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Clove Extract Reverses Hematological Deficits, Hormonal Imbalances and Dyslipidemia in Streptozotocin-Diabetic Rats

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Abstract

This research explores the therapeutic potential of a water-soluble extract from clove (*Syzygium aromaticum*) to alleviate multiple diabetic complications in a rat model. Materials and Methods: Over 21 days, we administered clove extract (500 mg/kg/day, orally) to a group of streptozotocin-induced diabetic male Wistar rats, comparing them to untreated diabetic and healthy control groups (n=7 per group). We evaluated the complete blood count (CBC), serum concentrations of reproductive hormones (LH, FSH, testosterone), and key metabolic indicators such as blood glucose and lipid profiles. Results: Clove extract treatment prompted a significant recuperation of erythrocyte counts, hemoglobin, hematocrit and platelet levels. Furthermore, it markedly increased the circulating levels of luteinizing hormone, follicle-stimulating hormone, and testosterone ($p < 0.05$). We also observed a pronounced (approx. 30%) reduction in blood glucose and a reversal of dyslipidemia, evidenced by decreased triglycerides, total cholesterol, and LDL, and an elevation in HDL by approximately 38%. Conclusion: The outcomes demonstrate that clove extract provides multi-faceted protection against diabetes-induced pathologies through the hematological, endocrine, and metabolic systems, an effect likely driven by its antioxidant and hypoglycemic properties. The exact molecular pathways involved require further exploration.

Keywords: clove, diabetes, streptozotocin, hematology, reproductive hormones, lipid profile, antioxidant

1. Introduction

The global burden of diabetes mellitus, a chronic condition marked by persistent hyperglycemia due to insulin deficiency or resistance, continues to grow. Beyond impaired glucose homeostasis, diabetes establishes a systemic environment of elevated oxidative stress and inflammation. This milieu is a key driver of secondary complications, including dyslipidemia, hormonal axis disruption, and damage to cardiovascular, renal, and neurological systems, often extending to reproductive dysfunction (American Diabetes Association, 2023; Boles et al., 2022).

While conventional pharmaceuticals are a cornerstone of diabetes management, their long-term use can be limited by side effects and cost. This has accelerated the investigation of natural products, particularly medicinal plants, which offer a reservoir of bioactive compounds with historically recognized efficacy and safety (Gupta et al., 2021).

Clove (Syzygium aromaticum) is a common culinary spice rich in phytochemicals like eugenol, flavonoids, and phenolic acids. These compounds are credited with potent antioxidant, anti-inflammatory, and glucose-regulating activities (Duke et al., 2021; Kumar et al., 2021). Specifically, eugenol has been implicated in enhancing insulin sensitivity and modulating metabolic pathways related to glucose (Manjamalai et al., 2012; Bhardwaj et al., 2023). Research also indicates clove's beneficial influence on lipid metabolism (Lima et al., 2019) and its potential to shield various tissues, including hematopoietic and endocrine organs, from oxidative damage (Gao et al., 2022; Ahmed et al., 2018). Given this background, our objective was to determine whether a water-based clove extract could restore hematological indices (red and white blood cell counts, hemoglobin, hematocrit, platelet count), reproductive hormone balance (LH, FSH, testosterone), and metabolic profiles (blood glucose, triglycerides, total cholesterol, LDL, HDL) in an experimental model of streptozotocin-induced diabetes.

2. Materials and Methods

2.1. Ethical Statement and Animal Model

All procedures involving the twenty-one adult male Wistar rats in this study were conducted under controlled laboratory conditions and received full approval from the institutional ethics board at Umm Al-Qura University (Approval No: HAPO-02-K-012-2024-I2-123), in strict accordance with international animal welfare standards.

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2.2. Diabetes Induction and Study Groups

To induce diabetes, rats were fasted and then received a single intraperitoneal injection of streptozotocin (STZ) at 50 mg/kg. Seventy two hours post-injection, animals with confirmed hyperglycemia (blood glucose >250 mg/dL) were randomized into three experimental cohorts (n=7 each):

Group I (Control): Non-diabetic, healthy rats receiving no intervention.

Group II (Diabetic Control): Diabetic rats that remained untreated.

Group III (Treatment): Diabetic rats receiving daily oral clove extract (500 mg/kg) for 21 consecutive days.

2.3. Water Extraction of Cloves

Authenticated dried clove buds (*Syzygium aromaticum*) were sourced locally. A 500-gram batch was mechanically pulverized into a fine powder. This powder was then infused in 2 liters of distilled water within a glass vessel, heated to a temperature range of 70–80°C for 50 minutes with constant agitation. After cooling to ambient temperature (~25°C), the solution was filtered through a double layer of cheesecloth (approx. pore size = 150-200 µm). The clarified filtrate was stored in sterile amber glass bottles at 4°C to ensure chemical stability and prevent microbial contamination. To guarantee consistent bioactivity, a fresh batch of extract was prepared weekly for the duration of the study.

2.4. Biochemical and Hematological Sampling

At the conclusion of the 21-day treatment, all the rats were anesthetized for terminal blood collection via cardiac puncture. Blood for hematology was anti-coagulated with EDTA, while samples for serum biochemistry were collected in plain tubes, allowed to clot and centrifuged at 3,000 rpm for 15 minutes to harvest serum.

2.4.1. Analysis of Blood Cells

A full CBC was automatically generated by a hematology analyzer. Parameters assessed included:

Hemoglobin (Hb), Hematocrit (HCT), Red blood cell count (RBC), Total and differential white blood cell count (WBC), Platelet count (PLT), and erythrocyte indices (MCV, MCH, MCHC).

2.4.2. Evaluation of Hormonal Status

Serum concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone were quantified using specific, commercially available enzyme-linked immunosorbent assay (ELISA) kits, adhering strictly to the manufacturers' protocols.

2.4.3. Assessment of Metabolic Markers

Fasting serum was utilized to determine glucose concentration and lipid profile. The lipid panel comprised measurements of: total cholesterol, triglycerides, low-density lipoprotein (LDL) and high-density lipoprotein (HDL). All assays were performed with validated commercial enzymatic colorimetric kits.

2.5. Data Analysis

All numerical data are expressed as mean ± standard deviation (SD). To determine statistical significance across the three groups, a one-way analysis of variance (ANOVA) was applied, followed by Tukey's post-hoc test for pairwise comparisons. A p-value of less than 0.05 was deemed statistically significant.

3. Results

Impact on Hematological Parameters: Relative to the healthy control group, the untreated diabetic rats developed significant hematological deficits, including anemia and thrombocytopenia (Table 1). Intervention with clove extract effectively restored these parameters, bringing red blood cell count (a 45% increase from 5.6 to 8.1 $x10^6/\mu L$), hemoglobin (a 38% increase from 10.3 to 14.2 g/dL), hematocrit (a 50% increase from 30% to 45%), and platelet count (a 25% increase from 522 to 652 $x10^3/\mu L$) back to levels statistically indistinguishable from normal ($p < 0.05$).

Impact on Reproductive Hormones: The diabetic condition led to a significant suppression of the hypothalamic-pituitary-gonadal axis, reflected in markedly lower serum levels of FSH, LH and testosterone (Table 2). Oral administration of the clove extract produced a significant upregulation in the concentrations of all three hormones (FSH increased by 38%, LH by 134%, and testosterone by 166%) ($p < 0.05$).

Impact on Metabolic Parameters: The diabetic control group exhibited severe hyperglycemia and an adverse lipid profile (Table 3). Treatment with clove extract resulted in a significant ($p < 0.05$) 30% decrease in fasting blood glucose and a comprehensive improvement in the lipid panel, characterized by a 22% reduction in triglycerides, a 23% reduction in total cholesterol, a 22% reduction in LDL, and a 38% increase in protective HDL cholesterol.

Table 1: The effect of clove on Complete Blood Count (CBC) in both normal and diabetic rats

Parameter	Literature Reference Values	Untreated Rats (Control)	Diabetic Rats Before Treatment	Diabetic Rats After Treatment
White Blood Cells (WBC) /µL x 10^3	6.0 - 10.0	6.0 ± 2.88	8.2 ± 2.16	7.1 ± 2.18*
Lymphocytes/µL x 10^3	2.5 - 5.0	2.5 ± 1.68	2.4 ± 1.85	2.6 ± 1.97
Neutrophils/µL x 10^3	1.5 - 4.0	3.0 ± 1.83	3.1 ± 2.86	3.5 ± 1.88
Monocytes %	1 - 4	2.5 ± 1.2	6.5 ± 2.1	4.0 ± 1.5
Eosinophils%	0.5 - 3	1.0 ± 0.5	1.2 ± 0.7	1.1 ± 0.6
Basophils%	0 - 1.5	0.5 ± 0.3	0.6 ± 0.4	0.5 ± 0.3
Red Blood Cells (RBC) /µL x 10^6	7.0 - 9.0	7.0 ± 1.66	5.6± 1.60	8.1± 1.75*
Hemoglobin (Hb) g/dL	12.0 - 15.0	13.0 ± 2.98	10.3 ± 2.41	14.2 ± 1.44*
Hematocrit (HCT) %	38 - 48	40 ± 3.82	30 ± 1.47	45 ± 2.65*
Mean Corpuscular Volume (MCV) fL	50 - 60	55 ± 4.44	51 ± 2.32	60 ± 2.46*
Mean Corpuscular Hemoglobin (MCH) pg	17 - 21	18 ± 2.55	16 ± 1.65	19 ± 2.35
Mean Corpuscular Hemoglobin Conc.(MCHC)%	30 - 35	30 ± 1.96	24 ± 3.18	31 ± 2.13
Platelets (PLT) /µL x 10^3	550 - 800	600 ± 5.37	522 ± 2.35	652 ± 4.39*

Note: *Significant difference at $p < 0.05$ level.

Table 2: The effect of clove on Sex Hormones in normal and diabetic rats

Parameter	Literature Reference Values	Untreated Rats (Control)	Diabetic Rats Before Treatment	Diabetic Rats After treatment
FSH (IU/mL)	12.0 - 15.0	13.57±0.07	11.27±0.01	15.53±0.20*
LH (IU/mL)	1.5 - 2.0	1.76±0.01	1.07±0.08	2.50 ±0.16*
Testosterone (ng/mL)	3.0 - 5.0	3.64±0.03	2.23±0.09	5.94±0.66**

Note: *Significant difference at $p < 0.05$ level. **Testosterone showed a highly significant increase beyond control levels ($p < 0.01$).

Table 3: The effect of clove on Glucose and Lipid Level in normal and diabetic rats

Parameter	Literature Reference Values	Normal Rats (mg/dL)	Diabetic Rats Before treatment (mg/dL)	Diabetic Rats After Treated with clove (mg/dL)
Glucose	80 - 110	85.6±5.3	198.8±8.5	139.6±6.7*
Triglycerides	100 - 150	138.7±6.3	190.6±11.5	148.4±8.13*
Cholesterol	130 - 170	150.2±9.5	200.2± 13.40	155± 9.18*
LDL	70 - 110	94.3±4.2	129.5± 16.10	101.5± 8.60*
HDL	45 - 60	50.9±2.9	41.6± 3.2	57.3± 3.22*

Note: *Significant difference at $p < 0.05$ level.

4. Discussion

Our findings reveal that diabetic rats developed significant hematological and endocrine disturbances, which were substantially reversed by clove extract supplementation.

The observed anemia and low platelet count in the diabetic group align with the known suppressive effects of chronic hyperglycemia and associated oxidative stress on bone marrow activity and red blood cell longevity (Hussain et al., 2020). The ability of clove extract to normalize these parameters underscores its hematoprotective potential, most plausibly attributable to its high concentration of antioxidant compounds like eugenol, which mitigate oxidative damage to cellular components, including erythrocyte membranes (Srinivasan, 2014; Abdel-Mawgoud et al., 2019).

The suppression of FSH, LH, and testosterone in diabetic rats indicates a dysfunction at the level of the hypothalamic-pituitary-gonadal (HPG) axis. The successful restoration of these hormones by clove extract points to a central mechanism of action. We posit that the extract alleviates the oxidative stress and inflammation that disrupt the pulsatile secretion of Gonadotropin-Releasing Hormone (GnRH) from the hypothalamus. This restoration of central signaling would subsequently normalize pituitary release of LH and FSH, thereby reactivating testosterone production in the Leydig cells of the testes, a process known to be impaired by diabetes (Ahmed et al., 2018; Gianatti & Grossmann, 2020). The fact that hormone levels were not just normalized but elevated beyond control values suggests a potent, possibly stimulatory, effect of clove's bioactive components on the HPG axis, potentially through enhanced steroidogenic enzyme activity or receptor sensitivity (Huang et al., 2014; Elgharib et al., 2025). The potent antioxidant activity of eugenol, the primary bioactive molecule in clove, is a likely cornerstone of this restorative effect. By scavenging the excess reactive oxygen species (ROS) generated in the diabetic state, eugenol protects sensitive neuroendocrine and testicular tissues from damage, thereby permitting the

resumption of normal hormonal synthesis and regulation (Khosravi Z et al., 2021). Finally, the significant amelioration of hyperglycemia and dyslipidemia in the treated group confirms the extract's metabolic benefits. The glucose-lowering action is consistent with proposed mechanisms of improved insulin sensitivity and peripheral glucose uptake (Manjamalai et al., 2012; Bhardwaj et al., 2023). The corrected lipid profile--reduced triglycerides, cholesterol, and LDL, and increased HDL--likely results from a dual action: the suppression of hepatic cholesterol and fatty acid synthesis, coupled with the promotion of lipid catabolism and clearance (Lima et al., 2019; Hamri et al., 2022).

5. Conclusion

This study provides strong evidence that a water-based extract of clove effectively counteracts diabetes-induced hematological deficits, hormonal imbalances, and dyslipidemia in rats. These findings position clove as a promising candidate for adjunct therapy, potentially offering a natural means to improve quality of life by addressing multiple diabetic complications simultaneously.

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