Effect of Zucchini (*Cucurbita pepo L.*) and Clove Blossom (*Syzygium aromaticum*) Powders, Individually and in Combination, on Biochemical Indicators in Diabetic Male Rats.

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SUMMARY

This investigation focused on examining the effects of clove, zucchini blossom, and their combination on the metabolic markers of diabetic rats. Forty-eight adult albino rats, weighing about 120 ± 5 g each, were split into eight groups of six animals. The first group served as the negative control, while the other groups were injected with alloxan (150 mg/kg body weight) to induce diabetes mellitus (DM). One diabetic group was kept as a positive control, and the remaining groups received 28 days of treatment with either clove blossom, zucchini blossom, or their combination at dietary levels of 5% and 10%. After the treatment, biochemical markers like serum glucose, insulin, lipid profile, liver enzymes, kidney function markers, cardiovascular risk factors, and antioxidant enzymes were measured. In the diabetic rats (positive control group), serum glucose, total cholesterol, LDL-C, VLDL-C, triglycerides, malondialdehyde (MDA), 8-hydroxydeoxyguanosine (8-OH-dG), liver enzymes, kidney function indicators, and cardiovascular risk indices increased significantly (p < 0.05). Meanwhile, insulin, HDL-C, catalase (CAT), and superoxide dismutase (SOD) levels decreased significantly (p < 0.05) compared to the normal control group. The group that received the mixture of clove and zucchini blossoms, especially at the 10% dose, showed the greatest protective effects against diabetic complications across all treatments. These results indicate that moderate intake of clove and zucchini blossoms, whether alone or combined, may provide notable therapeutic and nutraceutical benefits for managing DM and related conditions.

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INTRODUCTION

Over 150 million people worldwide suffer from DM (DM), one of the most common endocrine conditions. Globally, the prevalence of diabetes is predicted to increase from 4% in 1995 to 5.4% by 2025, with an estimated 300 million people living with the illness, mostly in developing nations (**Jayaprasad *et al*., 2011**). Type I, type II, and gestational diabetes are the three main forms of the illness.

Chronic hyperglycemia caused by deficiencies in insulin secretion, action, or both is the main feature of DM. Long-term effects include vascular damage, neuropathy, and high blood glucose levels resulting from these disruptions, which affect the metabolism of proteins, fats, and carbohydrates (ADA, 2008). In cases of diabetes, persistent hyperglycemia occurs due to inefficient glucose entry into cells. Additionally, lipid metabolism is often disrupted; common abnormalities include increased levels of triglycerides, LDL-C, total cholesterol, and HDL-C (Dufurrena et al., 2017). Secondary effects, such as polyuria, polydipsia, polyphagia, ketosis, retinopathy, and cardiovascular diseases, are caused by these imbalances.

Although several hypoglycemic medications are currently used to treat diabetes, many have adverse side effects such as lactic acidosis, liver damage, and gastrointestinal issues **(Samar and others 2023).** Therefore, there is growing interest in complementary natural therapies and functional foods alongside traditional treatment options.

Through a variety of methods, such as increasing insulin secretion, boosting peripheral glucose absorption, and blocking hepatic gluconeogenesis, several therapy approaches seek to reduce blood glucose. Vegetables, fruits, and especially edible flowers are now considered essential parts of a balanced diet. A wide range of bioactive substances, including phenolic acids, flavonoids, anthocyanins, carotenoids, chlorophylls, alkaloids, betalains, and sulfur-containing compounds, are abundant in flowers, which are the reproductive organs of flowering plants (**Lysiak, 2022; Dujmović and colleagues, 2022**). These substances have a variety of medicinal qualities, such as immunomodulatory, neuroprotective, hepatoprotective, cardioprotective, anti-inflammatory, and antioxidant activities **(Lu et al., 2021).**

Given the drawbacks and adverse effects of oral antidiabetic medications and insulin, eating edible flowers is seen to be a potential approach to managing diabetes. Some edible flowers may induce β-cell regeneration or contain chemicals that mimic insulin **(Prawej et al., 2023).**

Because of its potent flavor and aromatic qualities, clove *(Syzygium aromaticum Linn.*), a member of the Myrtaceae family, is frequently used in Asian and African cuisines. Up to 18% essential oil can be found in the flower buds, of which 89% is eugenol. The antioxidant, anti-inflammatory, antibacterial, anti-fungal, antiviral, and antidiabetic properties of eugenol have been the subject of much research **(Batiha and the rest 2018; Vicidomini et al., 2021).**

Widely cultivated for its edible fruit, zucchini (Cucurbita pepo L.) is also known for its blossoms, which have recently been found to be high in polyphenolic compounds that may offer health benefits **(Krimer-Malešević and others 2020; Adnan et al., 2017).** According to **Luigi, among others (2023),** these polyphenols, including flavonoids like rutin and quercetin glycosides, significantly enhance antioxidant activity. The bioavailability of these compounds is affected by intestinal microbiota and digestive enzymes, which are essential for their effectiveness **(Wojtunik-Kulesza and co-authors, 2020; Kumar Singh and co-authors, 2019).** Therefore, this study aims to examine how food supplementation with 5% and 10% clove and zucchini blossom, both separately and combined, influences biochemical markers in male mice with alloxan-induced diabetes.

MATERIALS AND METHODS

Materials

Plant material

The Agriculture Crops Department, Agriculture Faculty, Menoufia University, characterized the clove bloom *(Syzygium aromaticum Linn.)* and zucchini blossom *(Cucurbita pepo)* that were acquired from a local in Cairo, Egypt.

Experimental animals

Healthy male albino rats (Sprague-Dawley strain) were acquired from the Research Institute of Ophthalmology in Giza, Egypt.

Chemical kits and alloxan

El-Gomhoria Company for Trading Drugs, Chemicals, and Medical Instruments, Cairo, Egypt, provided the chemical kits for biochemical measurements and alloxan.

Methods

Determination of total phenols, total flavonoids, and DPPH activity

The Folin-Ciocalteu reagent and the **Singleton and Rossi (1965)** method were used to measure the total phenols in alcoholic and aqueous extracts. The aluminum chloride colorimetric approach was used to determine the total flavonoids **(Park and others, 1997). Xu and Chang's (2007)** method was used to assess DDP's capacity to scavenge DPPH free radicals,

Preparation of clove and zucchini blossom powder

After being detached from the fresh fruits, the clove and zucchini flowers were cleaned with clean water, chopped into small pieces, and dried in an air dryer set at 50±5°C for 48 hours, or until their moisture content reached 11%. Before being employed, the dehydrated pieces were kept at 4°C after being finely powdered in a laboratory disc mill (Braun AG, Frankfurt Type: KM 32, Germany) **(Russo, 2001)**.

Injured rats with DM

Using the technique outlined by **Desai and Bhide (1985**), 150 mg/kg of body weight of alloxan was injected intraperitoneally into healthy male albino rats for three days. Following **NDDG (1994),** fasting blood samples were taken one week after the alloxan injection to determine fasting serum glucose levels in rats at 200 mg/dl.

Biological experiment

A week before the start of the adaptation studies, 48 albino rats in good health were housed separately in wire cages with adequate ventilation and a hygienic environment (temperature of 25 ± 2°C and 12 h light and 12 h dark), and normal ambient humidity.

They were fed the basal diet according to **Pell et al., (1992).** Glass tubes that protruded to provide the rats with water were checked every day.

Biological design

Following the adaptation phase, eight cohorts of six rats each were randomly selected. As a negative control cohort, the first six rats were given a baseline diet. The first subgroup was given a basal diet as a positive control cohort, while the second major cohort (42 rats) was split up into seven cohorts and given an injection of alloxan to cause DM. During the 28-day testing period, the third and fourth diabetic cohorts were given clove blossom powder at levels 5 and 10%, the fifth and sixth cohorts were given zucchini bloom at levels 5 and 10%, and the seventh and eighth cohorts were fed a mixture of clove blossoms at levels 5 and 10%. The study was carried out in the Animal House at Menoufia University. Department of Nutrition and Food Science, Faculty of Home Economics.

Ethical approbation:

The National Hepatology and Tropical Medicine Research Institute in Cairo, Egypt, granted ethical approval for the study (Approval No. AI-225; NIGHTMARE).

Biological evaluation

The following formulas were used to determine the body weight gain (BWG g/day), feed efficiency ratio (FER), and organ weight (liver, kidney, and pancreas) by **Chapman and colleagues (2023)** to conduct a biological evaluation of the various diets:

BWG = Final weight (g) – Initial weight (g).

FER = Body weight gain / Feed intake.

Blood tests

Following the experiment's conclusion, the rats were fasted for two hours and without water for the entire night before being killed under light anesthesia. In sterile, dry centrifuge tubes, blood samples were drawn from the hepatic portal and allowed to coagulate. Following **Schemer (1967),** the samples were centrifuged for 10 minutes at 3000 revolutions per minute to separate the serum. It was kept frozen at -20°C till examination**.**

Biochemical evaluation

The procedure outlined by **Brăslasu and Co-authors (2007)** was used to determine serum glucose. The Enzyme Linked Immunosorbent Assay (ELISA) was used to measure insulin **(King et al., 2002),** and the **Mari and others (2005)** methods were used to measure insulin resistance.

While protein content, urine volume, uric acid clearance, and urine creatinine concentration were measured in the urine samples as described by **Mutakin and so forth (2018); Corder and the rest (2021); Milani and Jialal (2023); Jaffe (1987),** renal function was estimated using serum urea, uric acid, and creatinine as per **Patton and Crouch (1977); Fossati et al. (1980).**

Bergmeyer (1974) was used to determine the liver enzymes aspartate amino-transaminase (AST) and alanine aminotransferase (ALT), whereas **Moss and Henderson (1999)** were used to determine alkaline phosphatase.

The methods of **Lopez (1990)** were used to test high-density lipoprotein cholesterol (HDL-c), triglycerides (TG), and total cholesterol (TC) by **El-Anany and Ali (2012).** However, LDL-c and VLDL-c (very low-density lipoproteins) were computed using **Friedwald et al.'s (1972)** approach as follows: LDL-c (mg/dl) = Total cholesterol - (HDL-c + VLDL-c), and VLDL-c (mg/dl) = Triglycerides/5.

The formulas of **Bhardwaj et al. (2013)** were used to determine the atherogenic ratios, such as the Atherogenic Index (AI), Cardiac Risk ratio (CRR), and Atherogenic Coefficient (AC), while **Aguilar and colleagues (2011)** were used to create the Atherogenic Fraction (AF).

In the case of antioxidant enzymes, the procedures of **Sun and other (1988), Aebi (1983); Ohkawa et al., (1979); Boonla and co-author (2007)** were followed in the case of superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and 8-hydroxy-deoxyguanosine (8-OH-dG).

Statistical analysis

One-way analysis of variance (ANOVA) was used to assess the effects of different treatments, Duncan's multiple range test was used for post hoc comparisons, the Statistical

Package for the Social Sciences (SPSS; 25) was used to analyze the data, and the results are displayed as means ± standard deviation (SD). A p-value of less than 0.05 is deemed to be significantly different **(Snedecor and Cochran, 1967).**

OUTCOMES AND INTERPRETATION.

Total phenols, total flavonoids, and the antioxidant activity as measured by DPPH in clove and zucchini blossoms are displayed in **Table 1.** The results were statistically significant, showing that the total flavonoid content in both blossoms was higher than the total phenol content, but the flavonoid content in the zucchini blossom was higher than that in the clove blossom. Additionally, the same table's results showed that both plant blooms had high percentages of antioxidant activity as a percentage of DPPH, with zucchini blossoms having higher antioxidant activity than clove blossoms, at 63.34 and 75.09%, respectively. Since the DPPH approach has been effectively utilized to assess the antioxidant activity and oxidative stability of various plant components, the results of this investigation indicate that both of them demonstrated free radical scavenging activity **(Mokashi and Others 2017).** The existence of a particular molecule may be the reason for the variation and high content in the antioxidant activity values in zucchini, depending on the water solubility of the sample **(Aquino-Bolaños, 2013).**
Furthermore, **Morittu and the rest (2021)** discovered that the high value in zucchini was caused by their high concentration of several types of bioactive substances (antioxidants), such as flavonoids and phenolics.

In Table 2, data indicated that there were significant differences in BW between the experimental cohorts ($p \leq 0.05$) about the effects of feeding clove, zucchini flower, and their mixture on the body weight, feed intake, and feed efficiency ratio of mice with DM. The diabetic untreated cohort (G2) displayed the lowest values for body weight, FI, and FER, whereas the normal control cohort (G1) recorded the highest values. This suggests that, because of poor glucose utilization and catabolic conditions, diabetes has a detrimental effect on biological parameters. Although still below the healthy control and a successful intervention, cohorts G6, G7, and G8, possibly higher-dose or recovery cohorts, showed progressive increases in their parameters, with G8 reaching, suggesting significant restoration, perhaps as a result of enhanced glucose metabolism and anti-oxidant activity from the dietary supplements. In contrast to untreated diabetic controls, it was discovered that giving clove, zucchini blossom, and particularly their combination to diabetic mice improved BW, feed intake, and feed efficiency. According to the findings, these combinations may be used as functional dietary therapies to control weight loss and metabolic deterioration associated with diabetes. The antioxidant, anti-inflammatory, and hypo-glycemic qualities of the bioactive substances found in clove (e.g., eugenol) and zucchini blossom (e.g., flavonoids, carotenoids) are probably what caused these changes (**Fedchenkova *et al,* 2015; Cortés-Rojas *et al.,* 2014).**

Table 3 shows that there was a significant difference in liver weight between the cohorts ($p \leq 0.05$). The weight of the organs was lowest in the normal control group (G1). The diabetic untreated cohort (G2), on the other hand, had the largest weight of their organs, suggesting that they may have hepatomegaly as a result of fat buildup or liver inflammation, which are frequent side effects of poorly managed DM (**Mukhtar *et al.*, 2020)** and also indicating possible nephropathy or renal hypertrophy, which are frequent in diabetes (**Danilova and other 2017)** and perhaps as a result of compensatory hypertrophy or inflammation brought on by β-cell failure **(Jayaprasad *et al.*, 2011)**. Although it remained higher than usual, the liver weight significantly decreased after treatment with clove and zucchini blossoms, as well as their combination, as compared to the diabetic control. This implies a potential hepatoprotective impact and a partial recovery of liver health. Further improvements were shown in cohorts G6, G7, and G8, with G8 showing the strongest recovery and treatment impact, as their liver weight almost returned to normal. Kidney weights were dramatically decreased by clove (G3), zucchini blossom (G4), and the combo (G5).

Additionally, G6, G7, and G8 showed progressive improvements, confirming the beneficial effects of the therapies' anti-inflammatory and antioxidant qualities **(Cortés-Rojas and So on, 2014; Fedchenkova *et al.,* 2015).** When compared to the normal cohort, pancreatic weights varied significantly between cohorts, with G8 diabetic mice exhibiting the best recovery. Thus, it may be concluded that the most successful intervention, perhaps as a result of the combined actions of anti-inflammatory and antioxidant substances such as flavonoids (from zucchini blossom) and eugenol (from clove) (**Vicidomini *et al.,* 2021; Rudrapal *et al.*, 2022)**.

Blossom-induced hypoglycemia is most likely caused by either an extra-pancreatic mechanism or increased insulin release from the beta-cells of the pancreatic islets. Furthermore, some substances from the zucchini blossom may have acted on pancreatic β-cells to have a regenerative impact, which could explain lower blood glucose levels. Numerous compounds with antioxidant properties are responsible for the medicinal benefits of zucchini flowers (**Fedchenkova *et al,* 2015).** Flavonoids, terpenoids, alkaloids, and glycosides are all present in clove bloom. Eugenol made up about 89% of the 18% essential oil that was present. According to certain research, eugenol has antioxidant properties. Antioxidants found in S. aromaticum lessen the impact of inflammatory cytokine production in diabetes, which may be one of the factors contributing to insulin resistance (**Mishra and Singh, 2008; Cortés-Rojas *et al.,* 2014).**

The mean levels of serum insulin hormone, insulin resistance, and blood glucose are illustrated in **Table 4.** The data showed that the positive control cohort's blood glucose and insulin resistance levels were noticeably greater than those of the negative control cohort. When diabetic mice were fed a diet containing varying amounts of clove flower, zucchini blossom, or both, their blood glucose levels significantly decreased over time ($P < 0.05$). The combination cohort at various levels recorded significant values lower than the positive control cohort, while the other cohorts displayed nonsignificant differ-rences between the same degree of insulin resistance. In the case of insulin resistance, the positive control cohort was considerably higher than the negative control cohort. When compared to the level of 5%, the high-level percentage (10%) showed a significant decrease. The levels of clove and zucchini blossoms were followed by the levels of mixture blossoms, which significantly reduced both parameters. On the other hand, it was discovered that the positive control cohort had lower levels of the insulin hormone than the normal cohort, and that adding bloom levels greatly increased these levels. The mixed cohorts had the highest values, followed by the levels of zucchini blossoms.

Alloxan injection damaged the ß-cells in the pancreas, which control the release of insulin and raise blood sugar levels. By oxidizing ketoaldehydes and superoxide anion radicals, alloxan harms the pancreas that causing rapid oxygen free radicals in diabetes. The superoxide anion then dismutates to hydrogen peroxide and produces extremely reactive hydroxyl radicals, which significantly raises the concentration of calcium in the cytosol (**Danilova *and Other* 2017).**

According to **Table 5,** rats treated with alloxan to induce diabetes mellitus showed a significant ($p \leq 0.05$) increase in serum renal function indicators such as creatinine, uric acid, and urea levels compared to the normal cohort. On the other hand, there was a substantial ($p \leq 0.05$) increase in the levels of blood renal function biomarkers following treatment with clove blossoms or zucchini blossoms and their combo. Although the kidney functions of the tested blossoms were reduced, there were no appreciable changes in the mean kidney function values between cohorts 5 and 7, which were fed a mixture of 5% zucchini blossoms and 5% blooms.

Diabetic nephropathy is mostly linked to abnormal renal function. In the pathophysiology, glucose forms irreversible bonds with kidney circulation proteins to produce advanced glycosylation end products, which can then combine to form complexes that stimulate fibrotic growth factors and cause renal damage (**Danilova *et al.*, 2017).** Oxidative stress may be the cause of renal damage and dysfunction in diabetes **(Forbes et**

al., 2008). Diabetes also raises serum levels of creatinine and urea, according to **Sharma et al., (2006).** According to the findings of the present investigation, S. aromaticum medication can shield renal tissue from oxidative damage, lowering kidney weight, glomerular diameter, and basement membrane thickness. It may also lower urea and creatinine levels. Nonetheless, it can cause diabetic mice' urinary spaces to have more glomeruli and a wider width. The strong antioxidant properties of the plant's blossom may be the reason for this **(Cortés-Rojas et al., 2014). Bakour and Co-author (2018)** demonstrated that S. aromaticum can lower urea levels in the blood. According to **Goel (2013),** the observed findings were consistent with the use of zucchini flowers as medicine since they contain a wide range of molecules that scavenge free radicals, including vitamins, phenolic compounds, and other endogenous metabolites with antioxidant properties. In the past, people mostly ate flowers for their medicinal properties rather than their nutritional content. Prebiotics and probiotics, dietary fiber, carotenoids, fatty acids, phenolic acids, flavonoids, isothiocyanates, polyols, sterols, vitamins, essential mineral elements, amino acids, and phytoestrogens are among the significant bioactive and nutraceutical compounds found in edible flowers, according to several recent studies (**Koike *et al.,* 2015).**

In comparison to the control group, alloxan substantially boosted urine volume and urine protein levels ($P \leq 0.05$) (Table 6). Alloxan-treated cohorts with the studied flowers and their mixture showed a substantial decrease ($P \leq 0.05$) in urine protein levels, but there was no discernible difference in renal performance between cohorts 5 and 7. Except for cohorts 5 and 7, the diabetic cohorts that administered varying quantities of zucchini blossom and the mixture exhibited a steady decrease ($P \leq 0.05$) in urine protein as compared to the positive control cohort. The positive control cohort's mean creatinine and uric acid clearance values were lower than those of the negative control cohort. While uric acid clearance, bloom mixture levels, and zucchini flower levels produced the highest effect with nonsignificant differ-rences at the same levels, the cohort treated with a mixture of blossom levels showed the largest effect on creatinine clearance. As a result of increased urine albumin excretion, creatinine levels, and serum ammonia levels, as well as impaired creatinine and uric acid clearance, diabetic nephropathy damages the kidney and causes more protein to accumulate in the urine than is typical (**Samar *et al*., 2023**). The results were comparable to those of a study by **Vicidomini and colleagues (2021),** which found that clove blossom contained soluble flavonoids and phenolic compounds that had a strong antioxidant impact against oxidative damage. Additionally, **Rudrapal and Others (2022)** discovered that *zucchini blossom* may be linked to a number of processes, such as anti-inflammatory and antioxidant /scavenging properties, decreased levels of free radicals in renal tissues, and decreased urine protein and volume.

The effects of 5 and 10% clove, flower, or their combination were shown in **Table 7.** As a result, the positive control cohort had considerably greater levels of TC, LDL-c, and TG than the negative cohort. Compared to the negative control cohort, the HDL-c concentration in the positive control cohort was considerably lower; however, the tested blossom changed these findings. While TC, TG, LDL-c, and VLDL-c levels were dramatically reduced ($p \leq 0.05$), HDL-c values were significantly raised by blossom supplementation. Significant changes occurred when blossom levels increased, indicating a significant drop in cholesterol levels as compared to the positive control cohort. The blossom mixture had a large impact on the lipid profile, followed by the influence of zucchini levels. According to earlier research, dyslipidemia has been linked to diabetes, as shown by elevated triglycerides, total cholesterol, low-density lipoprotein, and a decrease in high-density lipoprotein **(Elleuch et al., 2010).** Additionally, it was demonstrated that hyperglycemia raises LDL-C's glycation and atherogenicity **(Ochuko et al., 2013).** The observed hypolipidemic action, as demonstrated by decreased levels of total cholesterol, LDL-C, triglycerides, and HDL-C, suggests that the cake supplemented with zucchini blossoms may protect against diabetes (**Mohamed, 2018).**

One factor contributing to insulin resistance or low insulin levels is a decrease in the activity of lipoprotein lipase, the enzyme essential for hydrolyzing lipoproteins containing TG **(Fayad, among others, 2017).** According to current findings, S. aromaticum blossom raises serum HDL levels while lowering LDL, TC, and TG levels. Furthermore, by reducing MDA levels, S. aromaticum can stop lipid peroxidation (**Adefegha *et al.,* 2014**). Therefore, treatment with the herb's extract may lower TG, LDL, and TC levels while raising serum HDL levels in diabetics because S. aromaticum blossoms can restore beta cell activities, improve serum insulin levels, and subsequently increase the activity of the enzyme lipoprotein lipase **(Seyd *et al.*, 2021).**

The AI was used to predict cardiovascular risk, which is log TG/HDL-c. In comparison to the diabetic cohort (positive control cohort), it was lower in all treatment cohorts **(Table 8).** The negative control cohort's CRR, or LDL-c/HDL-c ratio, was 0.52, whereas the positive control cohort was 3.13, and the AC for that cohort was 3.90. In particular, the cohorts treated with the blossom mixture, followed by the amounts of zucchini blossoms, were able to reduce their cardiovascular risk in comparison to the positive control cohort. When the blossom level was raised by 10%, this impact was greatly enhanced. With a 10% blossom mixture, the negative control cohort showed no discernible changes in AI metrics, nor did cohorts 6 and 7. The values of TC, HDL-c, and LDL-c were found to be connected to cardiovascular risk, while cohorts 4 and 5 showed nonsignificant differences in all parameters save the AI parameter from the earlier results (**Samar *et al.*, 2023)**. Due to the presence of several phytochemicals with antioxidant properties, such as flavonoids and phenols, the addition of zucchini blossom improved these values and decreased the cardiovascular risk (**Fedchenkova *et al*, 2015)**. Additionally, clove flower can raise serum HDL levels, which contain carotenoids, flavonoids, terpenoids, alkaloids, and glycosides that may have anti-diabetic and antilipidemic properties, while lowering TG, LDL, and TC levels (**Seyd *et al.*,2021).**

It was shown that diabetes causes the free radicals that lead to inflammation, as evidenced by the rise in malonaldehyde and 8-hydroxydeoxyguanosine in **Table 9** and the considerable decrease in antioxidant enzymes ($P \leq 0.05$). In contrast, when compared to the mean values of the positive control cohort, the diabetic cohorts treated with 5 and 10% of zucchini or clove blossom, or their combination, demonstrated a decrease in malonaldehyde and 8-hydroxydeoxyguanosine while increasing antioxidant enzymes. When compared to the other treatment cohorts and the positive control cohort, the effect of zucchini was the next most significant effect observed in both mixture levels. Cohorts 4 and 5 showed nonsignificant differences in SOD, whereas cohorts 6 and 7 showed no significant differences in CAT, MDA, or 8-hydroxydeoxyguanosine. It was evident from the results that diabetes significantly raised MDA and 8-OH-dG while lowering the levels of the antioxidant enzymes SOD and CAT. These results could be explained by free radicals and the oxidative stress caused by diabetes. The primary scavengers are antioxidant enzymes, which can also function as compensatory mechanisms by boosting their activity in different tissues. According to **Onyema et al. (2005),** antioxidants are crucial for scavenging free radicals and shielding the body from oxidative stress. The MDA concentrations were measured to assess oxidant damage to lipids in each cohort. Following alloxan-induced diabetes, higher MDA levels indicated increased lipid peroxidation, which either sustained an intense production of ROS or decreased the activity of antioxidant defense systems. Lipid peroxidation is reflected in the markedly elevated MDA level in the brain tissues of the diabetic (untreated) mouse cohort. This suggests that diabetic (untreated) mice have diminished enzymatic antioxidant defense systems. These findings are consistent with previous research by **Erukainure et al. (2011),** which discovered that the antioxidant properties of cakes enhanced with various powdered zucchini blossoms can raise CAT levels in the brain tissues of the diabetic cohorts receiving treatment. The diabetic (untreated) cohort's enhanced synthesis is consistent with earlier research showing that these enzymes are produced in response to oxidative stress. The polyphenols and antioxidants found in zucchini flowers may be responsible for this **(Fedchenkova et al., 2015).** According to reports, zucchini blossoms have antioxidant qualities. This could be because the flower's polyphenols, carotenoids, and vitamin C serve as antioxidants by scavenging reactive oxygen species **(Urrutia-Hernández, 2011).**

Alternatively, **Ogata and others (2005)** observed comparable findings. The increase in reactive oxygen species, such as eugenol, which can inhibit the peroxidation system and act as a natural antioxidant to protect active species like (OH•) and (O2), may be the cause of clove blossom's decreased effect on antioxidant enzyme activity. Eugenol's antioxidant, radical-scavenging, and metal-chelating properties may shield the organs from oxidative damage **(Seyd *et al*., 2021).** The immune system is strengthened by cloves and clove oil. Clove, or *Syzygium* aromaticum, is a classic spice with a variety of pharmacological properties that have been used to preserve food. Sesquiterpenes, monoterpenes, hydrocarbons, and phenolic compounds are among the several phytochemicals abundant in S. aromaticum. Of the phytochemicals found in clove oil, eugenyl acetate, eugenol, and βcaryophyllene are the most important. S. aromaticum has been studied pharmacologically against a number of harmful parasites and microorganisms, such as hepatitis C viruses, Plasmodium, Babesia, Theileria parasites, Herpes simplex, and pathogenic bacteria. Numerous studies have shown that eugenol has antibacterial, antiviral, antifungal, anticancer, antiseptic, antidepressant, antispasmodic, anti-inflammatory, and analgesic properties against a variety of harmful bacteria, including methicillin-resistant Staphylococcus aureus and S. epidermidis. Additionally, it was discovered that eugenol protected against CCl4-induced hepatotoxicity and demonstrated potentially fatal effectiveness against the growth of a number of parasites, such as Schistosoma mansoni, Haemonchus contortus, Fasciola gigantea, and Giardia lamblia (**Ghanem et al., 2024**).

CONCLUSION

This study examined how the biochemical parameters of diabetic mice were affected by clove or zucchini blossoms and their mixture at levels of 5 and 10%. Because both blossoms included flavonoids and phenols, it was possible to deduce that they had substantial antioxidant activity. In contrast to the positive control cohort, these substances can increase HDL-c, CAT, and SOD while decreasing serum glucose, serum lipids, liver enzymes, kidney functions, MDA, and 8-OH-dG. Therefore, it can be said that incorporating the tested flower into one's regular diet will help prevent complications from DM.

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Table (1): Total phenols, total flavonoids, and the antioxidant activity of clove blossom and zucchini blossom

Content (mg/ml)	**Antioxidant activity (%) measured as percent scavenging of DPPH radical**	**Total phenols**	**Total flavonoids**
Clove blossom	$63.34^{b} \pm 3.02$	$50.11^{b} \pm 6.23$	$69.04^{b} \pm 1.08$
Zucchini blossom	$75.09^{a} \pm 5.63$	$59.1^{a} \pm 2.87$	$80.13^{a} \pm 4.17$

Data are expressed as mean ± standard deviation. Values within a column having different superscripts are significantly different (p≤0.05)

Table (2). The effect of feeding *clove, zucchini blossom, and their mixture* on the body weight, feed intake, and feed efficiency ratio of rats with DM.

Parameters	**(G1)**	**(G2)**	**(G3)**	**(G4)**	**(G5)**	**(G6)**	**(G7)**	**(G8)**
Body weight	39.76 a ± 1.74	19.32 f ±1.04	21.32^{e} ±2.03	25.09 d ±1.91	23.17 e ±1.85	28.11^{c} ±2.04	25.11^{d} ±3.01	**32.27b ±1.87**
Feed intake	14.95^{a} ±1.84	9.58^{f} ±4.06	10.29^{e} ±0.96	11.64^{d} ±1.04	11.03^{e} ±1.53	12.71^{c} ±0.15	11.49^{d} ±1.38	**13.89^{b} ±2.63**
Feed efficiency ratio	**0.095a ±0.005**	**0.072^{f} ±0.002**	**0.074d ±0.01**	**0.077c ±0.008**	**0.075^{d} ±0.006**	**0.079^{c} ±0.02**	**0.078 c ±0.003**	**0.083 ±0.007 b**

Data are expressed as mean ± standard deviation. Values within a row having different superscripts are significantly different (p≤0.05)

Table (3). The effect of feeding *clove, zucchini blossom, and their mixture* on the liver, kidney, and pancreas of rats with DM.

Parameters	(G1)	(G2)	(G3)	(G4)	(G5)	(G6)	(G7)	(G8)
Liver	4.46 f ±1.74	5.78a ±1.04	5.55[b] ±2.03	5.34 [c] ±1.91	5.47 [b] ±1.85	5.21[d] 2.04	5.19[d] ±3.01	**4.87[e] ±1.87**
Kidney	1.05[g] ±1.84	1.49[a] ±4.06	1.44[b] ±0.96	1.40[c] ±1.04	1.36[d] ±1.53	1.31[e] ±0.15	1.29[e] ±1.38	**1.21[f] ±2.63**
Pancreas	**0.35e ±0.005**	**0.47 [a] ±0.002**	**0.46a ±0.01**	**0.44b ±0.008**	**0.43 [b] ±0.006**	**0.40 [c] ±0.02**	**0.41 c ±0.003**	**0.38[d] ±0.007**

Data are expressed as mean ± standard deviation. Values within a row having different superscripts are significantly different (p≤0.05)

Table (4). The effect of feeding *clove, zucchini blossom, and their mixture* on blood glucose and insulin hormone of rats with DM.

Parameters	(G1)	(G2)	(G3)	(G4)	(G5)	(G6)	(G7)	(G8)
Blood glucose mg/dl	90.34[h] ±1.87	272.7[a] ±5.67	250.09[b] ±8.34	230.1[c] ±6.24	223.97[d] ±5.69	201.4[e] ±4.97	180.56[f] ±9.02	**157.39[g] ±6.22**
Insulin hormones (U/ml)	12.73 [a] ±1.02	6.21 [f]±0.89	6.51 [e]±2.14	6.9 [d]±0.74	6.6 [e]±1.23	6.9[d] ±2.01	7.2 [c]±2.77	**7.9 [b] ±1.53**
Insulin resistance	**2.72 [h] ±0.62**	**4.18 [a] ±0.67**	**4.02 [b] ±0.05**	**3.92 [c] ±0.24**	**3.65 [d] ±0.65**	**3.43 [e] ±0.18**	**3.21 [f] ±0.43**	**3.07 [g] ±1.03**

Data are expressed as mean ± standard deviation. Values within a row having different superscripts are significantly different (p≤0.05)

Table (5). The effect of feeding *clove, zucchini blossom, and their mixture* on creatinine, uric acid, and urea of rats with DM.

Parameters	(G1)	(G2)	(G3)	(G4)	(G5)	(G6)	(G7)	(G8)
Creatinine (mg/dl)	0.68 ^{g}±0.06	1.32^{a} ±0.09	1.22^{b} ±0.8	1.11^{c} ±0.12	1.01d ±0.27	0.89^{e} ±0.06	0.91^{d} ±0.05	**0.73^{f} ±0.04**
Urea (mg/dl)	21.78 ^{g} ±1.23	42.19^{a} ±2.51	39.01 ^{b} ±1.62	36.75^{c} ±3.01	32.94^{d} ±2.17	28.24^{e} ±2.03	30.75^{d} ±1.75	**25.74^{f} ±1.64**
Uric Acid (mg/dl)	**2.02^{g} ±0.12**	**4.06^{a} ±0.05**	**3.74^{b} ±0.35**	**3.43^{c} ±0.41**	**3.11^{d} ±0.29**	**2.71^{e} ±0.15**	**3.05^{d} ±0.18**	**2.32^{f} ±0.39**

Data are expressed as mean ± standard deviation. Values within a row having different superscripts are significantly different (p≤0.05)

Table (6). The effect of feeding *clove, zucchini blossom, and their mixture* on urine volume, urine protein, creatinine clearance, and uric acid clearance of rats with DM.

Parameters	(G1)	(G2)	(G3)	(G4)	(G5)	(G6)	(G7)	(G8)
Urine Volume (ml/24 h)	2.12^{g} ±0.52	4.23^{a} ±0.76	4.04^{b} ±0.27	3.81^{c} ±0.55	3.67^{d} ±0.44	3.39^{e} ±0.75	3.54^{d} ±0.32	**3.19^{f} ±0.17**
Urine Protein (g/24 h)	0.0053^{g} ±0.001	0.028^{a} ±0.005	0.021^{b} ±0.006	0.018 ^{c} ±0.008	0.015^{d} ±0.001	0.011^{e} ±0.003	0.013^{d} ±0.001	**0.009^{f} ±0.004**
Creatinine Clearance (ml/min)	0.188^{a} ±0.012	0.084^{g} ±0.009	0.087^{f} ±0.008	0.092^{e} ±0.001	0.097^{d} ±0.004	0.12^{c} ±0.002	0.099^{d} ±0.006	**0.15^{b} ±0.003**
Uric Acid Clearance (ml/min)	**0.021^{a} ±0.005**	**0.005^{f} ±0.0004**	**0.008^{e} ±0.0003**	**0.011^{d} ±0.0005**	**0.014^{c} ±0.0006**	**0.017^{b} ±0.0009**	**0.015^{c} ±0.0008**	**0.018^{b} ±0.0007**

Data are expressed as mean ± standard deviation. Values within a row having different superscripts are significantly different (p≤0.05)

Table (7). The effect of feeding *clove, zucchini blossom, and their mixture* on the lipid profile of rats with DM.

Parameters	(G1)	(G2)	(G3)	(G4)	(G5)	(G6)	(G7)	(G8)
TG	95.25[g] ±2.65	134.09[a] ±5.32	128.51[b] ±3.51	121.43 [c] ±6.21	115.07 [d] ±3.93	109.93[e] ±8.54	103.48[f] ±7.44	**97.54[g] ±2.92**
TC	108.73[h] ±5.11	169.24[a] ±4.06	151.55[b] ±7.53	140.73[c] ±5.93	147.74[d] ±7.59	132.82[e] ±4.15	123.58[f] ±8.26	**117.99[g] ±5.14**
HDL-c	59.09[a] ±2.71	34.51[g] ±3.88	38.03[f] ±4.21	42.46[e] ±2.52	46.26[d] ±5.81	51.09[c] ±1.98	50.23 [c] ±4.39	**55.11b ±2.19**
LDL-c	30.59g ±2.46	107.91a ±6.07	87.82 b ±2.91	73.98 c ±1.02	78.47C ±1.28	59.74d ±2.28	52.22e ±2.52	**43.37f ±3.82**
VLDL-c	**19.05[g] ±4.64**	**26.82[a] ±3.65**	**25.7b ±4.07**	**24.29[c] ±5.92**	**23.01[d] ±3.22**	**21.99[e] ±2.48**	**20.69[f] ±1.66**	**19.51[g] ±3.64**

Data are expressed as mean ± standard deviation. Values within a row having different superscripts are significantly different (p≤0.05)

Table (8). The effect of feeding *clove, zucchini blossom, and their mixture* on cardiovascular risk factors of rats with DM.

Parameters	(G1)	(G2)	(G3)	(G4)	(G5)	(G6)	(G7)	(G8)
AI	0.21 f ±0.05	0.59 a ±0.03	0.53 b ±0.006	0.46 c ±0.12	0.39 d ±0.001	0.33 e ±0.11	0.31 e ±0.007	**0.25 f ±0.008**
CRR	0 .52 g ±0.07	3.13 a ±0.86	2.31 b ±0.94	1.74 c ±0.66	1.69 c ±0.53	1.17 d ±0.005	1.04 e ±0.23	**0.79 f ±0.04**
AC	0.84 g ±0.04	3.90 a ±1.05	2.99 b ±0.38	2.31 c ±0.24	2.19 c ±0.98	1.60 d ±0.003	1.46 e ±0.61	**1.14 f ±0.72**
AF	**49.64 g ±2.53**	**134.73a ±4.51**	**113.52 b ±5.71**	**98.27 c ±2.96**	**101.48 c ±6.81**	**81.73 d ±4.68**	**73.35 e ±9.02**	**62.88 f ±6.33**

Data are expressed as mean ± standard deviation. Values within a row having different superscripts are significantly different (p≤0.05)

Table (9). The effect of feeding clove, zucchini blossom, and their mixture on serum antioxidant enzymes of rats with DM.

Parameters	(G1)	(G2)	(G3)	(G4)	(G5)	(G6)	(G7)	(G8)
CAT (Mmol/ml serum)	77.34[a] ±3.95	41.98[g] ±5.91	47.04[f] ±3.66	53.01[e] ±1.84	59.56[d] ±6.92	65.23[c] ±4.01	62.21[c] ±7.93	**69.42b ±6.55**
SOD (u/ml serum)	65.71[a] ±4.87	37.02g ±7.72	41.05f ±4.77	45.16e ±1.94	44.87[e] ±3.55	49.87[d] ±6.54	54.83[c] ±4.03	**59.03b ±2.88**
MDA (Mmol/ ml serum)	21.45[g]± 1.65	76.45a ±5.34	70.14b ±2.72	63.99c ±5.81	57.54d ±5.03	52.76[e] ±8.43	48.91[e] ±5.43	**40.01f ±6.33**
8-OH-dG (pg/ml serum)	**51.23[g] ±5.76**	**113.65[a] ±6.48**	**106.45[b] ±8.32**	**99.56c ±7.13**	**92.44d ±7.45**	**83.33e ±6.99**	**87.43e ±8.26**	**74.25[f]± 7.01**

تأثير مسحوق الكوسا (*Cucurbita pepo L*) وزهور القرنفل (*Syzygium aromaticum*)، بشكل فردي ومختلط، على المؤشرات الكيميائية الحيوية في ذكور الفئران المصابة بمرض السكري

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الملخص العربي :

أُجري هذا البحث لدراسة تأثير زهور القرنفل أومسحوق الكوسا وخليطهما على المعايير الكيميائية الحيوية للفئران المصابة بداء السكري . استُخدمت ثمانية وأربعون فأرا ألبينو بالغًا بوزن ١٢٠ ± ٥ جم. قُسِّمت إلى ثماني مجموعات (٦ فأر لكل مجموعة)، احتفظت المجموعة الأولى كمجموعة ضابطة سلبية، وحُقِنت المجموعات الأخرى بـ ١٥٠ ملجم ألوكسان/كجم لتحفيز داء السكري، وكانت إحداها مجموعة ضابطة إيجابية، وعولجت المجموعات الأخرى بزهور الكوسة والقرنفل بمستويات ٥ و١٠٪ لمدة ٢٨ يومًا. بعد العلاج، تم تحديد مستوى الجلوكوز في المصل، وهرمون الأنسولين، ومستوى الدهون، وإنزيمات الكبد، ووظائف الكلى، ومخاطر القلب والأوعية الدموية، وإنزيمات مضادات الأكسدة. أظهرت النتائج أن الفئران المعالجة بالألوكسان سجلت زيادة ملحوظة في نسبة الجلوكوز في المصل، والكوليسترول الكلي، وLDL-C، وVLDL-C، والدهون الثلاثية، ، وإنزيمات الكبد، ووظائف الكلى، ومستويات خطر الإصابة بأمراض القلب والأوعية الدموية (p≤0.05). في المقابل، انخفضت مستويات هرمون الأنسولين، وHDL-C، وCAT، و SOD (p<٠,٠٠٥) مقارنة بالمجموعة الطبيعية. كان علاج الفئران المصابة بداء السكري بخليط الأزهار، متبوعًا بزهور الكوسا بمستوى ١٠٪ أكثر فعالية في الحماية من مضاعفات داء السكري. لذلك، يمكن الاستنتاج أن الكوسا أو زهر القرنفل وخليطهما بكمية معتدلة يمكن الاستفادة منهما في المشروبات العادية والنظام الغذائي اليومي لإثبات الفوائد العلاجية الغذائية القوية في علاج اضطرابات داء السكري.

الكلمات المفتاحية: الفئران المصابة بداء السكري؛ إنزيمات الكبد؛ مسحوق زهرة الكوسا؛ إنزيمات مضادة للأكسدة؛ الألوكسان.