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Original Research Article

Datura Metel Leaf Extracts Potential on Antioxidant Enzymes in Alloxan-Induced Diabetic Albino Rats

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ABSRTACT

Public water systems must provide reliable, safe drinking water 24/7 to prevent contamination and illness. Regular testing for coliform bacteria is crucial to ensure clean, dependable water for the public. This study aimed to find coliforms in the public water supply in the Sokoto Metropolis. Eight (8) sterile bottles were used to collect eight (8) water samples, two samples from each of the four water treatment plants in Gagi, Arkilla, Wamakko, and Tashar Illela. As directed by the manufacturer, nutrient agar media, MacCkonkey, and Eosine methylene blue were produced. Standard procedures were followed for the isolates' verification and isolation. The result of this study has shown that the sample obtained from Gagi water production had the highest total mean count (25.9×10^6 CFU/ml). The sample obtained from Arkilla had the lowest total mean count of 4.7×10^5 CFU/ml, and the total coliform (MPN) recorded indicated that the samples obtained from Wamakko had the highest Most probable number (14/100ml). In contrast, the samples obtained from Arkilla had the lowest coliform count (6/100ml). The bacteria identified include *Shigella spp* (12.5%), *Pseudomonas aeruginous* (37.5%), *Staphylococcus aureus* (12.5%), *Enterobacter spp* (12.5%) and *Salmonella spp (25%).* Improvements to the environmental conditions in the research location and regular testing by the treatment facilities for coliforms are advised to create better water.

Keywords: Public water, *Shigella spp., Salmonella spp., Staphylococcus aureus,* Environmental

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Introduction

Medicinal plants have been a vital source of therapeutic agents due to their bioactive components.[1] The WHO Expert Committee on Diabetes recommended further investigation of medicinal herbs as they are often considered less toxic, more affordable, and associated with fewer side effects.[2] Plants are rich sources of antidiabetic compounds such as alkaloids, flavonoids, phenolics, and tannins, improving pancreatic tissue function by increasing insulin secretion or decreasing intestinal glucose absorption.[3,4] Numerous medicinal plant extracts were evaluated for their ability to influence metabolic pathways such as glycolysis, gluconeogenesis, the Krebs cycle, glycogen synthesis and degradation, insulin production and secretion, cholesterol synthesis, carbohydrate metabolism, and absorption.[5]

Diabetes is a chronic metabolic disorder that affects the metabolism of proteins, lipids, and carbohydrates.[6-7] It is characterised by high blood glucose levels following meals and is the sixth most significant cause of mortality. Diabetes occurs when the secretion of insulin, a hormone essential for glucose metabolism by the β-Langerhans islet cells of the pancreas, diminishes.[8]

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After consuming a meal rich in carbohydrates, blood sugar levels increase, which triggers the release of insulin that activates glucose metabolism in the liver. It also prompts adipose and muscle cells to extract glucose from the bloodstream, markedly reducing blood sugar levels to a normal range. In individuals with diabetes, blood glucose levels persistently rise due to inadequate insulin secretion by the pancreas.[9] The International Diabetes Federation reports that some 463 million persons aged 20 to 79 years are currently living with diabetes, a figure projected to increase to 700 million by 2045. The prevalence of type 2 diabetes is rising in numerous countries, with around 79% of persons affected residing in low and middle-income nations. [10] Despite considerable advancements in diabetes treatment, further research is necessary due to the limitations of existing therapies, including insulin therapy, medication, and dietary management.[3] The drawbacks of these therapeutic modalities encompass medication resistance (diminished efficacy), adverse effects, and potential toxicity. An illustration is the sulfonylureas, which diminish effectiveness after six years of treatment, as noted in 44% of patients.[11] Moreover, these medications have demonstrated negligible efficacy in managing hyperlipidemia related to diabetes.[12]

A study by Halliwell[13] identified reactive oxygen species (ROS) as contributors to diseases and disorders such as diabetes, cancer, and liver cirrhosis. Reactive oxygen species (ROS), including superoxide anion, hydrogen peroxide, and singlet oxygen, induce oxidative stress by disrupting the equilibrium between their generation and the neutralisation by antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD), resulting in a significant elevation of malondialdehyde, a biomarker indicative of oxidative stress levels.[13-14] Nevertheless, an elevation in antioxidant levels might mitigate the oxidative damage induced by chronic diabetes.[14] Alloxan induces diabetes by elevating reactive oxygen species (ROS) production, leading to partial degradation of pancreatic islet beta (β) cells, causing diabetes mellitus.[16-17]

Recent studies demonstrate that extracts from medicinal plants are more effective in controlling diabetes.[18] This is attributable to their antioxidant constituents, including carotenoids, flavonoids, terpenoids, alkaloids, and glycosides, often demonstrating antidiabetic effects.[19] Metformin is an oral hypoglycemic agent produced from the medicinal plant *Galega officinalis*. Historically, it served as a treatment for diabetes in medieval Europe. [20] The anti-hyperglycemic properties of botanical therapies are mostly ascribed to their ability to improve pancreatic tissue function by increasing insulin secretion or reducing intestinal glucose absorption.[3]

Datura metel is a herbaceous plant belonging to the Solanaceae family. The common names for *D. metel* are Devil's Apple and Thorn Apple. It can reach a height of up to 200 centimeters. [21] The leaves are placed alternately and can attain dimensions of up to 21 cm in width and 26 cm in length. The fruit can produce up to three hundred seeds and usually dehisces to release the seeds upon ripening. The leaves and seeds of *Datura metel* are employed for multiple purposes owing to their alleged psychotropic properties.[22] The phytochemical screening results reveal the presence of alkaloids, flavonoids, tannins, saponins, glycosides, and non-protein amino acids in the crude leaf extracts of *Datura metel*; however, steroids and terpenoids were absent in all extracts.[23] Notwithstanding progress in diabetes therapy, existing medicines frequently prove inadequate owing to their adverse effects, resistance, and restricted efficacy. Investigating medicinal herbs such as *Datura metel* presents a viable option, as these plants are abundant in bioactive chemicals with antioxidant and antidiabetic attributes. This study examines the effectiveness of *Datura metel* in alleviating oxidative stress in diabetic circumstances, thereby addressing a notable deficiency in the existing therapy landscape.

Materials and methods

Plant material and extract preparation.

The leaves of *Datura metel* were collected in Ihiagwa, Owerri, Imo State, Nigeria, in March 2023. Dr. D. I. Edet, a plant taxonomist from the Department of Forestry and Wildlife at the School of Agricultural Technology, Federal University of Technology Owerri, made the identification. The specimen was subsequently placed at the University Herbarium under voucher FUTO/HERB/2023/052 for reference.

The collected leaves samples were rinsed with distilled water, shade-dried under aseptic conditions, and then pulverised using an electric blender. The extract was prepared using a method previously reported.[27] Powdered *Datura metel* leaf (1 kg) was soaked in 3 L of ethanol for 24 hours. The extract was filtered through sterile muslin fabric and Whatman No. 1 filter paper. The filtrate was then evaporated in a regulated water bath at 40℃. The residual ethanol in the extract evaporated in open air, resulting in a dark greenish extract. 20 g of the extract was dissolved in 5 mL of 3% polysorbate 80 and diluted to 100 mL with distilled water. The extract was stored in the refrigerator at 4℃ until it was evaluated.

Ethics statement

The Ethics Committee of the School of Health Sciences, Federal University of Technology, Owerri, approved the study (FUTO/SOHT/2023/66). All animals received care following the WHO's ethical code for animal experimentation.[24]

Chemicals and Dosage Selection

All chemicals and reagents used in this experiment were Analytical-grade chemicals from Randox Laboratories Ltd, UK, and Teco Diagnostics USA. The doses of *Datura metel* leaf extract (250 mg/kg and 500 mg/kg) were selected based on previous studies demonstrating their efficacy in similar experimental models.[25] The use of alloxan to induce diabetes was chosen due to its well-documented ability to selectively destroy pancreatic β-cells, thereby mimicking the oxidative stress associated with diabetes.[26]

Experimental animals

Twenty-five male albino rats weighing 160 and 220 grams were used for this study. They were procured from the animal facility, Department of Physiology, College of Medicine, University of Nigeria, Enugu campus. The rats were housed in the animal facility at the Department of Biochemistry, Federal University of Technology, Owerri. The animals acclimatised for 14 days under standard laboratory conditions before the commencement of this research with unrestricted access to commercially produced feed (Grower's feed from Vita Feed Nig. Ltd.) and clean drinking water. The commercial rat feed came from Okigwe in Imo State and comprises the following constituents, as shown in Table 1.

Table 1: Composition of rat feed

Parameter	Value
Crude protein	16.00%
Calcium	1.0%
Crude fiber	7.0%
Fat/oil	5.0%
Phosphorus	0.4%
Lysine	0.75%
Methionine	0.36%
Salt (min)	0.30%
Energy	2,450 kcal/kg

Acute toxicity (LD_{50} Determination).

The acute toxicity index was evaluated using the LD_{50} method described by Lorke. [27] LD_{50} refers to the dose of a chemical that causes mortality in 50% of a population of animals after exposure to the substance. This methodology comprises two distinct components. In the preliminary stage, nine (9) rats were randomly allocated into three (3) groups, each including three animals. The groups received a single oral administration of ethanol leaf extract of *Datura metel* at dosages 10, 100, and 1000 mg/kg of body weight.

The animals were meticulously observed for the first hour and, subsequently, every 30 minutes during the following 24 hours to detect any immediate signs of toxicity, abnormal reactions, or fatality. In the second phase, nine rats were randomly divided into three groups, each containing three rats. The groups received single oral doses of ethanol leaf extract of *Datura metel* at 1600, 2900, and 5000 mg/kg of body weight. The animals were carefully observed for the first hour and every 30 minutes to detect any immediate signs of toxicity, abnormal reactions, or mortality. The extract's lethal dose (LD_{50}) was determined by calculating the geometric mean of the maximum dose that caused 0% mortality and the minimum dose that resulted in death. [27]

$$LD_{50}=\sqrt{\frac{Geometric\ mean\ of\ Maximum\ dose\ with\ 0\%\ mortality}{Geometric\ mean\ of\ Minimum\ dose\ with\ mortality}}$$

Experimental Design

Following a 14-day acclimation period, the rats were categorised into five groups, each comprising five rats. The subsequent treatment was administered: Group one (control group) was administered food and regular saline. Conversely, group two was the untreated diabetic control group. Group three was administered 20 mg/kg of glimepiride and metformin, serving as the standard control group. Group four was administered 250 mg/kg bw of ethanolic leaf extract of *Datura metel*. Group five was administered 500 mg/kg bw of ethanolic leaf extract of *Datura metel*.

Induction of diabetes and sample collection

Diabetes was induced in the rats with a single intraperitoneal injection of alloxan at a dose of 120 mg/kg. The administration of *Datura metel* extract and the positive control drug via oral injection began three days post-diabetes induction and continued for 14 days. Following the 14th day of extract administration, the rats were euthanised, and blood samples were obtained in plain and EDTA collection tubes. The rats'

organs were collected following cervical dislocation, weighed, and fixed in 10% formal saline for histological examination.

Determination of stress enzyme activity
A 10% homogenate of each stomach tissue was prepared using an ice-cold homogenisation buffer consisting of 125 mM sucrose, 125 mM mannitol, 1 mM Ethylene Glycol-bis (*β*-aminoethyl ether)-N, N, N', N'-Tetraacetic acid (EGTA), and 5 nM 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) at pH 7.2, 0.25 M. The homogenisation was performed with 15 strokes in a Teflon pestle homogeniser, followed by centrifugation at 3000 rpm for 15 minutes at 4°C. The supernatant was preserved for subsequent examination.

Determination of superoxide dismutase activity (SOD)
The superoxide dismutase activity of the homogenate was evaluated using the method developed by Marklund & Marklund. [28] Two test tubes, labelled Blank (B) and Test (T), was placed in a test tube rack. In tube T, 100 µL of potassium phosphate buffer, 830 µL of distilled water, and 50 µL of samples were combined, while tube B contained 150 µL of buffer and 830 µL of distilled water. The contents of the tubes were incubated for 10 minutes at 25ºC, after which 20 µL of pyrogallol was added to each tube. The components were inverted to facilitate mixing, and absorbance at 420 nm was recorded for 3 minutes. The variation between the initial and final absorbance, together with the mean absorbance difference, was calculated (Δ420/min).
The activity of superoxide dismutase was quantified as
% inhibition = $((\Delta A\ 420nm/min\ blank - \Delta A\ 420nm/min\ test)(100))/(\Delta A\ 420nm/blank)$
Units of SOD=$(\%\ inhibition)/((100 - \%inhibition))$
Units/ml = Units × Dilution factor.
Units/g tissue = units/mL or g tissue/mL.

Determination of tissue glutathione peroxidase (GPx)
The homogenate's peroxidase activity was assessed using the method established by Chance and Maehly.[29] Two test tubes, designated as blank (B) and Test (T), were positioned in a test tube rack. In tube T, 120 µL of potassium phosphate buffer, 700 µL of distilled water, 50 µL of hydrogen peroxide, and 100 µL of pyrogallol were pipetted, whereas tube B contained 150 µL of buffer, 700 µL of distilled water, 50 µL of hydrogen peroxide, and 100 µL of pyrogallol. The tubes were incubated for 10 minutes at 25ºC, after which 20 µL of pyrogallol was introduced to each tube. Incubation occurred for 3 minutes at ambient temperature, after which 30 µL of samples was introduced into tube T. The samples were combined by inversion, and absorbance was measured at 420 nm over 5 minutes. The change between the initial and final absorbance and the average absorbance difference was computed as Units/mL enzyme.

$=((\Delta A\ 420nm/20s\ test - \Delta A\ 420nm/20S\ blank)(DF))/(12 \times 0.03)$
Where:
DF = dilution factor
12 = millimolar extinction coefficient of pyrogallol
0.03 = volume (in millilitres) of enzyme used
Units/g tissue = units/ml or g tissue/mL

Determination of tissue lipid peroxidation
Lipid peroxidation in the tissue was assessed using Buege and Aust's established method. [30] Thirty microliters of homogenate and 0.05 mL distilled water (as a blank) were combined with 1.0 mL of TBA reagent and incubated in a boiling water bath for 15 minutes. The tube was promptly positioned under a tap for cooling and centrifuged at 1000 rpm for 10 minutes. The absorbance of the transparent supernatant was measured against the blank at 535 nm. TBARS (MDA) concentration was determined using the formula
Concentration (M) = Absorbance/Ɛ.
$1.56 \times 10^5\ M^{-1}\ cm^{-1}$ = extinction coefficient (Ɛ) of the MDA-TBA complex at 535 nm.

Determination of catalase activity
The catalase activity was determined using Kanu's method.[31] This approach relies on measuring the reduction in absorbance of the test sample due to the induced breakdown of H_2O_2. The rate was determined by assessing the decrease in absorbance during 3 minutes at 240 nm in 1.5 mL of the reaction mixture. The reaction mixture comprised 13.2 nM H_2O_2 in 50 nM phosphate buffer (pH 7.0) and 0.1 mL of cell homogenate. The control combination comprised 50 nM phosphate buffer (pH 7.0) and 0.1 mL of cellular homogenate. Catalase activity was quantified in micromoles of H_2O_2 decomposed per minute per gram of cell weight.

Statistical Analysis
All data were presented as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) for a completely randomised block design and Tukey's multiple comparison tests were conducted using Minitab (Version 14.0) software to evaluate the data. Values were deemed statistically significant when $p<0.05$.

Results and Discussion

The crude extract of *D. metel* leaf was subjected to acute toxicity screening using albino rats. The screening results revealed no fatalities in rats administered the crude leaf extract, even at the maximum dose of 5000 mg/kg body weight (Table 2). Consequently, based on the study findings, the plant extract can be classified as safe.
In the antioxidant screening, diabetic rats that received no treatment had a statistically significant difference ($p<0.05$) in antioxidant capacity compared to the normal control groups (Table 3). The administration of the standard drug did not affect superoxide dismutase (SOD) activity compared to the normal control; however, a statistically significant increase ($p<0.05$) in SOD activity when comparing the normal control to the negative control. The catalase activity of the standard control with that of the normal control and between the catalase activity of the standard control and the negative control showed significant differences ($p<0.05$). There was also a significant difference in the glutathione peroxidase (GPX) activity of the standard control (35.36±1.23) to that of the normal control ($p<0.05$) at 37.42±0.80, as well as the negative control (33.64±1.87). There was an increase in the MDA levels in the normal control group (0.63±0.66) compared to the standard control (1.08±0.82), which was statistically significant ($p<0.05$), as well as between the MDA levels of the standard control and the negative control (Table 3). Administration of 250 mg/kg of *Datura metel* leaf crude extract increased SOD activity when compared to the normal control group and test groups. However, no statistically significant difference ($p>0.05$) was noted between the SOD activity of this test group and the negative control. This may be attributed to factors identified by Kakimoto and Ogino[32, 33], including the high instability of SOD, its elevated immunogenicity, low cellular uptake leading to rapid renal clearance in its intact form, and diminished *in vivo* circulation, which may limit the capacity of the leaf extract to enhance endogenous SOD activity.
A comparison of the CAT activity in this test group with the standard control revealed a statistically significant difference ($p<0.05$). Additionally, there was an increase ($p<0.05$) in the CAT value of this test group relative to the negative control. This may be attributed to deficient catalase levels in pancreatic cells, as observed by Goth[34] and Takemoto[35], who indicated that reduced catalase activity in the blood could be linked to diabetes mellitus resulting from alloxan administration, which destroys pancreatic *β*-cells. Given that pancreatic cells exhibit low catalase activity and alloxan administration further compromises them, the leaf extract did not enhance catalase production to the levels observed in the normal control, as evidenced by this study.
A comparison of the glutathione peroxidase activity between the test group and the normal control group revealed a statistically significant difference ($p<0.05$). However, no significant difference ($p>0.05$) was found between the test and negative control groups. In a study, Bienert observed that the half-life of hydrogen peroxide (H_2O_2) exceeds that of superoxide, allowing hydrogen peroxide to traverse membranes via diffusion or through channels such as aquaporins.[36] Consequently, a

greater quantity of GPX would be required to neutralise the hydrogen peroxide in the rats, as evidenced by my results. Consequently, the scavenging of hydrogen peroxide by GPX may be contingent upon the dosage.

In the 250 mg/kg bw treatment group, there was a significant difference (p<0.05) in the malondialdehyde levels compared with those of the normal group and a marked reduction (p<0.05) between this experimental group and the negative control. A study on the atomic absorption spectroscopy of *Datura metel* extract revealed the presence of several trace elements, including iron, calcium, manganese, titanium, magnesium, and potassium, which act as essential co-factors for numerous enzymes in their catalytic functions.[37] Magnesium is a key mineral in glucose and fat metabolism, and insufficient magnesium levels have been associated with impaired insulin release.[38] Manganese enhances insulin activity in animals with diminished insulin levels and facilitates glucose transfer into adipose tissue[39], augmenting lipid peroxides' catalysis and subsequently upregulating antioxidant enzyme activities (Table 3).

Table 2: Result for acute toxicity evaluation of *Datura metel* leaf extract

Groups	No of mice	Mortality
Phase 1		
I	3	None
II	3	None
III	3	None
Phase 2		
IV	3	None
V	3	None
VI	3	None

Table 3: Effect of Datura metel leaf extract on antioxidant enzymes of alloxan-induced diabetic albino rats.

GROUP	SOD	CAT	GPX	MDA
Normal control	29.12±1.25b	13.36±1.05c	37.42±0.80c	0.63±0.66a
Negative control	27.38±0.99a	10.98±0.37a	33.64±1.87a	1.44±0.40c
Standard control	28.06±0.96b	11.24±0.67b	35.36±1.23b	1.08±0.82b
250 mg/kg leaf extract	27.14±0.38a	11.38±0.44b	34.96±0.31a	1.14±0.67b
500 mg/kg leaf extract	27.18±0.27a	11.68±0.80b	35.06±1.62b	1.14±0.47b

All values were compared at p<0.05.
Values with different alphabets vertically differ statistically (p<0.05)
Values with the same alphabet do not differ statistically (p > 0.05).

Administration of 500 mg/kg of *Datura metel* leaf crude extract resulted in a significant difference (p<0.05) increase in SOD activity compared to the normal control; however, no statistically significant difference (p>0.05) was noted in this test group compared to the negative control. This may be attributed to factors identified by Kakimoto and Ogino,[32, 33] including the high instability of SOD, its considerable immunogenicity, low cellular uptake, rapid renal clearance in its intact form, and diminished *in-vivo* circulation, which may limit the leaf extract's capacity to enhance endogenous SOD activity. Also, in the 500 mg/kg bw group, there was a statistically significant difference (p<0.05) in the CAT activity compared to the normal control as well as the negative control group attributed to the low catalase levels exhibited by pancreatic cells of diabetic animals induced with alloxan.[34-35] Given that pancreatic cells inherently possess low catalase activity, alloxan administration further impairs this function, rendering it improbable that the leaf extract can enhance catalase production to the levels observed in the normal control, as evidenced by this study.

Similarly, in the evaluation of glutathione peroxidase (GPx) in the 500 mg/kg bw treated group, there was a statistical increase in the GPx activity in the treated animals compared to the normal control as well as the negative control (p<0.05). This may correlate with Bienert's observations that the half-life of hydrogen peroxide (H_2O_2) exceeds that of superoxides.[36] Moreover, hydrogen peroxide can traverse membranes by diffusion or be facilitated through channels like aquaporins; hence, an increased quantity of GPX would be required to neutralise the hydrogen peroxide in the rats. The results indicate that the scavenging of hydrogen peroxide by GPX may be dose-dependent, and this test group enhances the activity of GPX in comparison to the 250 mg/kg test group. Also, MDA levels decreased in the treated groups compared to the negative and normal controls (Table 3) (p<0.05). The several trace elements, including iron, calcium, manganese, titanium, magnesium, and potassium, are essential co-factors for several enzymes in facilitating their catalytic functions.[37] Magnesium is one of the major minerals involved in the metabolism of carbohydrates and fat, and a low level of magnesium has been implicated in the defective release of insulin.[38] Manganese also increases the action of insulin in animals with low insulin levels and increases glucose transport into adipose tissue,[39] as shown in this study. The administration of 500 mg/kg of the extract upregulates the catalysis of lipid peroxides, [39] which would potentiate the antioxidant capacity of the antioxidant enzymes (Table 3). The presence of various phytochemicals such as flavonoids, saponins, alkaloids, glycosides, and amino acids in *Datura metel* helps elevate the antioxidant capacity of the different enzymatic antioxidants, reducing oxidative stress caused by diabetes mellitus.

Conclusion

The administration of alloxan to rats, which partially destroys pancreatic β-cells and produces reactive oxygen species to cause hyperglycemia, leads to a marked increase in oxidative stress indicators and a reduction in both enzymatic and non-enzymatic antioxidant defence mechanisms. This study indicates that the ethanolic extract of *Datura metel* leaf can enhance the activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) while reducing the levels of malondialdehyde (MDA) in the liver of diabetic rats. Antidiabetic medicinal herbs can effectively address the complexities of diabetes.

Conflict of Interest

The authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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