



Evaluation of the Anti-diabetic Properties of Clove Essential Oil

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ABSTRACT

The study evaluated the anti-diabetic properties of clove essential oil on streptozotocin-induced diabetic rats. Clove (*Syzygium aromaticum*) is considered as one of the most valuable spices. It is mainly processed into clove oil. It has wide uses in medicine due to its antioxidant, antimicrobial, antinociceptive, antiviral, and anaesthetic properties which are attributed to the presence of eugenol its major constituent. Diabetes was induced in rats by intraperitoneal injection of streptozotocin (60 mg/kg). After induction of diabetes, rats were treated with clove essential oil (100, 200 and 250 mg/kg) and metformin (100 mg/kg) for 14 days. Blood samples were collected and analysed for glucose. Administration of clove essential oil caused significant reduction from 255.05±3.18 to 152.00±5.57 for metformin while 154.00±2.08, 133.00±1.00 and 128.33±2.08 for essential oil (100 mg/kg, 200 mg/kg and 250 mg/kg) respectively. These results indicated that essential oil showed significant anti-diabetic effects in streptozotocin-induced diabetes rats.

Introduction

Clove (*Syzygium aromaticum*) stands out as one of the most prized spices, primarily processed into clove oil, cherished for its numerous medicinal benefits. Its richness in eugenol, a major constituent, contributes to its antioxidant, antimicrobial, antinociceptive, antiviral, and anesthetic properties [1]. Hydro distillation is the most common methods for extracting essential oil from plant materials. Hydro distillation involves heating the plant material

to just below water's boiling point to decompose its outer structure, which subsequently will release the molecules contained by the plants [2]. Another common method is maceration, which involves dissolving plant materials and deriving the essential oil via evaporation [3]. The Soxhlet extraction method is also considered as a reliable method. It is based on fat extraction using organic solvent that is heated, volatilized and condensed above the samples [4].

Clove oil has been widely used as spice and is well known for its medicinal properties. It is active against oral bacteria and fungi [5]. Previous studies also have reported antifungal [6;7], antioxidant [8], antibacterial [9], and anti-inflammatory [5;10] properties of clove oil. The discovery of type 2 diabetes (T2DM) is common in ambulatory medicine; this epidemic is linked to a reduced life expectancy, lack of physical activity, obesity, and

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due to an unbalanced diet particularly food rich in fats and refined sugars [11]. According to Grimaldi et al. [12] the disease has a significant genetic and frequently associated predisposition to obesity, hypertension and dyslipidemia.

The prevalence of type 2 diabetes (T2DM) is a significant concern, often linked to factors such as obesity, sedentary lifestyle, and poor diet. T2DM is characterized by chronic hyperglycemia and some complex physiological factors such as insulin resistance, inadequate insulin secretion, and dysregulation of glucagon and incretin hormones [13].

Clove oil has shown promise in modulating physiological responses in diabetic rats [14], while spice mixtures have improved glucose metabolism and lipid profiles in experimental models [15]. The reduced plasma glucose and insulin levels, together with the favourable lipid profile, were possibly brought about through improved insulin-sensitising actions of the active constituents. The spices improved the biomarkers of oxidative stress in the tissues of fructose-fed insulin-resistant rats [16]. Culinary, herb and spice extracts also inhibited protein glycation in vitro and the most potent inhibitors were extracts of cloves, ground Jamaican allspice, and cinnamon [17]. Insulin-like biological activity of clove and other culinary or medicinal plant aqueous extracts were also proven in vitro [18].

Shukri et al. [19] show that dietary supplementation with cloves significantly reduced blood sugar increases and lipid peroxidation in diabetic rats and restored the antioxidant enzyme levels. Dietary cloves also reduced tissue injuries in the lens, cardiac muscles, and livers of these rats. Clove oil was emphasized to adjust physiological responses in streptozotocin-induced diabetic rats.

The administration of clove essential oil (*Syzygium aromaticum*) will significantly reduce blood glucose levels in STZ-induced diabetic rats due to its antioxidant properties and potential insulin-sensitizing effects, thereby demonstrating its efficacy as a therapeutic agent for the management of diabetes mellitus.

Materials and Method

Plant Material

Dried flower bud of clove plant was purchased from Wukari market.

Preparation of Plant Material

In the Soxhlet extraction method [20], dried flower buds of clove are first ground into a coarse powder using a grinder. The powdered clove is then placed in a filter paper thimble, which is inserted into the Soxhlet extractor. The Soxhlet extractor is attached to a round-bottom flask containing n-hexane, and a condenser is connected to the extractor. The extraction process begins by heating the round-bottom flask with a heating mantle or water bath. As the solvent (n-hexane) evaporates, it passes through the Soxhlet extractor and condenses in the condenser. The condensed solvent drips onto the plant material, dissolving the essential oils. When the Soxhlet chamber is full, the solvent is siphoned back into the boiling flask. This cycle is repeated several times, typically for a duration of 6 to 24 hours, until the extraction is complete. Once the extraction is finished, the solvent in the round-bottom flask, now containing the dissolved essential oil, is subjected to evaporation using a rotary evaporator to obtain the essential oil. The collected essential oil is then stored in an airtight container and refrigerated to prevent oxidation [4].

Experimental Animals

25 Male rats of Wistar strain weighing between 100 and 150 g were procured from National Veterinary Research Institute (NVRI) Vom and allowed to acclimatize for a week before commencement of treatments. Animals were fed with commercial rat chows and given portable water ad libitum.

Chemical and Reference Drug

All chemicals to be used in this research are of analytical grade and was purchased from commercial market. Metformin was used as reference drug and was obtained from a pharmacy shop in Wukari, Taraba State, Nigeria. The drug is an anti-diabetic drug and helps to lower the level of diabetes level in a diabetic patient.

Experimental Protocol

25 albino rats were randomly grouped into five groups, this include normal group, positive control group while three groups for extracts dosage, except the normal all the groups were induced with diabetes, the animals was starved 24 hours before the commencement of the experiment [31]. Diabetes level was checked before, after induction of diabetes and also after administration for two weeks.

Group 1: Normal control (diet/water)

Group 2: Rats (induced diabetes 25mg/kg/bwt +diet/water + Metformin)

Group 3: Rats (induced diabetes 25mg/kg/bwt +diet /water +100mg/kg/bwt extracts)

Group 4: Rats (induced diabetes 25mg/kg/bwt +diet /water +200mg/kg/bwt extracts)

Group 5: Rats (induced diabetes 25mg/kg/bwt +diet /water +250mg/kg/bwt extracts)

Determination of Diabetic Activities

After 2 weeks of the administration, the rats were sacrificed based on National Research Council Guide for the care of laboratory animal (2011) and blood samples was collected to check for the level of diabetes. In the glucose estimation method, blood samples are collected from the rats through cardiac puncture or tail vein puncture and then centrifuged to separate the serum or plasma. The reagents are prepared according to the instructions provided in a glucose assay kit, commonly based on the glucose oxidase-peroxidase (GOD-POD) method [21].

A specific volume of serum or plasma, typically 15 μ L, is pipetted into test tubes, to which the glucose reagent (approximately 1 mL) is added. The contents are mixed thoroughly and incubated at 37°C for 10-15 minutes. Following incubation, the absorbance of the samples is measured at a specific wavelength, usually 505 nm, using a spectrophotometer. A standard curve is prepared using known concentrations of glucose to determine the glucose concentration in the samples. The glucose concentration in the blood samples is then calculated using the standard curve and expressed in mg/dL or mmol/L [22].

Data Analysis

Data were analysis using descriptive statistical analysis.

Results and Discussion

The research conducted by Abel-Salam [23] highlighted that diabetes mellitus is characterized by impaired carbohydrate, fat, and protein metabolism due to insufficient insulin secretion or decreased tissue sensitivity to insulin. According to Nangle et al. [14] and Zari and Al-Attar [24], clove has been reported to modulate physiological responses in diabetic rats, leading to a reduction in glucose levels, attributed to the antioxidant and hypoglycemic effects of clove essential oil components, particularly eugenol and eugenyl acetate [25]. These compounds facilitate glucose

utilization through pathways independent of pancreatic function [26]

In this study, diabetes was induced in rats using a 60 mg/kg dose of STZ, a compound known to selectively destroy pancreatic islet β -cells, leading to hyperglycemia and glycosuria, mimicking type 1 diabetes. Metformin hydrochloride, a commonly used treatment for diabetes, promotes glucose uptake by GLUT4 transporters and reduces glucose production in the liver, with potential antioxidant properties [27].

The increase in blood glucose levels observed may be attributed to insulin deficiency resulting from STZ-induced pancreatic damage. STZ promotes ATP dephosphorylation, leading to the generation of reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, and hydroxyl radicals, which damage pancreatic islets [28;29]. Antioxidants are essential under hyperglycemic conditions to repair damaged extracellular matrix proteins and promote cell growth [30].

Administration of STZ (60 mg/kg) to the diabetic control group resulted in elevated blood glucose levels compared to the normal control group. However, all groups treated with clove essential oil exhibited significant reductions in blood glucose levels, potentially due to β -cell regeneration.

Table 4.4 presents the effect of clove essential oil on blood glucose levels. Treatment with clove essential oil led to a significant ($P < 0.05$) decrease in glucose levels compared to the positive control group, consistent with findings by Naeem et al. [31]. The greatest reduction in glucose levels was observed in groups treated with 250 mg/kg of clove essential oil. Animals treated with 100 mg/kg of standard diabetic drugs showed a lower reduction compared to those treated with 100 mg/kg of clove essential oil, indicating a dose-dependent effect of clove essential oil on glucose levels.

Conclusion

This study together with existing reports demonstrates that the administration of cloves (*Syzygium aromaticum*) essential oil has antidiabetic effect. Therefore, high concentration of clove essential oil could be an excellent adjuvant support in the therapy of diabetes mellitus and its complications. On the basis of this results, clove essential oil showed significant anti-diabetic effect on STZ-induced diabetic rats due to its antioxidant activity. The present study indicated the usefulness of clove essential for further development as a therapeutic agent for treatment of diabetes. Moreover, further study is required to illustrate the

Table 1. Animal Grouping and Administration.

S/N	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
1	135	260	259	254	258
2	115	251	257	253	260
3	121	255	252	253	254

Table 2. Blood Glucose level before the induction of Diabetes.

S/N	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
1	135	127	88	84	114
2	121	130	129	100	117
3	115	110	131	113	100
4	106	99	116	123	99
5	125	107	114	101	105

Table 3. Blood Glucose level after the induction of Diabetes.

S/N	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
1	135	251	257	253	258
2	121	260	252	254	260
3	115	255	259	254	253
4	106	277	265	274	286
5	125	281	275	267	262

Table 4. Lowering Ability of the Extract.

Experimental	14days
Normal control	128.00±3.00
Induced Diabetes level	255.05±3.18
Standard treatment (Meformin)	152.00±5.57
Treatment group 1	154.00±2.08
Treatment group 2	133.00±1.00
Treatment group 3	128.33±2.08

Values are expressed as mean±SEM. Number of animals in each group was 3. Data was compared with diabetic control.

mechanism of action of clove essential oil at the cellular and molecular level.

Contribution of authors

Solomon Samuel Ruyati: Conceptualization, Writing, Original Draft Preparation. Sunny S. Ruyati: Methodology, Collection, Analysis, Writing, Review & Editing

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Conflict of Interest

The authors declare no conflict of interest.

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