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Original Article

Assessment of the Secondary Metabolites of the Aerial Extract of Ocimum Tenuiflorumfor Anti-Diabetic Potential.

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Abstract

Background: *Ocimum tenuiflorum*is an important plant in Africa with diverse medicinal properties. This study investigates the anti-diabetic effects of *Ocimum tenuiflorum* methanol-ethyl acetate (2:1) extract (OTE) on alloxan-induced diabetic rats.
Methodology: Thirty rats (90-100g) acclimatized for one week were grouped into 5 (n=6). Group 1 (Normal control) received distilled water. The other groups were administered a single dose of 100 mg/kg alloxan intraperitoneally and treated as follows: Group 2 (olive oil), Groups 3 and 4 (200mg/kg and 400mg/kg OTE, respectively), and Group 5 received 5mg/kg glibenclamide. After twenty-one days of treatment, the rats were fasted overnight and sacrificed. Blood and liver samples were collected for analysis. Blood glucose status was measured using a glucometer. Serum insulin concentration was measured using an Enzyme-linked immunosorbent assay. Antioxidant assays were assessed via spectrophotometry. $P<0.05$ was considered statistically significant.
Results: Blood glucose level increased while insulin concentration reduced in diabetic controls relative to normal rats. Conversely, a dose-dependent reduction was observed in the glucose level, while insulin concentration returned to near normal with increasing doses of OTE compared to the normal control. It was observed that GSH level, CAT, and GST activities decreased significantly in diabetic control relative to normal control. Meanwhile, treatment with doses of OTE caused a dose-related increase in these antioxidant parameters, significantly compared to the normal control. MDA showed a significant increase in the untreated group relative to the normal control. However, MDA concentration declined drastically ($P<0.01$) in a dose-dependent manner upon treatment with OTE relative to normal rats.
Conclusion: It could be inferred from the results above that OTE may exhibit its anti-diabetic potential via enhancing the synthesis of insulin and scavenging of free radicals. This study showed that the extract can be used in the treatment of diabetes and can be formulated into a novel drug or supplement.

Keywords: *Ocimum Tenuiflorum;* Secondary Metabolites; Anti-Diabetic; Therapeutic Potential.

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Introduction

Natural products from plants, rich in phytochemicals such as flavonoids, terpenoids, and polyphenols, have health-promoting properties. They are traditionally used for maintaining health and managing various diseases, including diabetes and oxidative stress-related conditions [1,2]. Polyphenols (flavones and flavonoids,etc) could reduce oxidative stress and hyperglycemia and prevent diabetic complications [3]. They are good natural flavoring and preservative agents. Medicinal plants have been proven to be the best alternative for this purpose and have been used to cure numerous infectious and noninfectious diseases throughout history, and play a vital role in various fields, including health foods, phytoremediation, bioenergy/biodiesel production, herbal teas, etc.[4,5]. *Ocimum tenuiflorum*, a member of the *Lamiaceae* family, has been traditionally used in folk medicine for its various health benefits [6]. *O.tenuiflorum*is an odoriferous vegetable commonly known as *Holy Basil* or *Tulsi* and identified as two common cultivars, *Rama Tulsi* with green leaves and *Krishna Tulsi* with purple leaves [7,8]. The essential oil was also effective in decreasing the footpad thickness and the percentage of leukocyte migration inhibition, suggesting the induction of a cell-mediated immune response. Diabetes mellitus (DM) is a chronic metabolic disorder characterized by high blood glucose levels, which can lead to various complications such as cardiovascular disease, kidney damage, and nerve damage [9,10]. DM is a group of physiological dysfunctions characterized by hyperglycemia resulting directly from insulin resistance, inadequate insulin secretion, or excessive glucagon secretion [5]. The International Diabetes Foundation (IDF) estimated that in 2019, 463 million adults lived with diabetes worldwide, representing 9.3% of the adult population. It projects an increase to approximately eight hundred million adults by 2045, or 10.9% [11].

The increasing prevalence of diabetes worldwide has led to a growing interest in finding alternative treatments, particularly from plant-based sources. Several interventions have been employed in the management of DM. One of these is the use of orthodox drugs, which helps to regulate blood glucose levels by increasing the release of insulin and decreasing glucose production [12,13]. Some of these anti-diabetic drugs include metformin and Januvia, which work by decreasing the secretion of glucagon. However, some of these drugs have side effects. The most common adverse effects of Januvia are upper respiratory infection and swelling of the hands and legs [14,15]. The prevalence of these side effects brought about a shift of attention from the use of these orthodox medicines to other alternatives, such as plant biomass [16,17]. Therefore, this study was designed to investigate the effect of the methanol-ethyl acetate (2:1) extract of the whole aerial part of *Ocimum tenuiflorum*for its anti-diabetic potential and possible mechanisms in the alloxan-induced diabetic rat model.

Materials and Methods

Collection and Identification of the Sample

The aerial part (whole of the leaf, stem, bark, and flower) of the plant was collected during the rainy season in Ondo, Nigeria, where it was grown as a vegetable. The sample was identified and authenticated at the UNIMED herbarium as *Ocimum tenuiflorum* Linn (*Krishna Tulsi* with green-purple aerial part) with voucher specimen number (UNIMED PBTH 0190).

Preparation of the Leaf Extract

The plant extract was prepared using the method of Ololade *et al* [18]. The leaves were air-dried for three weeks, ground using a blender into powdery form, and extracted with methanol and ethylacetate (2:1) for 72 hours with intermittent shaking, after which it was subjected to filtration and the filtrate was concentrated using a rotary evaporator. The extract obtained was thereafter refrigerated until use.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis of the extract was conducted by means of a GCMS-QP2010 Plus (Shimadzu) system equipped with an AOC-20i auto sampler. The separations were conducted using Restek Rtx-5MS fused silica capillary column (5%-diphenyl-95%-dimethylpolysiloxane) of 30 m× 0.25 mm internal diameter (di) and 0.25 mm in film thickness. The conditions for the analysis were set as previously reported by Ololade *et al* [1].

Experimental Animals and Designs

The study utilized male Wistar rats, locally bred at the University of Medical Sciences, Ondo State Animal House. The rats were acclimatized for one week in standard environmental conditions, including a 12-hour day/night cycle, and were provided with rat feed and water *ad libitum*. Thirty rats (95.0±5.0 g) acclimatized for one week were grouped into 5, (n=6). Group 1 (Normal control) received distilled water. Other groups were induced with diabetes using 100 mg/kg alloxan intraperitoneally and treated as follows: Group 2 (olive oil), Groups 3 and 4 (200mg/kg and 400mg/kg OTE, respectively), and Group 5 received 5mg/kg glibenclamide. After twenty-one days of administration, the rats were fasted overnight and sacrificed. Blood and liver samples were collected for analysis. Glucose status was measured using a glucometer. Serum insulin concentration was measured using an Enzyme-linked immunosorbent assay. Antioxidant assays were conducted using a spectrophotometric method [19, 20]. The rats were kept in good environmental conditions as prescribed by ethical conditions.

Induction of Diabetes and Blood Samples Collection: Diabetes was induced according to the previous method used by Ghasemi and Jeddi [21]. Alloxan induces diabetes by selectively destroying pancreatic β-cells through the generation of reactive oxygen species (ROS), leading to insulin deficiency and hyperglycemia. The blood sample was collected as described in the work done by Ololade et al [18].

Blood glucose measurement

This method operates on the principle of amperometry, in which the peak current generated during an electrochemical reaction, under a constant potential applied between electrodes, serves as a measure of the analyte's concentration. A glucometer uses this technique to quantify the glucose concentration in each solution [22].

Insulin

An insulin assay is a blood test designed to assess insulin production by pancreatic beta cells [18].

Procedure

Serum insulin extracted from whole blood was utilized for this assay. A microplate was coated with a capture antibody specific to insulin. Following this, 25 µl of the capture antibody solution was added, and the plate was rinsed with 400 µl of buffer. To prevent non-specific binding, the plate was blocked with Bovine Serum Albumin (BSA) and incubated for 1–2 hours. Insulin standards were prepared, and both the standards and samples were diluted using a dilution buffer. A detection antibody conjugated with horseradish peroxidase (HRP, 100 µl) was introduced to bind the captured insulin. The plate was incubated to allow binding between the capture and detection antibodies and the insulin in the samples. To remove unbound material, the plate was washed with a suitable wash buffer. A substrate solution (200 µl) was then added, initiating a reaction that produced a colour change proportional to the insulin concentration. Finally, a stop solution (50 µl of an acidic solution) was added to terminate the colour development. The absorbance of each well was measured at a wavelength of 450 nm using a plate reader.

Antioxidant Enzyme Assay

Evaluation of catalase activity, lipid peroxidation, reduced glutathione level, and Glutathione S-transferase activity was determined according to the previous method used [18,23].

Determination of Catalase Activity

The enzyme activity was done by modification of the method previously used by Oseni et al [23].

Principle

The method is based on the loss of absorbance observed at 240 nm as catalase splits hydrogen peroxide. Even though hydrogen peroxide has no absorbance maximum at this wavelength, its absorbance correlates well enough with concentration to allow its use for a quantitative assay. An extinction coefficient of 0.0436 $mM^{-1}cm^{-1}$ was used.

Procedure

Hydrogen peroxide (2.95 ml of 19 mM solution) was pipetted into l cm quartz cuvette, and 50µl of the sample was added. The mixture was rapidly inverted to mix and placed in a spectrophotometer. Change in absorbance was read at 240 nm every minute for 5 minutes.

Calculation

$$\text{Catalase activity} = \frac{\Delta A240/\text{min} \times \text{reaction volume} \times \text{dilution factor}}{0.0436 \times \text{sample volume} \times \text{mg protein/ml}} = \mu\text{mole } H_2O_2/\text{min/mg protein}$$

Assessment of Lipid Peroxidation

Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) present in the test sample according to the method previously used by Oseni et al [23].

Principle

Under acidic conditions, malondialdehyde (MDA) produced by peroxidation of fatty acids reacts with the chromogenic reagent 2-thiobarbituric to yield a pink-coloured complex with maximum absorbance at 532nm.

Procedure

An aliquot of 0.4ml of the test sample was mixed with 1.6ml of Tris-KCl buffer to which 0.5ml of 30% TCA was added. Then 0.5ml of 0.75% TBA was added and placed in a water bath for 45 minutes at 80°C. This was then cooled in ice to room temperature and centrifuged at 3,000 rpm for 10 minutes. The clear supernatant was collected, and the absorbance was measured against a reference blank of distilled water at 532nm.

Calculation

The MDA level was calculated using an extinction coefficient of 0.156$\mu M^{-1}cm^{-1}$.

$$\text{Lipid peroxidation}\left(\text{nmol}\frac{\text{MDA}}{\text{mg}}\text{protein}\right) = \frac{\text{Absorbance} \times \text{Volume of mixture}}{\text{E532nm} \times \text{Volume of sample} \times \text{mg protein/ml}}$$

Estimation of Reduced Glutathione (GSH) Level

The estimation of the level of reduced glutathione (GSH) was carried out based on the method previously used by Oseni et al [23].

Principle

This method is based upon the development of a relatively stable yellow-coloured product when 5, 5-dithiobis-2-nitrobenzoic acid (DTNB, Ellman's reagent) is added to sulfhydryl compounds, of which glutathione comprises the bulk in tissues. The resulting chromophoric product possesses maximum absorbance at 412 nm.

Procedure for the sample

The sample (0.4ml) was added to 0.4ml precipitating solution, which was vortexed and centrifuged at 4000rpm for 5 minutes. Thereafter, 0.5 ml of the supernatant was added to 1.5 ml of Ellman's reagent. The absorbance of the reaction was read at 412nm against a reagent blank.

Estimation of Glutathione S-Transferase Activity

Glutathione-S-transferase activity was determined according to Oseni et al [23].

Principle

The assay is based on the principle that all known glutathione-S-transferase isotypes demonstrate a relatively high activity with 1-chloro-2, 4-dinitrobenzene (CDNB) as the second substrate. When CDNB is conjugated to reduced glutathione, its absorption maximum shifts to a longer wavelength, and the absorption increase at the new wavelength of 340 nm provides a direct measurement of the enzymatic reaction.

Procedure

The medium for the estimation was prepared as shown in the table below, and the reaction was allowed to run for 3 minutes with readings taken every 60 seconds against a blank at 340nm.

Calculation

The extinction coefficient of CNDB at 340nm = 9.6mM-1cm-1

$$\text{Glutathione} - \text{S} - \text{transferase activity factor} = \frac{\Delta A340/\text{min} \times \text{reaction volume} \times \text{dilution}}{9.6 \times \text{sample volume} \times \text{mg protein/ml}} = \mu\text{mole/min/mg protein}$$

Ethical Approval

The University's ethical committee approved and evaluated this study before any data were collected and ensured that all areas of animal experimentation were done in line with the highest ethical standards. The ethical approval reference number is UNIMED-AREC/Apv/2025/76.

Statistical analysis

Results were analyzed using one-way analysis of variance and reported as mean ± standard deviation. Mean differences were determined using the Tukey test. $P< 0.05$ was considered statistically significant.

Results

From GC-MS analysis, a total of thirty-eight (38) chemical components were identified in the aerial extract of *O. tenuiflorum*, accounting for (97.9%), of the total components in the extract (Table 1).

Table 1: Chemical Composition of the Aerial Extract of *Ocimum tenuiflorum*

Compound	**Percentage Composition**
methyleugenol	27.5
3-carene	11.0
1-(4,5-dimethyl-2-nitrophenyl)-1Htetraazole	7.0
2-methyl-3,5-dodecadiyne	7.0
o-nitrocumene	7.0
2-phenyl-1-hexanol	7.0
caryophyllene	5.5
humulene	3.7
patchoulane	3.3
D-limonene	2.5
(+)-4-carene	2.0
β-elemene	1.8
β-copaene	1.5
aromadendrene	1.0
(5E,9E)-12-methyl-1,5,9,11-tridecatetraene	1.0
β-cis-caryophyllene	1.0
trans-7-hydroxymethyl-3- cyclopropylbicyclo[4.1.0]heptane	0.9
2,4-diisopropenyl-1-methyl-1-vinylcyclohexane	0.9
β-myrcene	0.8
4-methylene-1-(1-methylethyl)bicyclo[3.1.0]hexane	0.5
(+)-*δ*-cadinene	0.5
1,9-decadiyne	0.5
3-[(1Z)-1-butenyl]-4-vinyl-1- cyclopentene	0.5
1-(1-ethylvinyl)-1-(2-methylene-3- butenyl)cyclopropane	0.5
8- methylenedispiro[2.1.2.4]undecane	0.5
1-(2-nitro-2-propenyl)-1- cyclohexene	0.5
2,3,3-trimethyl-1,4-pentadiene	0.3
α-phellandrene	0.2
α-4-dimethylbenzenemethanol	0.2
2,3,4-trimethyl-1,4-pentadiene	0.2
1,3-dimethyl-1-cyclohexene	0.2
iso-borneol	0.2

α-cubebene	0.2
2,3,5-trimethyl-4-methylene-2-cyclopenten-1-one	0.1
copaene	0.1
megastigma-7(E),9,13-triene	0.1
nopol	0.1
2-(1-methyl-2-propenyl)bicyclo[2.2.1]heptane	0.05
Percentage Total	**97.9**

Anti-Diabetic Biochemical Assays

The results on the blood glucose revealed a significant increase in the blood glucose level of diabetic control relative to normal control. However, there was a dose-dependent decrease in groups treated with OTE and glibenclamide compared to normal rats. Moreover, there was no significant difference between the blood glucose level of the group administered 400 mg/kg OT extract and normal rats (Figure 1).

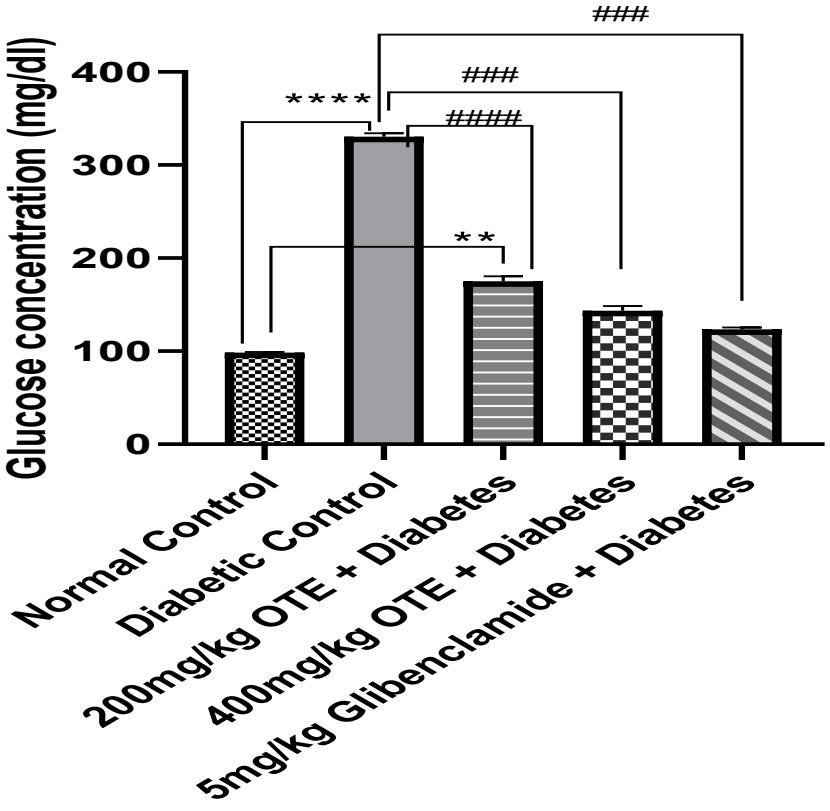

Figure 1: Effect of OTE on glucose concentration in alloxan-induced diabetic rats.

OTE: *Ocimum tenuiflorum* Extract; **P< 0.01: Test groups vs normal control; ****P< 0.0001: Test groups vs normal control; P< 0.001: Other groups vs Glibenclamide group; P< 0.0001: Other groups vs Glibenclamide group

A significant decrease was observed in the insulin level of the diabetic control group relative to the normal control. Conversely, there was a significant dose-dependent increase in insulin concentration of groups treated with OT extract and glibenclamide in comparison with the normal control group (Figure 2).

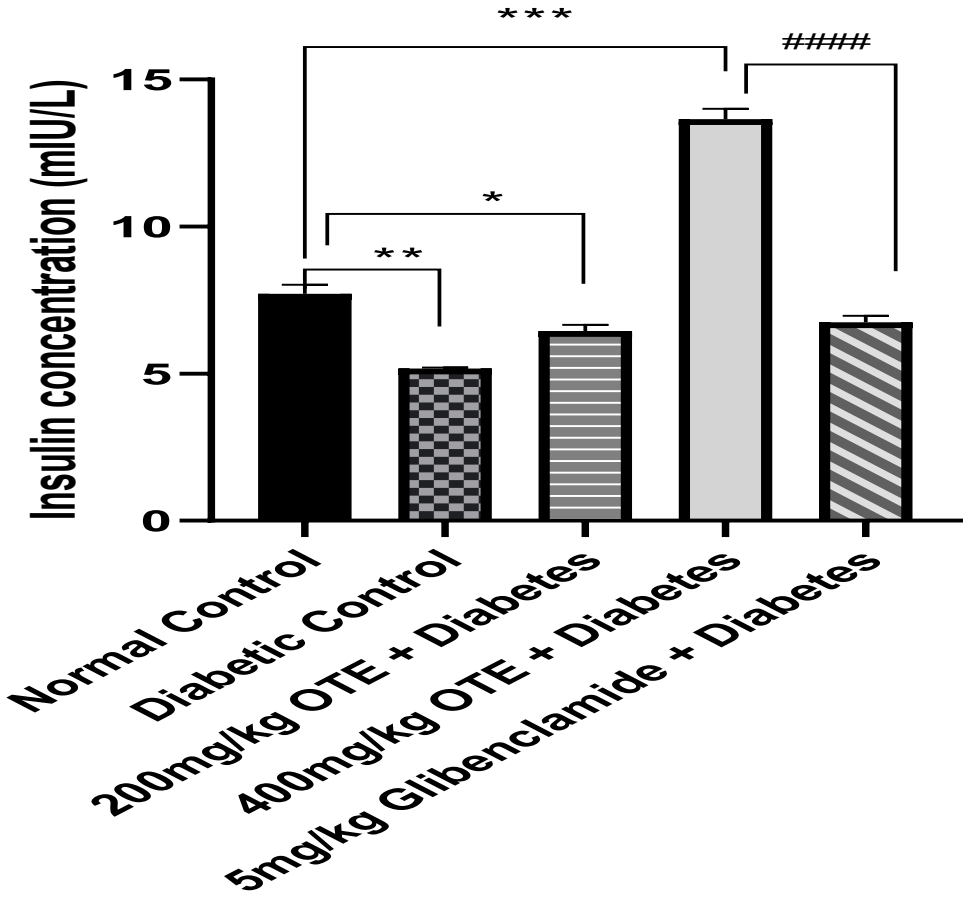

Figure 2: Effect of OTE on insulin concentration in alloxan-induced diabetic rats.

OTE: *Ocimum tenuiflorum* Extract; *P<0.05: Test groups vs normal control; **P< 0.01: Test groups vs normal control; ***P< 0.001: Test groups vs normal control; P< 0.0001: Other groups vs Glibenclamide group.

In an experiment that evaluated the effect of *Ocimum tenuiflorum* leaf extract (OTE) on alloxan-induced diabetic rats, notable changes in malondialdehyde (MDA) levels were observed after four weeks of treatment. While the diabetic control group showed elevated MDA levels compared to healthy rats, the groups treated with OTE displayed a dose-dependent reduction in oxidative stress markers. Specifically, higher doses of OTE exhibited lower MDA levels (Figure 3).

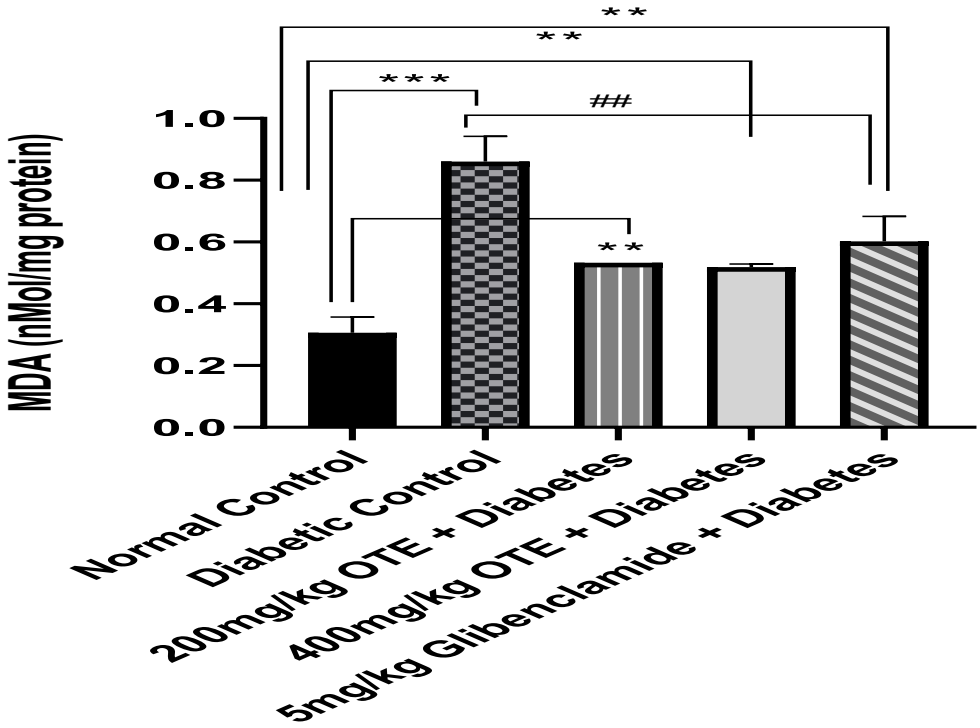

Figure 3: Effect of OTE on Lipid peroxidation in alloxan-induced diabetic rats.

OTE: *Ocimum tenuiflorum* Extract; MDA: Malondialdehyde; **P< 0.01: Test groups vs normal control; ***P< 0.001: Test groups vs normal control; P< 0.001: Other groups vs Glibenclamide group.

The results, as depicted in Figure 4, showed that while diabetic control rats exhibited a significant decline in GSH levels relative to normal rats, groups treated with OTE or the standard antidiabetic drug, glibenclamide, showed a dose-dependent elevation in GSH levels. Notably, the OTE-treated groups exhibited similar activity to glibenclamide.

The results in Figure 5 revealed that diabetic control rats had a significant decline in GST activity compared to normal rats. Groups treated with OTE or glibenclamide demonstrated a dose-dependent restoration of GST activity, but not to normal activity.

The study revealed a noticeable contrast between the catalase activity of the diabetic controls and normal control rats: diabetic controls exhibited a significant decline in enzyme activity compared to normal rats (Figure 6). Conversely, OTE-treated groups were observed to display elevated catalase activityrelative to normal rats. Interestingly, the observed increase in catalase activity of OTE-treated groups exceeded that of glibenclamide-treated rats.

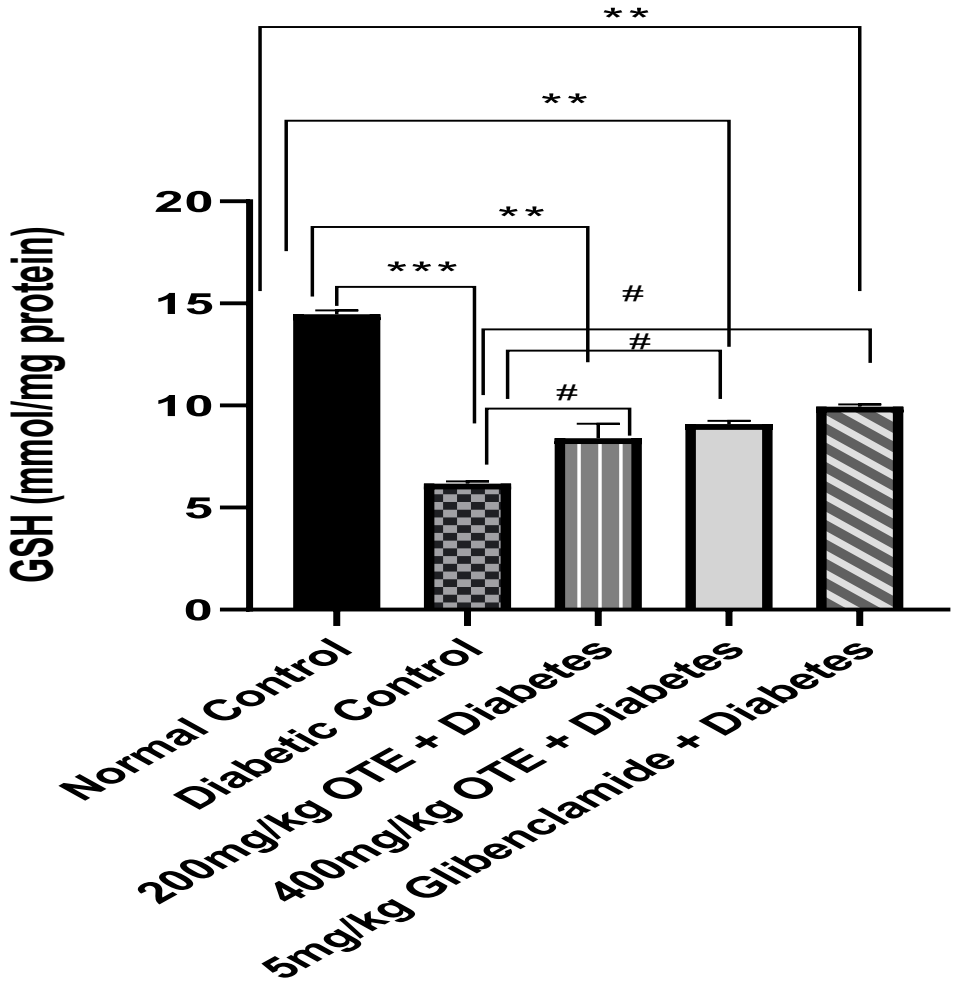

Figure 4: Effect of OTE on GSH status in alloxan-induced diabetic rats.

OTE: *Ocimum tenuiflorum* Extract; **P< 0.01: Test groups vs normal control; ***P< 0.001: Test groups vs normal control; P< 0.05: Other groups vs Glibenclamide group.

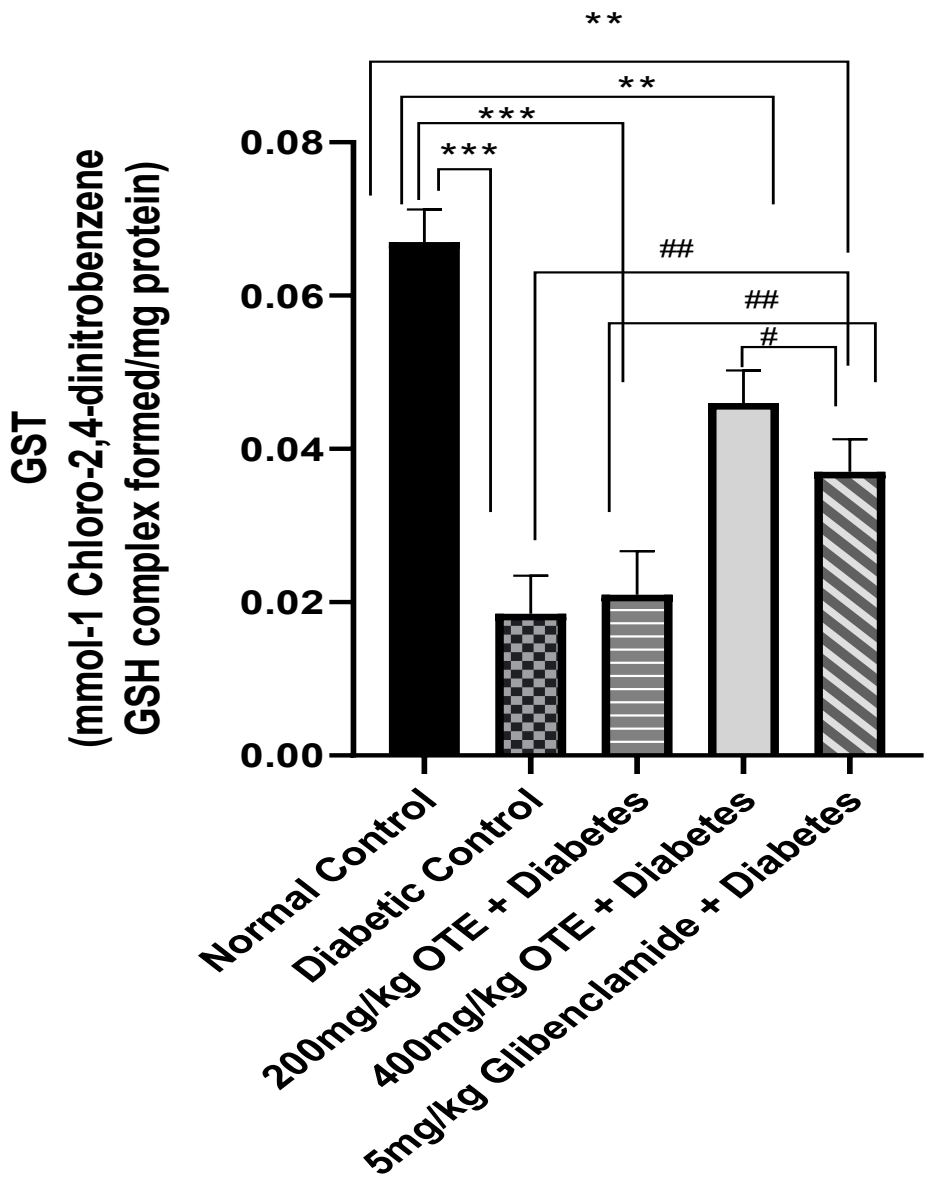

Figure 5: Effect of OTE on GST activity in alloxan-induced diabetic rats.

OTE: *Ocimum tenuiflorum* Extract; **P< 0.01: Test groups vs normal control; ***P< 0.001: Test groups vs normal control; P< 0.05: Other groups vs Glibenclamide group; P< 0.01: Other groups vs Glibenclamide group.

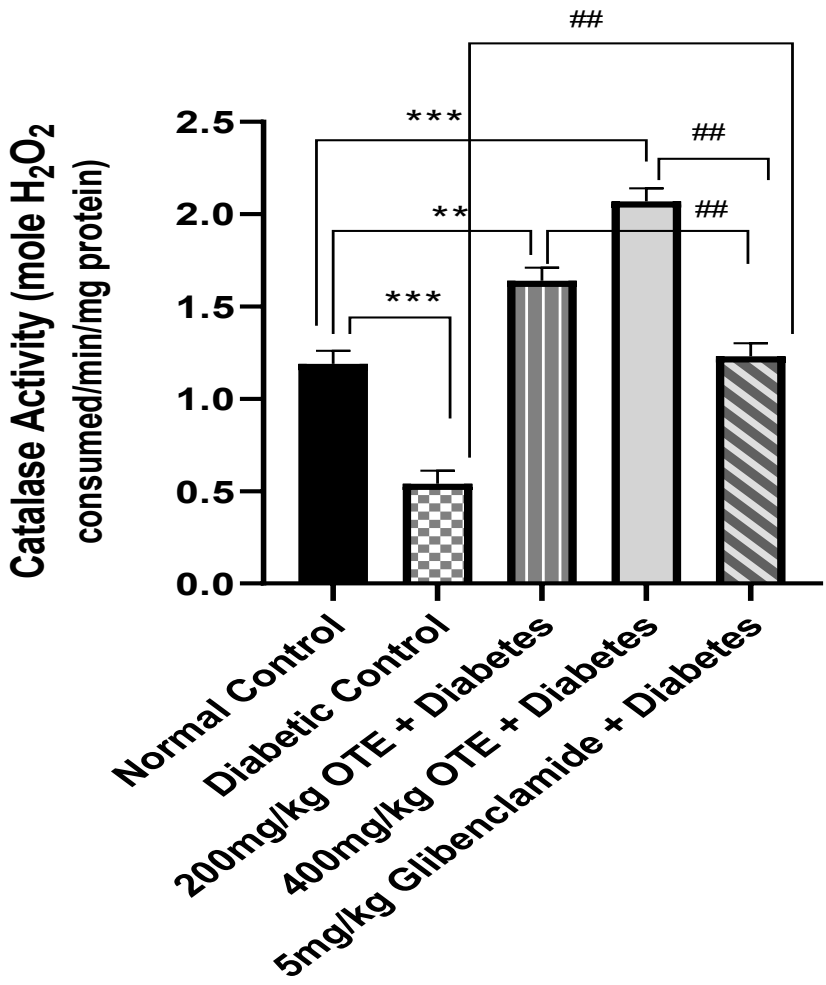

Figure 6: Effect of OTE on Catalase activity in alloxan-induced diabetic rats.

OTE: *Ocimum tenuiflorum* Extract; **P< 0.01: Test groups vs normal control; ***P< 0.001: Test groups vs normal control; P< 0.01: Other groups vs Glibenclamide group.

Discussion

Alloxan, a well-known diabetogenic agent, selectively destroys pancreatic β-cells, leading to insulin deficiency and hyperglycemia, thus establishing an experimental model for type 1 diabetes mellitus.

The significant elevation in blood glucose levels in the diabetic control group compared to the normal control confirms the successful induction of diabetes by alloxan, which severely impairs glucose homeostasis due to compromised insulin secretion. In contrast, the observed dose-dependent reduction in blood glucose levels of OT-treated groups suggests that it possesses potent antihyperglycemic properties that may be attributed to its bioactive compounds, such as flavonoids, polyphenols, and terpenoids, which have been reported to enhance glucose uptake and modulate insulin secretion [24].

Furthermore, the observed decline in blood glucose levels in OT-treated groups parallels the effects seen in the standard drug-treated group (glibenclamide). Glibenclamide, a well-established sulfonylurea, functions by stimulating pancreatic β-cells to enhance insulin secretion [25], thereby lowering blood

glucose levels. The similarity in response between OT-treated groups and the glibenclamide group indicates that OT leaf extract may share similar mechanisms of action, probably through the insulin-mimetic effects or by enhancing β-cell regeneration. The presence of methyl eugenol in some plants has previously been reported for its insulinotropic activity.

Notably, the group receiving 400 mg/kg OTE demonstrated blood glucose levels comparable to those of normal, non-diabetic rats. This finding is particularly significant as it suggests that at an optimal dosage, OTE can effectively restore glycemic control to near-normal levels.

The observed significant reduction in insulin levels of diabetic rats compared to the normal control group confirms the β-cell destruction caused by alloxan. This severe decline in insulin production further supports the hyperglycemic state. Conversely, a significant increase in insulin concentration observed in groups treated with OTE and glibenclamide, in comparison with the diabetic control group, suggests that OTE may have a protective or regenerative effect on pancreatic β-cells, potentially facilitating insulin secretion [26,27]. The similarity between OTE and glibenclamide-treated groups indicates that the bioactive compounds in OTE may act via mechanisms that enhance insulin production, β-cell repair, or insulin sensitivity.

Additionally, the increased insulin levels in OTE-treated groups surpass those of the normal control group, suggesting a stimulatory effect on insulin synthesis, which could contribute to the significant blood glucose reductions. This aligns with previous studies on medicinal plants with antihyperglycemic properties, where bioactive compounds enhance insulin secretion and β-cell function. The observed effects of OTE further underscore its potential as a natural therapeutic agent for diabetes management. It was also found to function as an insulin secretagogue by enhancing the synthesis of insulin [5].

Malondialdehyde (MDA) is a well-known biomarker of lipid peroxidation and oxidative stress, which are significantly elevated in diabetic conditions due to persistent hyperglycemia and the subsequent generation of reactive oxygen species (ROS) [28,29]. As observed, the diabetic control group exhibited a significant increase in MDA levels compared to the normal control, indicating heightened oxidative stress and cellular damage in untreated diabetic rats.

In contrast, the dose-dependent decrease observed in MDA levels of groups treated with OTE relative to the diabetic control group is suggestive of the potent antioxidative properties inherent in the extract, which potentially mitigated oxidative stress-induced damage by scavenging of free radicals and inhibiting lipid peroxidation [30].

The significant decline in MDA levels in OTE-treated groups aligns with previous studies on medicinal plants rich in flavonoids, polyphenols, and other antioxidant compounds. These bioactive constituents play a crucial role in enhancing endogenous antioxidant defenses, reducing oxidative stress markers, and protecting cellular structures from oxidative damage. Additionally, the observed reduction in lipid peroxidation may contribute to improved pancreatic *β*-cell function, thereby supporting insulin secretion and glucose homeostasis.

The significant reduction in GSH level of the diabetic rats relative to normal control is consistent with the previous results on the probable reason for elevated MDA level in the untreated diabetic rats [31,32].However, an increase in GSH level upon treatment with doses of OTE could suggest that it may enhance the endogenous antioxidant defense system by boosting GSH synthesis or preventing its depletion.

Significant decline in GST activity of the diabetic control group relative to normal rats' highlights compromise in the cellular defense mechanisms [33]. In contrast, the observed dose-dependent increase in GST activity of the rats treated with OTE could indicate that the extract may contribute to the restoration of detoxification pathways and enhancement of cellular resistance to oxidative damage.

Catalase is an essential enzyme that decomposes hydrogen peroxide into water and oxygen, thereby preventing oxidative damage. A decrease in catalase activity of the diabetic control group is suggestive of impaired antioxidant defense. However, a dose-dependent increase observed in groups treated with OTE further substantiates its antioxidant potential. Studies have shown that 3-carene, one of the bioactive substances of OTE, is known to have other therapeutic effects such as anti-inflammatory, antioxidant, and anti-stress [34].

Conclusion

The aerial part extract of *O. tenuiflorum* was found to have excellent anti-diabetic potential. The aerial part of the plant indeed possessed valuable secondary metabolites, which could be exploited to control diabetic problems. The mechanism of the anti-diabetic activity of the sample investigated is due to the synergetic reactions of the phytochemicals, such as terpenoids and phenolic compounds, present in the medicinal plant. The observed medicinal effects of OTE further underscore its potential as a natural therapeutic agent for diabetes management. It could be inferred from the results above that *O. tenuiflorum* exhibits its anti-diabetic potential via enhancing the synthesis of insulin and scavenging of free radicals. Therefore, the leaves of the plant studied are a potential source of a dietary anti-diabetic agent and demonstrate the importance of this plant in medicine and in assisting primary health care in this part of the world.

Conflict of interest: We have no conflict of interest.

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