin levels of the animals in the diabetic and non-diabetic groups. Expectedly, the mean insulin level of mice in the diabetic group was lower than the non-diabetic group with a mean insulin level of Table 6. Within the diabetic groups, the following mean insulin levels were recorded: Negative Diabetic Control group (26.95±2.68 mIU/mL), Essential Oil 50 mg/kg (16.47±2.31 mIU/mL), Essential Oil 100 mg/kg (23.78±2.27 mIU/mL), Essential Oil 200 mg/kg (20.68±3.32 mIU/mL), and Positive Diabetic Control (21.24±2.15 mIU/mL) (Figure 3).

Insulin resistance in diabetic and non-diabetic mice

There was no significant difference in the insulin resistance profile between animals in the diabetic and non-diabetic groups. Although the mice in the diabetic groups recorded a higher mean HOMA-IR value (8.23±0.83 pg/mL) compared to the non-diabetic group (7.78±0.78 pg/mL), there was no significant difference ($p = 0.760$) (Table 7). Within the diabetic groups, the mean insulin resistance levels obtained were: Negative Diabetic Control group (14.78±1.84 pg/mL), Essential Oil 50 mg/kg (5.15±1.07 pg/mL), Essential Oil 100 mg/kg (6.59±0.74 pg/mL), Essential Oil 200 mg/kg (5.63±0.88 pg/mL), and Positive Diabetic control 5 mg/kg (10.25±1.39 pg/mL) (Figure 4).

Comparison of effects of essential oil of D. tripetala on insulin levels in mice

In this study, there was no significant difference in insulin levels of mice in all groups, with animals treated with 50 mg/kg of essential oil recording the lowest mean Insulin levels of 16.47±2.31 mIU/mL. However, among the treated groups, 100 mg/kg dosage produced the highest mean Insulin level of 23.78±2.27 mIU/mL, followed by 200 mg/kg, which produced 20.67±3.32 mIU/mL (Table 8). Moreover, the results showed that the insulin levels of mice treated with 50 mg/kg and 200 mg/kg of the essential oil of *D. tripetala* were lower when compared to the Positive Diabetic Control group ($p > 0.05$).

Comparison of effects of essential oil of D. tripetala on insulin resistance in mice

Insulin resistance was found to be lowest in animals treated with 50 mg/kg dose. (mean HOMA IR = 5.15±1.07 pg/mL), followed by 200 mg/kg (mean HOMA IR = 5.63±0.88 pg/mL), and 100 mg/kg (mean HOMA-IR of 6.60±0.74 pg/mL) (Table 8). The results further showed a significantly lower insulin resistance of mice treated with 50mg/kg, 100 mg/kg and 200 mg/kg of essential oil of *D. tripetala* when compared to the Negative Diabetic Control group ($p < 0.0001$) and Positive Diabetic Control group ($p < 0.05$), with most notable effect at 50 mg/kg when compared with both negative and positive diabetic control groups.

Discussion

Following diabetic induction, the animals displayed classical symptoms of diabetes mellitus, except for weight loss. Observed symptoms included hyperglycemia, along with increased food and water consumption (polyphagia and polydipsia, respectively). These findings are consistent with previously reported typical symptoms of diabetes mellitus [2,3]. The observed polydipsia could be a result of excessive urination and dehydration brought about by plasma hyperosmolality and glucose levels exceeding the tubular maximum for glucose reabsorption, which is usually 10 mmol/L. The resultant osmotic gradient causes net inward movement of fluid into the renal tubule and polyuria. Polyphagia could result from the cells' inability to utilize glucose in the blood (starvation in abundance), therefore stimulating the hypothalamus to cause hunger [26,27]. Lifestyle, especially a high-fat diet, provides the greatest impetus to the development of obesity, especially visceral adiposity, which is a harbinger of insulin resistance and type 2 diabetes. In this study, diabetic mice recorded a higher weight than the non-diabetic mice. Although animals in both groups were fed with HFD, the development of diabetes appears to correlate with greater increases in weight observed in the diabetic group, thus suggesting a possible insulin-resistant state in the diabetic mice that probably resulted from fat accumulation and obesity. This observation was also seen in diabetic mice treated with Tween 80, reinforcing a strong correlation between insulin resistance and obesity, which has been variously described in humans and diabetic mouse models. Therefore, it may be suggested that induction of insulin-resistant-type diabetes, rather than treatment with the essential oil, led to the observed weight gain in the diabetic mice. This presupposition is supported by contrasting reports of the effect of essential oil on the weight of animals in previous studies. While Anoike et al. [28] reported a cumulative weight gain after a 10-day treatment period, Daniyan et al. [13] found a consistent weight reduction following repeated administration of the essential oil in rats. However, while the latter study was in normoglycemic animals, the former was in diabetic animals, and this may account for the observed difference in findings.

Given that weight management is crucial for glycemic control in diabetic patients, therapies that promote weight loss, such as GLIP-1 agonists and SGLT-2 inhibitors, are becoming integral to managing both obesity and type 2 diabetes [29-31]. Thus, the observed weight gain in diabetic mice may be primarily attributed to insulin resistance rather than the treatment with essential oils.

The study also investigated the glucose-lowering potential of variable doses of the essential oil of dried seed of *D. tripetala* in streptozocin-induced diabetic mice in comparison with a known oral glucose-lowering agent, Tab Glibenclamide. We also determined the insulin levels as well as insulin resistance of

diabetic mice following a 20-day treatment with graded doses of the essential oil of dried seed of *D. tripetala*. Results obtained in this study showed that the essential oil of dried seed of *D. tripetala* produced a significant reduction in blood glucose level in STZ-induced diabetic mice in a dose-dependent manner and a possible cumulative effect, given that the most notable reductions occurred in the latter days of the experimentation, especially at a dose of 100 mg/kg. This is in agreement with the findings of Anoike *et al.* [28] and Abonyi *et al.* [32]. However, the studies differ slightly in the choice of animal and plant materials used. While the latter used mice and crude methanol extract from the fresh leaves, the former employed rats and dried seeds of the plant. Notwithstanding, the anti-hyperglycemic effect of *D. tripetala* has been attributed to the presence of heterogeneous phytoconstituents such as alkaloids, saponins, tannins, and flavonoids, which are present in the leaves, fruits, and seed extracts of the plant. In a more recent study by Imo *et al.* [33], administration of a high dose of the dried seed extract (100 mg/kg) rather than fruit extract for a short period was found to produce a glucose-lowering effect in normoglycemic rats. In particular, the major chemical constituents of the essential oil of the dried seed, notably sesquiterpenes and 1-nitro-2-phenylethane, may have contributed singly or synergistically to the observed antihyperglycemic effect of the dried seed extract and essential oil in mice [11, 12, 16,34].

This study did not perform phytochemical analysis of the EO of the dried seeds of *tripetala,* thus precluding categorical inferences on the specific constituents responsible for the observed antihyperglycemic effect and lowering insulin resistance. However, the proven glucose-lowering potential in insulin-resistant diabetic mice strongly suggests the presence of such constituents. As our understanding of the pathophysiology of diabetes has improved, further studies are recommended to advance our knowledge of phytotherapeutics.

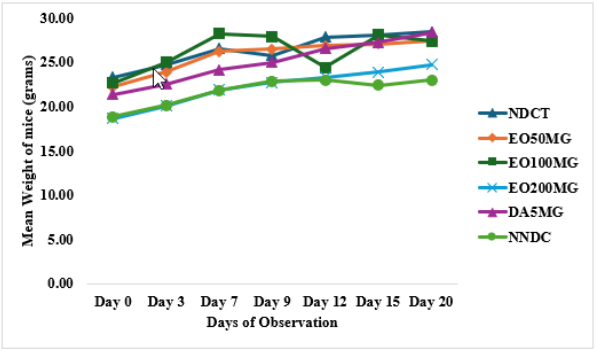

Figure 1. Comparative Effects of essential oil of *D. tripetala* on body weights of mice.
NDCT: Negative Diabetic Control, Tween 80; EO50MG: Essential oil 50 mg/kg; EO100MG: Essential oil 100 mg/kg; EO200MG: Essential oil 200 mg/kg; DA5MG: Glibenclamide 5mg/kg; NNDC: Negative Non-diabetic Control; *p < 0.05

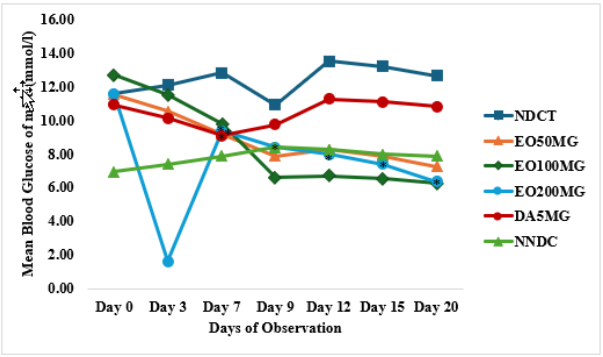

Figure 2. Effects of essential oil of *D. tripetala* on blood glucose of mice.
NDCT: Negative Diabetic Control Tween 80; EO50MG: Essential oil 50 mg/kg; EO100MG: Essential oil 100 mg/kg; EO200MG: Essential oil 200 mg/kg; DA5MG: Glibenclamide 5mg/kg; NNDC: Negative Non-diabetic Control; *p < 0.05

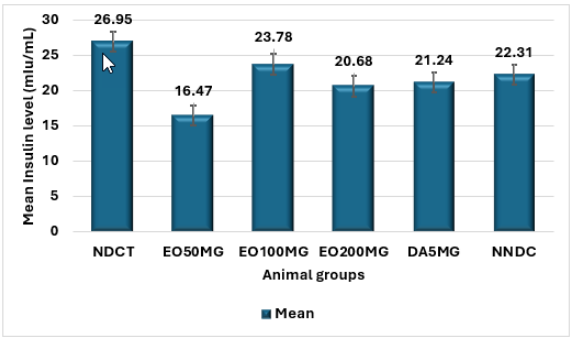

Figure 3. Summary statistics of insulin levels profile of mice.
NDCT: Negative Diabetic Control Tween 80; EO50MG: Essential oil 50 mg/kg; EO100MG: Essential oil 100 mg/kg; EO200MG: Essential oil 200 mg/kg; DA5MG: Positive Diabetic Contol (Glibenclamide 5 mg/kg); NNDC: Negative Non-diabetic Control

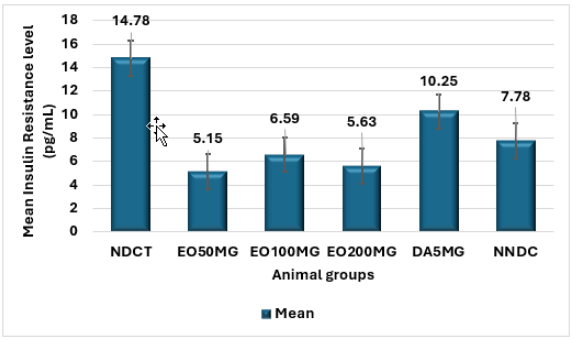

Figure 4. Summary statistics of HOMA-IR levels profile of mice.
NDCT: Negative Diabetic Control Tween 80; EO50MG: Essential oil 50 mg/kg; EO100MG: Essential oil 100 mg/kg; EO200MG: Essential oil 200 mg/kg; DA5MG: Glibenclamide 5mg/kg; NNDC: Negative Non-diabetic Control

Table 1. Composition of the controlled and high-fat diet for experimental animals.

Diet components	Control diet	High-fat diet (g)
Energy (Kcal/g)	3.00	6.4
Calorie percentage:		
• Carbohydrate	60	30
• Fat	15	65
• Protein	25	5
Weight Percentage		
• Carbohydrate	10	40
• Fat	25	40
• Protein	60	15
Materials	Standard chow diet	Maize (2.75), Wheat offal (0.25) Groundnut cake (6.75), Soya meal (10.25) Palm kernel (6.5) cake oil, Bone meal (0.25), Methionine (0.125), Lysine (0.125), Salt (0.03125), Finisher premix (0.03125), Cluppled (0.25)

Table 2. Weight profile of mice showing significant differences in body weights between the diabetic and non-diabetic groups.

	Negative Diabetic Control Tween 80	Essential oil (mg/kg)			Glibenclamide 5mg/kg	Negative Non-diabetic Control
		50	100	200		
Minimum	19.50	18.60	20.20	17.00	19.50	15.00
Maximum	30.00	32.00	32.00	26.00	31.00	26.00
Range	10.50	13.40	11.80	9.00	11.50	11.00
Mean	26.20	25.69	26.69	22.15	24.99	21.53
SEM	0.41	0.50	0.48	0.33	0.44	0.63
Diabetic Group mean±SEM	25.01±0.22					
Non-Diabetic Group mean±SEM	21.53±0.63					

Table 3. Summary statistics of the glycemic profile of mice.

	Negative Diabetic Control Tween 80	Essential oil (mg/kg)			Glibenclamide 5mg/kg	Negative Non-diabetic Control
		50	100	200		
Minimum	1.89	2.33	1.61	4.67	6.22	4.22
Maximum	16.61	13.44	16.17	14.11	14.00	10.33
Range	17.72	11.11	14.56	9.44	7.78	6.11
Mean	12.31	8.90	8.71	8.79	10.41	7.76
Std. Error of Mean	0.51	0.33	0.49	0.31	0.27	0.22
Diabetic Group mean±SEM	9.768±0.19					
Non-Diabetic Group mean±SEM	7.76±1.20					

Table 4. Effects of Essential oil of *D. tripetala* on blood glucose of mice.

	Negative Diabetic Control Tween 80			Essential oil 50mg/kg			Essential oil 100mg/kg		
	(Mean±SEM)	% Effect Change		(Mean±SEM)	% Effect Change		(Mean±SEM)	% Effect Change	
Day 0	11.54±0.39	-	-	11.49±0.52	-	-	12.65±0.82	-	-
Day 3	12.07±0.65	4.59	0.498	10.50±0.62	-8.62	0.250	11.47±0.69	-9.33	0.273
Day 7	12.79±1.01	10.8	0.275	9.13±0.90	-20.5	**0.046**	9.75±1.05	-22.9	0.054
Day 9	10.89±2.18	-5.63	0.775	7.86±0.15	-31.6	**<0.0001**	6.56±1.13	-47.4	**0.001**
Day 12	13.48±1.67	16.8	0.247	8.23±0.38	-28.4	**<0.0001**	6.69±0.55	-47.1	**<0.0001**
Day 15	13.16±1.65	14.0	0.325	7.85±0.55	-31.7	**0.001**	6.52±0.37	-48.5	**<0.0001**
Day 20	12.63±1.70	9.45	0.509	7.22±1.11	-37.2	**0.006**	6.24±0.32	-50.7	**<0.0001**

Table 5. Effects of Essential oil of *D. tripetala* on blood glucose of mice.

	Essential oil 200mg/kg			**Glibenclamide 5mg/kg**			**Negative Non-diabetic Control**		
	(Mean±SEM)	**% Effect Change**		**(Mean±SEM)**	**% Effect Change**		**(Mean±SEM)**	**% Effect Change**	
Day 0	11.53±0.69	-		10.88±0.31	-	-	6.93±0.54	-	-
Day 3	1.60±0.54	-86.1	0.308	10.10±0.30	-7.17	0.099	7.36±0.29	6.21	0.506
Day 7	9.37±0.43	-18.7	**0.020**	9.05±0.40	-16.8	**0.004**	7.86±0.27	13.4	0.168
Day 9	8.36±0.58	-27.5	**0.004**	9.72±1.13	-10.7	0.347	8.36±1.10	20.6	0.280
Day 12	7.96±0.48	-30.9	**0.001**	11.25±0.87	3.40	0.695	8.24±0.29	18.9	0.092
Day 15	7.35±0.44	-36.3	**<0.0001**	11.07±0.60	1.75	0.779	7.94±0.18	14.6	0.218
Day 20	6.34±0.43	-45.0	**<0.0001**	10.79±0.75	-0.83	0.911	7.83±0.17	13.0	0.266

Table 6. Insulin levels of mice showing significant difference between the diabetic and non-diabetic groups.

	Diabetic group	**N**	**Mean**	**Std. Dev.**	**SEM**	**Statistic**	**P-value**
Insulin	Diabetic group	29	21.54	6.91	1.28	Mann-Whitney U	0.903
	Non-diabetic group	3	22.31	3.53	2.04		

Table 7. Insulin resistance levels of mice showing significant difference between the diabetic and non-diabetic groups.

	Diabetic group	**N**	**Mean**	**Std. Dev.**	**SEM**	**Statistic**	**P-value**
Insulin Resistance	Diabetic group	29	8.23	4.47	0.83	Mann-Whitney U	0.760
	Non-diabetic group	3	7.78	1.34	0.78		

Table 8. Effects of Essential oil of *D. tripetala* on insulin levels and HOMA-IR of mice.

		N	**Mean**	**SEM**	**Statistic**	**P-value**
Insulin	Negative Diabetic Control Tween 80	5	26.94	2.68	*1.674*	*0.176*
	Essential Oil 50 mg/kg*	6	16.47	2.31		
	Essential Oil 100 mg/kg	5	23.78	2.27		
	Essential Oil 200 mg/kg	7	20.67	3.32		
	Glibenclamide 5 mg/kg	6	21.24	2.15		
	Negative non-Diabetic Control	3	22.31	2.04		
	Total	**32**	**21.613**	**1.173**		
HOMA IR	Negative Diabetic Control Tween 80	5	14.78	1.84	*8.980*	***<0.0001***
	Essential Oil 50 mg/kg	6	5.15	1.07		
	Essential Oil 100 mg/kg	5	6.60	0.74		
	Essential Oil 200 mg/kg	7	5.63	0.88		
	Positive Diabetic Control (Glibenclamide 5 mg/kg)	6	10.25	1.39		
	Negative non-Diabetic Control	3	7.78	0.78		
	Total	**32**	**8.189**	**0.754**		

Conclusion

In conclusion, the administration of *D. tripetala* EO to diabetic mice produced multifaceted effects on body weight and blood glucose regulation. The study demonstrated that diabetes induction and potentially the EO treatment resulted in dose-dependent weight gain in HFD-fed mice. Additionally, the EO exhibited a significant antihyperglycemic effect at a 100 mg/kg dose and a notable reduction in insulin resistance at a 50 mg/kg dose, highlighting its potential therapeutic impact in mitigating insulin resistance.

These findings emphasize the complex and pronounce effects of *D. tripetala* essential oil on metabolic parameters in diabetic mice, suggesting its potential as a promising therapeutic agent for diabetes management. Further investigations are warranted to explore its efficacy and additional underlying mechanisms of action.

Abbreviations

ADA: American Diabetes Association
BG: Blood Glucose
COVID-19: Coronavirus Disease 2019
D. tripetala: *Dennettia tripetala*
DM: Diabetes Mellitus
DNA: Deoxyribonucleic acid
DPP-IV: Dipeptidyl peptidase-IV
ELISA: Enzyme-linked immunosorbent assay
EO: Essential oil
FBG: Fasting Blood Glucose
GLP: Glucagon-like peptide
GM: Grams
HbA1C: Glycated hemoglobin
HDL: High-density lipoprotein
HFD: High-fat diet
HREC: Health Research Ethics Committee
HOMA-IR: Homeostasis Model Assessment of Insulin Resistance
OHA: Oral hypoglycemic agent
STZ: Streptozocin

T2DM: Type 2 diabetes mellitus
WHO: World Health Organization
RPG: Random Plasma Glucose

Authors' Contribution

TAA and IAO contributed to the concept, biochemical analysis, manuscript writing, data collation and interpretation; AKA and UFA contributed to the biochemical analysis, data collation, interpretation and editing of the manuscript. All authors read and approved of the final manuscript.

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Conflict of interest

The authors declare no conflict of interest

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