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Nelsonia canescens (*Acanthaceae*) aqueous extract and partitioned fractions ameliorates type-2 diabetes in alloxan-induced diabetic rats

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Abstract

Background: Diabetes mellitus is a metabolic disorder that affects the body's ability to produce or use insulin. There is a continuous rise of this disease particularly in developing countries due to changes in life style and poverty among the people. In this study, antidiabetic activities of aqueous extract of *Nelsonia canescens* and its partitioned fractions in alloxan-induced diabetic rats were evaluated. Male albino rats were divided into 9 groups (diabetic and non-diabetic) of 5 rats each. Diabetes was induced by single intraperitoneal administration of alloxan (90 mg/kgbwt). The experimental design consists of a diabetic control group (untreated), a normal control group (1 mL saline), a standard diabetic drug (Glibenclamide; 5 mg/kgbwt), two doses (50 and 300 mg/kgbwt) of aqueous extract, ethyl acetate and methanol fractions of *Nelsonia canescens* were orally administered for a period 21 days. Blood glucose of the rats was monitored at 3-days intervals while biochemical and in vivo antioxidant assays of serum and liver were determined after 21 days.

Results: The hypoglycemic effect of the extract observed was in a dose dependent manner with a significant reduction ($p < 0.05$) of blood glucose in ethylaceate fraction > aqueous extract > methanol fraction compared with the diabetic control group. A significant difference ($p < 0.05$) in lipid profiles and serum enzyme activity of rats in the diabetic control group was observed compared with the extract and fraction treated groups.

Conclusion: The results suggest that the aqueous extract and fractions of *N. canescens* showed hypoglycemic and hypolipidemic potentials and significantly ($p < 0.05$) reduced the progression of oxidative stress.

Keywords: Antioxidants, Fractions, Hypoglycemic, Hypolipidemic, *Nelsonia canescens*, Oxidative stress

Background

Diabetes mellitus (DM) is a non-communicable endocrine disorder characterized by an alteration in carbohydrate, protein, and fat metabolism [1, 2]. Globally, it is estimated that 463 million people are affected by DM and this figure may rise to 578 million by 2030 and 700 million by 2045 [3, 4]. DM is classified into three types, insulin dependent diabetes mellitus (IDDM) or type 1, non-insulin dependent diabetes mellitus (NIDDM) or type 2 and gestational diabetes mellitus (GDM). IDDM result from total loss of function of β-cell of islets of Langerhans which are present in the pancreas. NIDDM result from a temporary loss of β cell function which may be due to genetic predisposition that mostly occur in obese persons and associated with high blood pressure and high cholesterol levels while gestational diabetes is a type of diabetes with characteristic hyperglycemia in pregnant women in

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the 2nd or 3rd trimester [3–6]. Globally, DM is considered the most significant influencer for the development of various clinical conditions such as ischemic heart diseases, peripheral neuropathies, ulcerations, and delayed wound healings which consequently alters the life expectancy of affected patients [7]. Diabetes also affects the amount of lipid in the blood, a condition known as diabetic dyslipidemia [8]. Diabetic patients are characterized with lower serum levels of high-density lipoprotein cholesterol (HDL-C), higher serum levels of triglyceride (TG), and similar serum levels for low-density lipoprotein cholesterol (LDL-C) but with higher levels of small dense LDL when compared with nondiabetic patients [9, 10]. To date, the management of diabetes remains a major global challenge with no recorded success in complete treatment of the disease [2]. Many synthetic medicines have been developed to manage diabetes with no reported evidence of absolute recovery from using these drugs [10–14]. The modern oral hypoglycemic drugs produce undesirable and adverse side effects [2]. Therefore, alternative therapy with improved therapeutic potentials and less side effects is urgently needed [15]. There are many traditional herbal medicines recommended for the treatment and management of diabetes [15]. About 25% of the drugs prescribed across the world are derive from plants already used in traditional medicine [15]. Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost [11–13, 16]. Treatment of DM without any adverse side effects is a major challenge confronting physician [2]. More than 800 medicinal plants are currently used for the treatment and prevention of DM with over 450 that have successfully passed through the clinical trial stage from which 109 have their mode of action elucidated [17–20]. Traditional medicinal plants with their active constituents are used for treating different kinds of ailments such as heart diseases, cancer, and diabetes since ancient times [2, 20].

Nelsonia canescens (Lam). Spreng of the family *Acanthaceae* commonly known as the blue pussy plant is a wild medicinal plant which grow abundantly in various parts of the world including India and sub-Saharan Africa [21, 22]. This plant can be found in the rain forest, the savannah and often as secondary colonizers along the road, foot paths and mostly around uninhabited settlements [23]. The plant root is used for the treatment of various inflammatory and pain related ailments such as arthritis [22]. Metabolite analysis of the methanol extract of *Nelsonia canescens* has reported the presence of phenols, flavonoids, tannins, saponins and alkaloids, whereas steroids, glycoside and phlobatannins were not detected [24]. The aim of this study was to evaluate the antidiabetic activity of *N. canescens* aqueous extract and its partitioned fractions in alloxan-induced diabetic rats.

Methods

Chemicals and equipment

Gallic acid, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma-Aldrich Co., St Louis, USA. Sodium acetate anhydrous and Alloxan Monohydrate were obtained from BDH (BDH Laboratory Supplies, England), Formaldehyde Solution from (Laboratory Rasayan, Boisar); Folin–Ciocalteu phenol reagent (FC) from Merck (KGaA, Germany), Glibenclamide (Daonil; Aventis Pharma. Ltd., India), Cholesterol reagent (Teco Diagnostics., USA), Triglyceride GPO reagent (Teco Diagnostics., USA) and HDL Cholesterol reagent (Agape Diagnostics., Switzerland). Other chemicals were of analytical grade. All spectrophotometric measurements were taken using the double beam Shimadzu UV spectrophotometer, UV-1800 (Shimadzu Scientific Instruments Inc, Nakagyo-ku, Kyoto, Japan) while centrifugation was performed using High Speed Refrigerated Centrifuge, LR10-2.4A (Hunan Kecheng Instrument and Equipment Co., Ltd, Changsha city, China).

Processing and extraction of plant materials

The leaves of *Nelsonia canescens* (*Acanthaceae*) were harvested from the vicinity of Bosso campus of Federal University of Technology Minna, Niger State, Nigeria. The plant was identified and authenticated at the Plant Biology Department of Federal University of Technology Minna and a specimen of the plant sample was deposited at the herbarium with a voucher number: FUT/PLB/ACA/001. Plant sample was cleaned and washed with distilled water before air drying at room temperature for 2 weeks before crushing the leaves into smaller particles and blending into fine powder using a clean blender. A portion of the powdered sample (500 g) was refluxed at 40 °C with distilled water for 2 h to obtain the aqueous extract (AQ) which was filtered using a muslin cloth and Whatmann No 1 filter paper. The extract was further concentrated in a water bath at 30 °C and a semi-solid brownish like paste was finally obtained as the extract. The dried extract was stored in the refrigerator at 4 °C prior to usage [23, 25].

Partitioning of the crude extract

Fifty grams (50 g) of the aqueous extract was reconstituted in distilled water and solvent partitioned using ethylacetate (EA) and methanol (ME) in a separating funnel at 25 °C. The fractions were concentrated in a water bath at 30 °C to obtain a solid paste.

Quantitative phytochemical analysis of the extract

Quantitative phytochemical analysis of the crude aqueous extract (AQ), EA and ME fractions were performed

using the method described by Chang et al. [26] and Singleton et al. [27].

Total flavonoid content

A colorimetric method using aluminum chloride ($AlCl_3$) was used to quantify the flavonoid content in the crude extract, EA and ME fractions of *Nelsonia canescens* plants. A 1 mL aliquot of crude extract (1 mg/mL), fractions (EA and ME) or quercetin (12.5–100 mg/mL; standard) were mixed with 0.1 mL of 10% (w/v) $AlCl_3$ in methanol, 0.1 mL of 1 M sodium acetate and 2.8 mL of distilled water. The reaction mixture was incubated at 25 °C for 30 min and absorbance measured at 415 nm against a blank. The results were expressed as mg/g quercetin equivalents.

Total phenolic content

Total phenolic content of the crude extract and fractions (EA and ME) was determined using the Folin–Ciocalteu method. For the crude extract (1 mg/mL) and each fraction, a 0.5 mL aliquot was mixed with 2.5 mL of 10% Folin–Ciocalteu reagent (v/v). This mixture was oxidized with 2 mL of 7.5% Na_2CO_3 (w/v) and incubated at 45 °C for 40 min. The absorbance was read at 765 nm and the amount of phenols in the crude extract and fractions was calculated using the Gallic acid standard curve expressed as mg/g of gallic acid equivalents.

Alkaloids content

For the measurement of alkaloid content in the crude extract and separate fractions (EA and ME), 0.5 g of each sample was dissolved (separately) in 5 mL of 50% ethanol in sulfuric acid and filtered using Whatmann No 1 filter paper. An aliquot (1 mL) of the filtrate was mixed with 5 mL of 60% H_2SO_4 (v/v) and incubated for 5 min. After incubation 5 mL of 0.5% of formaldehyde solution (v/v) was added to the sample mixture and incubated for another 3 h and the absorbance measure at 565 nm. The molar extinction coefficient of Vincristine ($\varepsilon = 15{,}136$ mol/cm) was used to estimate the alkaloids content of the crude extract and fractions (EA and ME).

Saponin content

The total saponin content in crude extract and fraction of *Nelsonia canescens* leaves was determined using a colorimetric assay. 0.5 g of the crude extract and fractions was reconstituted separately in 20 mL of 1 M HCl, at 80 °C for 4 h and then filtered using Whatmann No 1 filter paper. Fifty milliliters of petroleum ether was added to the filtrate in a separating funnel to obtain the ether layer which was evaporated in a water bath. The sample was dissolved in 5 mL of 50% acetone in ethanol, 6 mL ferrous sulfate ($FeSO_4$) reagent and 2 mL of concentrated. H_2SO_4 were added to it and incubated for 10 min. The absorbance of each sample was measured at 490 nm and the amount of saponins extrapolated from a saponin standard curve.

Tannin acid content

The amount of tannins present in the extract and fraction was calculated using the tannic acid standard curve. 0.2 g each of the crude extract and fractions were weighed separately into a beaker and containing 20 mL of 50% methanol (v/v), covered with a foil paper and placed in a water bath at 80 °C for 1 h. 2.5 and 20 mL Folin–Ciocalteu reagent and distilled water, respectively, were added to the mixture and oxidized with 10 mL of 17% Na_2CO_3 (w/v). The mixture was incubated at room temperature for 20 min for color development and the absorbance was read at760 nm.

In vitro antioxidant activity of the crude extract and fractions

ABTS radical scavenging assay

This test was based on the samples' capacity to scavenge the radical cation produced when ABTS solution was reacted with a potent oxidizing agent like potassium permanganate ($KMnO_4$) or potassium persulfate ($K_2S_2O_8$) [28]. Equal volume of 2.45 mM $K_2S_2O_8$ and 7.4 mM ABTS stock solution were mixed together to generate the free radicals' persulfate in the dark for 12 to 16 h at room temperature. To get an absorbance of 0.700 ± 0.02 at 734 nm, the $ABTS^+$ solution was diluted with ethanol (0.90 mL ABTS and 25 mL of ethanol). Aliquot of different concentration of the samples (0.1 mL) and 0.9 mL of the $ABTS^{\bullet+}$ were mixed and allowed to react for 30 min in the dark before reading the absorbance at 734 nm. Percentage inhibition of the radicals was calculated using the formula below:

$$\%\ \text{inhibition} = \frac{A0 - A1}{A0} \times 100 \quad (1)$$

where $A0$ and $A1$ are the absorbance of control (without sample) and test samples, respectively.

DPPH radical scavenging assay

The DPPH radical scavenging potentials of the extract and fractions were evaluated as described by Gyamfi et al. [29]. A total of 1 mL of the crude extract and fractions in a concentration dependent dose (12.5–100 µg/mL) and 1 mL of 0.4 mM methanolic DPPH were mixed and incubated for 30 min in the dark. The absorbance was measured at 516 nm and the percentage inhibition of DPPH radical was calculated relative to the blank (0.4 mM methanolic DPPH) using the equation as follows:

$$\text{Inhibition (\%)} = \frac{A0 - A1}{A0} \times 100 \quad (2)$$

where $A0$ is the absorbance of control (without sample) and $A1$ is the absorbance of the extract and DPPH solution.

Experimental animals and ethics

Forty-five (45) male wistar rats (100–150 g) were obtained from the University of Jos animal farm. The rats were acclimatized for 2 weeks at 28 ± 2 °C, 45–55% of relative humidity on a 12 h light/12 h dark cycle, with access to water and pelletized standard guinea feed ad libitum. The rats were kept for 2 weeks to acclimatize to the environmental conditions. The present study was approved by the Ethical Committee on the use of animals for research at the Federal University Technology, Minna, Nigeria with NO: 000018EAU. Handling of the rats was in accordance with the standard principles of laboratory animal care of the United States National Institutes of Health (NIH, 1978) as reported by Belayneh et al. [10].

Induction of diabetes mellitus

A single intraperitoneal (i.p.) injection of 90 mg/kgbwt of alloxan monohydrate (Sigma, St. Louis, USA) in phosphate buffer saline (PBS) with a pH of 7.4 was used to induce diabetes mellitus in the rats. Animals were selected for the study if their fasting plasma glucose concentration (FPGC) was greater than 111 mg/dL for five consecutive days as determined by the Fine test Autocoding Premium Blood Glucose Monitoring System for self-testing. Nine groups of five male wistar rats each were created from a total of 45 male wistar rats. Before the start of the treatment, the animals were deprived of food and water for an extra 16 h [30].

Experimental design

The experimental design consists of 9 different groups which was based on the treatment received for 21 days as shown as follows:

Group I: normal control (non-diabetic) received1 mL PBS
Group II: positive control and treated with 5 mg/kgbwt of Glibenclamide (reference drug)
Group III: negative control (Diabetic control)
Group IV: treated with 50 mg/kgbwt of AQ
Group V: treated with 50 mg/kgbwt of EA
Group VI: treated with 50 mg/kgbwt of ME
Group VII: treated with 300 mg/kgbwt of AQ
Group VIII: treated with 300 mg/kgbwt of EA
Group IX: treated with 300 mg/kgbwt of ME

The rats were weighed every 7 days to monitor their body weight.

Collection and preparation of blood and tissues

After 21 days, rats who had been fasting overnight were sacrificed by heart puncture anesthetized with 150 mg/kgbwt of sodium pentobarbitone. Blood samples were then taken from each rat and put into a plain sample bottle. The blood samples were centrifuged at 2000 rpm for 10 min after being allowed to clot at room temperature for 2 h. With a fresh Pasteur pipette, the serum was separated. Prior to further biochemical analysis, serum samples were kept at − 80 °C. For liver enzyme analyses, the livers of the rats in each experimental group were removed, washed in normal saline, and stored in 10% formalin (v/v) [10].

Biochemical assays

Using different assay kits, the biochemical indices of the rats after 21-day of treatment with the extract and fractions to evaluate the level of high-density lipoprotein (HDL), cholesterol concentration Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total proteins, albumins, creatinine total cholesterol, and triglyceride. Additionally, the serum LDL-C concentration was determined according to the formula described by Kumari et al. [17] and previously reported by Oluba et al. [31].

$$[\text{LDL-C}] = [\text{TC}] - [\text{HDL-C}] - \frac{\text{TAG}}{5} \quad (3)$$

Serum VLDL-C concentration was estimated using the methods of Burnstein and Sammaille [32] where the ratio of serum VLDL-C to triglyceride concentrations was fixed at 1:5 in fasting animals.

$$[\text{VLDL-C}] = \frac{\text{TAG}}{5} \quad (4)$$

In vivo antioxidant enzyme activity

After sacrifice, the livers of the rats in each treatment group were immediately removed, washed with chilled saline solution, homogenized in ice-cold sucrose (10% w/v) and centrifuged at 10,000 g for 20 min. at 4 °C [33]. The activity of catalase (CAT), superoxide dismutase (SOD), and reduced glutathione (GSH) were assessed in the resulting supernatant.

Catalase activity

According to a method described by Luck (1974) and reported by Ahmad et al. [34], the activity of catalase (CAT) was assessed. 50 µL of the liver's supernatant was mixed with 2.5 mL of a buffer containing 30% H_2O_2 and

vortexed for two minutes. After 30 s and subsequently 90 s, the reaction mixture's absorbance at 240 nm was measured. The blank contained only 2.5 mL of 30% H_2O_2 buffer. The CAT activity was measured using the formula as follows:

$$\text{CAT activity (U/mins per mL of serum)} = \frac{\Delta\text{Abs}}{0.0008} \times 1 \text{ min} \quad (5)$$

where ΔAbs is the difference in sample absorbance after 30 and 90 secs.

Superoxide dismutase activity

The activity of the superoxide dismutase (SOD) enzyme was measured using the method described by Misra and Fridovich [35]. The reaction mixture consists of 20 µL of liver homogenate, 960 mL of sodium carbonate buffer (50 mM, pH 10.2), and 0.1 mM EDTA. Furthermore, 20 µL of 30 mM epinephrine, dissolved in 0.05% v/v acetic acid, were added to the mixture to initiate the reaction. The blank and control contained sodium carbonate buffer and distilled water, respectively. The increase in absorbance was measured at 480 nm for 4 min and activity calculated as follows:

$$\%\text{ inhibition} = 100 - \left(\frac{\Delta\text{Abs control} - \Delta\text{Abs sample}}{\Delta\text{Abs control}} \times 100\right) \quad (6)$$

$$\text{SOD Activity (U/mL)} = \%\text{ inhibition} \times 3.75 \quad (7)$$

where ΔAbs control is the difference in control absorbance at different times and ΔAbs sample is the difference in control absorbance at different times.

Reduced glutathione concentration

Reduced GSH content was assayed following the method described by Jollow et al. [36]. The liver homogenate (50 µL) was mixed with 150 µL of sulfosalicylic acid (SSA) and centrifuged at $5000 \times g$ for 10 min at 4 °C. The amount of GSH was determined by mixing 66 µL of supernatant with 66 µL of 0.01 M 5,5-dithiobis-2-nitrobenzoic acid (DTNB) and 865 µL of potassium phosphate buffer (0.1 M, pH 7.4). After 5 min, the absorbance was measured against SSA as blank at 412 nm and the concentration calculated as follows:

$$\text{GSH concentration (U/mL)} = \frac{\text{Abs}}{0.416} \times 2 \quad (8)$$

where Abs is the sample absorbance.

Statistical and data analyses

The data collected were analyzed using one-way analysis of variance (ANOVA) while treatment means was separated by the least significance difference (LSD) incorporated in the statistical package for social sciences (SPSS) version 20.

Results

Phytochemical constituents of *N. canescens* extract and fractions

The constituents of *N. canescens* extracts and fractions show a varying degree of phytochemicals present. Alkaloids (56.59 ± 1.40 mg/g) and tannins (76.21 ± 0.04 mg/g) were significantly higher in the ethylacetate fraction compared to the aqueous extract and methanol fraction. Phenols was significantly higher ($p < 0.05$) in the aqueous extract (49.65 ± 8.10 mg/g) compared to ethylacetate and methanol fractions while all the phytochemicals' constituents were generally distributed in low abundance in the methanol fraction. Flavonoid content (20.60 ± 0.20 mg/g) of aqueous extract was higher than the fraction while methanol fraction has the highest amount of saponins (17.61 ± 0.26 mg/g).

In vitro antioxidant potentials of *N. canescens* extracts and fractions

The antioxidant capacity of *N. canescens* aqueous extracts and EA and ME fractions was measured based on their ability of reducing ABTS and DPPH radicals. The results show that the radical scavenging capacity of *N. canescens* aqueous extract and fractions occurs in a dose dependent manner when compared to the standard ascorbic acid (Fig. 1). The IC_{50} values of the EA fraction (31.03 ± 0.89 µg/mL) and ME fraction (37.60 ± 1.20 µg/mL) were lower than that of the aqueous extract (50.10 ± 0.26 µg/mL) in the ABTS assay, with no significant changes observed relative to the standard ascorbic acid control standard (38.91 ± 0.25 µg/mL).

Similar to what was observed in the ABTS assay, the DPPH assay also shows a dose-dependent inhibition of the DPPH radicals by the *N. canescens* aqueous extract and fractions with no significant differences observed in the IC_{50} values of the same treatments relative to the standard ascorbic acid control (Fig. 1).

Bars with the same color and the same alphabet have no significance difference at $p < 0.05$.

Effect of *N. canescens* extracts on blood glucose levels of alloxan-induced diabetic rats

The antidiabetic properties of *N. canescens* extracts and fractions (EA and ME) in alloxan-induced diabetic rats were evaluated. The results show that the extract and respective fractions significantly reduced ($p < 0.05$) the blood glucose level in a dose-dependent manner. It is worth noting that the rats used in the diabetic control group did not survive beyond Day 12 of treatment due to

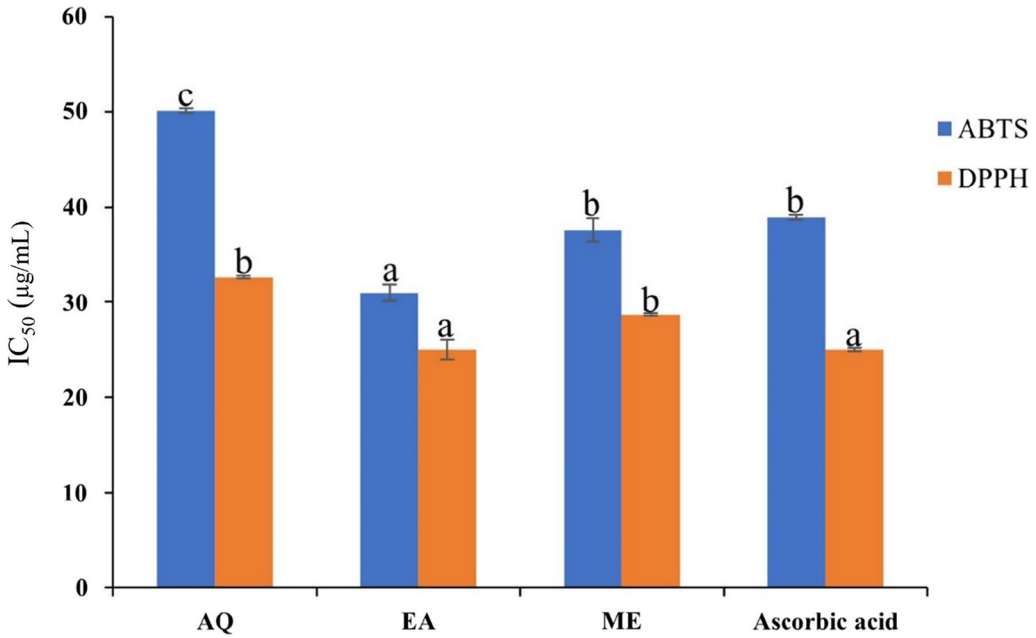

Fig. 1 Median inhibitory concentration (IC_{50}) of crude extract and fractions of *Nelsonia canescens*

a significant spike in blood glucose levels over a period of 6 days. Although a significant increase in glucose levels was observed in the Glibenclamide treatment for the first 3 days, this increase was gradually reduced over time and reached the levels of the normal saline control after 21 days.

Changes in blood glucose levels of alloxan-induced diabetic rats treated with N. canescens extracts and fractions

The aqueous extract of *N. canescens* reduced ($p < 0.05$) the blood glucose levels in alloxan-induced diabetic rats in a concentration dependent manner with the highest reduction of 74.35 ± 11.33 observed in the AQ 300 mg/kgbwt treatment group (Table 2). A similar phenomenon was observed in the EA and ME fraction treatment groups. The EA fraction (300 mg/kgbwt) reduced glucose levels by ($81.86 \pm 5.27\%$) whereas the ME fraction (300 mg/kgbwt) caused a reduction of $78.66 \pm 9.42\%$.

Effect of *N. canescens* extract and fractions on the lipid profile (mg/dL) of alloxan-induced diabetic rats

The effect of *N. canescens* extract and fractions (EA and ME) on the lipid profile of alloxan-induced diabetic rats was in a dose-dependent manner across all the groups; however, rats treated with 300 mg/kgbwt aqueous extract have a significant reduction ($p < 0.05$) in the total cholesterol; HDL and LDL cholesterol were compared with the diabetic control and the normal control groups (Table 3).

Effects of *N. canescens* extract and fractions on biochemical parameters and serum enzymes in alloxan-induced diabetic rats

The aqueous extract and fractions (EA and ME) of *N. canescens* differentially altered biochemical parameters and serum enzyme activity in alloxan-induced diabetic rats when compared to the normal and diabetic controls (Table 4). Apart from treatment with EA (300 mg/kgbwt) and ME (50 mg/kgbwt and 300 mg/kgbwt) fractions of *N. canescens*, no significant changes in total protein (mg/dL) were observed when compared to the normal and diabetic controls.

For the detection of Albumin (mg/dL) in alloxan-induced diabetic rats, no significant change was observed in all treatments except for the ME fraction (50 mg/kgbwt). Ethylacetate fraction at 50 and 300 mg/kgbwt significantly increased the creatinine level of the rats compared with the Glibenclamide treated groups. In the aqueous extract of *N. canescens*, respectively, there was an increase in the ALT levels in the animal's serum compared with the control groups. In addition, the aqueous extract (50 and 300 mg/kgbwt), EA fraction (300 mg/kgbwt) and ME fractions (50 and 300 mg/kgbwt) of *N. canescens* significantly increase the AST

level of rats relative to the normal and diabetic controls (Table 4).

Effects of *N. canescens* extracts and fractions on liver antioxidant enzyme activities in alloxan-induced diabetic rats

The hepatoprotective effects of *N. canescens* extract and fractions (EA and ME) were evaluated by monitoring changes in liver antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) as shown in Table 5.

The results show a dose-dependent regulation of antioxidant activity by *N. canescens* extract and fractions (EA and ME) relative to the Glibenclamide (5 mg/kgbwt of) and diabetic control groups. CAT activity for all treatments was significantly lower than that observed in the diabetic control (274.10 ± 16.76) and Glibenclamide treatment group (235.38 ± 27.66), respectively.

SOD activity in all treatments was significantly higher than observed in the normal saline control. The highest increase in SOD activity (75.09 ± 0.00) was observed in the ME (50 mg/kgbwt) treatment group relative to the diabetic and saline controls.

For GSH activity, no significant changes were observed in the ME fraction treatment groups (50 mg/kgbwt and 300 mg/kgbwt) when compared to the saline control. Both doses of the *N. canescens* extract (50 mg/kgbwt and 300 mg/kgbwt) showed an increase in GSH activity to levels higher than observed for the saline and diabetic control groups with the highest increase (103.31 ± 6.14) observed in the 50 mg/kgbwt treatment. A similar trend was observed in the EA fraction treatment group.

Discussion

The present study evaluates the antidiabetic activity of the aqueous extract of *Nelsonia canescens* and its partitioned fractions (EA and ME) in alloxan-induced diabetic rats. Quantitative phytochemical analysis of the extract and fractions revealed the presence of phenols, flavonoids, tannins, alkaloids and saponins at varying concentrations (Table 1). These results agreed with earlier reports by Daniel et al. [23] Haruna et al. [24] who showed the presence of these phytochemicals in methanol and chloroform extracts of *N. canescens.* The bioactivity or medicinal potentials of any plant is dependent on the presence and abundance of these phytochemicals [37]. Phenols, flavonoids, saponins, tannins and alkaloids possess good antidiabetic [37], anti-inflammatory [38], antibacterial [13], antiseptic and antioxidant [26], hemostatic, hypocholesterolaemic and anticarcinogenic activities [39]. Hence, the presence of these phytochemicals in the extract maybe attributed to its pharmacological importance of the plant as demonstrated in this study.

Table 1 Phytochemical contents (mg/g) of crude extract and fractions of *Nelsonia canescens*

Phytochemicals	AQ	EA	ME
Saponins	14.29 ± 0.39^{b}	0.21 ± 0.03^{a}	17.61 ± 0.26^{b}
Alkaloids	10.72 ± 0.13^{a}	56.59 ± 1.40^{b}	11.34 ± 0.43^{a}
Tannins	67.08 ± 1.50^{b}	76.21 ± 0.04^{c}	0.79 ± 0.08^{a}
Flavonoids	20.60 ± 0.20^{c}	11.68 ± 0.75^{b}	0.32 ± 0.01^{a}
Phenols	49.65 ± 8.10^{c}	19.20 ± 0.38^{b}	0.65 ± 0.02^{a}

Values are expressed in mean ± standard error of mean of triplicate determination. Values with the same superscript in the same row have no significant difference at $p < 0.05$

Key: AQ = Aqueous extract, EA = Ethylacetate fraction, ME = Methanol fraction

A major characteristic feature of a diabetic condition is the generation of free radicals in diabetic patients. Therefore, it is important to search for therapeutics that will not only manage the disease but also place emphasis on other complications associated with the disease. In vitro antioxidant activity of the aqueous extract of *N. canescens* and its fractions using DPPH and ABTS models (Fig. 1) shows a dose-dependent inhibition of the radicals in both assays. The median inhibition concentration (IC_{50}) which is the concentration required to inhibit 50% of the radicals is used to classify the antioxidant activities of chemicals as either very strong (< 50 µg/mL), strong (50–100 µg/mL) and weak (100–150 µg/mL) antioxidants [39, 40]. Based on this classification, the IC_{50} values of the aqueous extract and fractions of *N. canescens* are regarded as very active antioxidants except in the ABTS assay where the aqueous extract produced an IC_{50} value of 50.10 ± 0.26 µg/mL and is therefore classified as a strong antioxidant (Fig. 1). ABTS and DPPH are reliable models to evaluate the antioxidant potential of chemicals and natural products by arresting their radicals in solution [41]. The observed antioxidant properties demonstrated by the extract may also be attributed to phytochemicals such as phenols, flavonoids and tannins present in an appreciable amount in the extract which are natural antioxidants that exhibit their antioxidant potentials by donating the proton of their hydroxyl group hence arresting the free radicals in the system [42].

The use of alloxan monohydrate for the induction of type-2 diabetes is well documented [13]. Alloxan induces diabetes by altering the function of the β-cells of the islets of Langerhans in the pancreas leading to a decreased endogenous insulin thus affecting the ability of the tissue to utilize glucose [13, 43]. Antidiabetic activity of the extract and fractions shows a significant ($p < 0.05$) dose-dependent reduction of the blood glucose of rats which are significantly different to the diabetic control group (Fig. 2). The ethylacetate

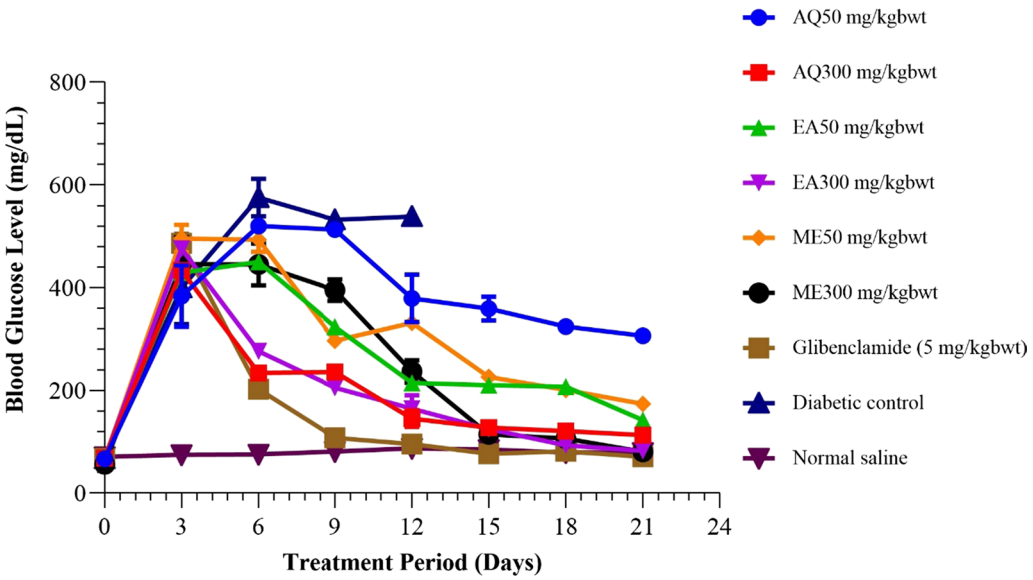

Fig. 2 Effect of *Nelsonia canescens* extract and fractions on blood glucose levels of Alloxan-induced diabetic rats

Table 2 Percentage glucose reduction of extract of *N. canescens* in alloxan-induced diabetic rats

Groups	% Glucose reduction
AQ 50 mg/kgbwt	40.75 ± 6.25^{b}
AQ 300 mg/kgbwt	74.35 ± 11.33^{c}
EA 50 mg/kgbwt	66.88 ± 9.33^{c}
EA 300 mg/kgbwt	81.86 ± 5.27^{cd}
ME 50 mg/kgbwt	59.14 ± 3.57^{b}
ME 300 mg/kgbwt	78.66 ± 9.42^{c}
Glibenclamide (5 mg/kgbwt)	86.11 ± 7.33^{d}
Normal saline	3.25 ± 0.59^{a}

Values are expressed at mean ± standard error of mean, $n = 5$. Values with the same superscript in the same column have no significant difference at $p < 0.05$

Key: AQ = Aqueous extract, EA = Ethylacetate fraction, ME = Methanol fraction

fraction at 300 mg/kgbwt showed the highest blood glucose reduction (81.86 ± 5.27%) followed by the 300 mg/kgbwt of the methanol fraction and the aqueous extract with a percentage reduction of 78.66 ± 9.42 and 74.35 ± 11.33%, respectively (Table 2). The observed level of activity in the extract and fractions may be attributed to the phytochemical constituents of the plants such as phenols, flavonoids, tannins and saponins which can mimic insulin or excite the secretion of β-cells by the islets of Langerhans [43, 44]. The antidiabetic properties of phenols and flavonoids are attributed to their ability to change the process of glucose and oxidative metabolisms in a diabetic condition and also by exerting a stimulatory effect on insulin secretion altering Ca^{2+} concentration [25, 38]. Also, flavonoids have been reported to modulate blood glucose levels and could regulate key rate-determining enzymes such as hexokinase, phosphofructokinase-1 and pyruvate kinase which are vital for metabolic pathways of carbohydrates [12]. Alkaloids, glycosides and saponins have similarly been implicated in the antidiabetic activities of plants [13].

In diabetic state, elevated blood glucose is often accompanied with dyslipidemia denoting risk factor for atherosclerosis [25, 45, 46]. Elevated levels of serum lipids are largely due to the uninhibited actions of lipolytic hormones on the adipose tissue as a result of insulin inactivity [25, 46]. Insulin is responsible for the activation of lipoprotein lipase an enzyme which hydrolyzes triglycerides (TGs) under normal circumstances [45]. Nevertheless, lipoprotein lipase is not activated in diabetic state because of insulin deficiency, leading to hypertriglyceridemia and hypercholesterolemia due to metabolic abnormalities [47]. TGs excite the secretion of very low-density lipoprotein cholesterol (VLDL); hence, increase in very low-density lipoprotein cholesterol particles reduces the high-density lipoprotein cholesterol (HDL-C) level and increases the low-density lipoprotein (LDL-C) particles [48]. A major feature of diabetic dyslipidemia is elevated level of serum triglycerides, total cholesterol, LDL-Cholesterol, and a decrease in HDL-Cholesterol levels [25, 49]. An altered serum lipid profile was observed in diabetic control group compared to the groups treated with extract and fractions of *N. canescens* (Table 3). The alteration in serum lipid profile however was reversed toward

Table 3 Effect of *N. canescens* Extracts and fractions on the Lipid Profile (mg/dL) of Alloxan-induced Diabetic Rats

Lipid Profiles	AQ 50	AQ 300	EA 50	EA 300	ME 50	ME 300	Glibenclamide	Diabetic control	Normal control
Total cholesterol	349.17 ± 34.54^{a}	338.06 ± 4.44^{a}	423.89 ± 56.39^{b}	474.17 ± 59.84^{b}	650.28 ± 0.00^{c}	438.89 ± 60.83^{b}	357.87 ± 37.73^{a}	835.28 ± 27.22^{d}	471.39 ± 55.45^{b}
HDL-Cholesterol	281.33 ± 69.37^{a}	354.00 ± 46.50^{b}	128.50 ± 39.00^{a}	512.63 ± 115.02^{d}	1315.00 ± 0.00^{e}	507.50 ± 5.00^{d}	326.83 ± 63.42^{b}	1414.25 ± 9.25^{e}	500.75 ± 100.72^{d}
LDL-Cholesterol	48.54 ± 37.09^{a}	31.68 ± 25.69^{a}	74.66 ± 18.91^{b}	107.38 ± 36.10^{c}	36.51 ± 0.00^{a}	96.24 ± 58.76^{c}	78.70 ± 16.43^{b}	251.34 ± 39.01^{e}	148.39 ± 43.55^{d}
Triacylglycerol	244.36 ± 19.82^{a}	235.58 ± 20.83^{a}	323.5 ± 89.68^{b}	264.26 ± 21.71^{ab}	350.77 ± 0.00^{b}	241.15 ± 3.08^{a}	213.80 ± 11.51^{a}	301.09 ± 9.94^{b}	222.85 ± 15.38^{a}

Values are expressed in men ± standard error of mean; $n = 5$. Values with the same superscript on the same row have no significant difference ($p < 0.05$)

Key: AQ = Aqueous extract, EA = Ethylacetate fraction, ME = Methanol fractions

Table 4 Effects of *N. canescens* extracts on biochemical parameters and serum enzymes in alloxan-induced diabetic rats

Parameters	AQ 50	AQ 300	EA 50	EA 300	ME 50	ME 300	Glibenclamide	Diabetic control	Normal control
Total Protein (mg/dL)	6.91 ± 0.36^{a}	6.76 ± 0.56^{a}	7.22 ± 0.06^{ab}	9.92 ± 3.10^{c}	9.61 ± 1.48^{c}	8.42 ± 1.14^{b}	6.24 ± 0.27^{a}	8.30 ± 0.10^{b}	7.01 ± 0.36^{ab}
Albumin (mg/dL)	4.09 ± 0.41^{a}	4.62 ± 0.36^{a}	4.33 ± 0.50^{a}	4.96 ± 0.33^{ab}	5.19 ± 0.29^{b}	4.53 ± 0.07^{a}	3.76 ± 0.35^{a}	4.75 ± 0.12^{a}	4.61 ± 0.13^{a}
Creatinine (mg/dL)	1.29 ± 0.57^{a}	1.81 ± 0.61^{a}	273.16 ± 10.31^{c}	211.28 ± 25.80^{c}	9.79 ± 1.84^{b}	2.67 ± 0.49^{a}	203.61 ± 43.64^{c}	401.56 ± 18.34^{d}	264.76 ± 40.17^{c}
ALT (U/L)	297.02 ± 16.06^{a}	237.50 ± 40.81^{a}	202.44 ± 17.63^{a}	364.21 ± 31.33^{b}	547.64 ± 65.42^{c}	247.74 ± 17.02^{a}	272.27 ± 13.83^{a}	383.66 ± 16.42^{b}	215.25 ± 46.11^{a}
AST (U/L)	179.16 ± 25.05^{b}	128.47 ± 33.71^{b}	0.41 ± 0.04^{a}	1.98 ± 0.86^{a}	6.39 ± 80.23^{d}	4.45 ± 92.60^{c}	2.41 ± 0.79^{a}	1.74 ± 0.21^{a}	2.30 ± 0.55^{a}

Values are expressed in mean ± standard error of mean, $n = 5$. Values with the same superscript on the same row have no significant difference at $p < 0.05$

Key: AQ = Aqueous extract, EA = Ethylacetate fractions, ME = Methanol fractions

Table 5 Effects of *N. canescens* EA fraction on liver antioxidant enzyme activities in alloxan-induced diabetic rats

Groups	Catalase (U/L)	SOD (U/L)	Glutathione (U/L)
AQ 50 mg/kgbwt	85.17 ± 5.37^{a}	35.56 ± 10.15^{ab}	103.31 ± 6.14^{d}
AQ 300 mg/kgbwt	162.22 ± 9.68^{b}	42.90 ± 5.72^{b}	86.18 ± 5.03^{c}
EA 50 mg/kgbwt	153.38 ± 19.44^{a}	55.45 ± 1.62^{c}	92.06 ± 3.11^{d}
EA 300 mg/kgbwt	188.13 ± 6.47^{b}	46.48 ± 0.49^{b}	85.24 ± 8.75^{c}
ME 50 mg/kgbwt	204.00 ± 3.92^{bc}	37.26 ± 1.55^{ab}	67.41 ± 1.4^{a}
ME 300 mg/kgbwt235.38	103.31 ± 2.97^{ab}	75.09 ± 0.00^{d}	65.29 ± 0.59^{a}
Normal saline	168.62 ± 23.84^{b}	23.32 ± 5.81^{a}	63.82 ± 2.01^{a}
Glibenclamide (5 mg/kgbwt)	235.38 ± 27.66^{c}	47.91 ± 0.63^{b}	76.41 ± 1.69^{b}
Diabetic control	274.10 ± 16.76^{c}	59.14 ± 17.83^{c}	75.06 ± 1.15^{b}

Values are expressed in mean ± standard error of mean, $n = 5$. Values with the same superscript on the same column have no significant difference at $p < 0.05$

Key: AQ = Aqueous extract, EA = Ethylacetate fraction, ME = Methanol fraction

normal levels in the extract and fractions treated groups; hence, the extract and fractions may be useful as cardioprotective agent in enhancing the breakdown of lipid which may subsequently help to prevent diabetic complications such as coronary heart diseases and atherosclerosis [12].

Creatinine, total proteins, albumin, ALT and AST levels are used as biochemical markers of liver and kidney injuries; thus, elevated levels of these markers may indicate liver or kidney dysfunction [50]. The result presented here shows a significant increase ($p < 0.05$) in creatinine levels in non-dose dependent manner when compared to the diabetic control (Table 4). Creatinine is a non-protein nitrogenous substance formed from creatine and phosphocreatine during muscle metabolism and excreted through glomerular filtration. The rate of excretion of

creatinine is influenced by the glomerular filtration rate (GFR), so any abnormality that decreases GFR will result in increased serum creatinine [51]. The significant increase ($p < 0.05$) in creatinine levels in the groups treated with 50 and 300 mg/kgbwt of the EA fraction may be an indication of kidney impairments due to the adverse effect of the treatment [52]. No significant difference ($p > 0.05$) in albumin content was observed between the treatments relative to the control groups. Total protein content was significantly increased ($p < 0.05$) in both EA and ME fractions (50 and 300 mg/kgbwt) when compared to the diabetic control group which suggest that these fractions may possess some hepatoprotective properties because albumin and total proteins are also used to determine a dysfunction in liver integrity [53].

The first line of defense against reactive oxygen and nitrogen species (ROS and RNS) induce damage in a living system is enzymatic and nonenzymatic antioxidants [54]. Three major scavenging antioxidants responsible for the removal of toxic free radicals in vivo are superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) [45]. By accelerating the elimination of superoxide radical and converting it to H_2O_2 and molecular oxygen, which both harm the cell membrane and other biological components, SOD protect tissues against free radicals [45]. A hemeprotein called CAT oversees detoxifying good amount of H_2O_2 in the body [55]. GSH catalyzes the detoxification of endogenous metabolic peroxides and hydroperoxides as well as the catabolism of H_2O_2 [56]. A crucial co-substrate for GSH-Px, glutathione serves as a free radical scavenger [45]. In comparison to the diabetic control group, the activity of these enzymes was substantially different ($p < 0.05$) in the extract and fractions treated groups and the Glibenclamide (reference group) (Table 5). This serves to further support the extract's safety as reported by Haruna et al. [24], who found that the extract's LD50 was above 2000 mg/kgbwt and that it may help treat diabetes and other health related conditions.

Conclusion

In conclusion, the outcome of this study shows that the extract and respective fractions (EA and ME) of *N. canescens* have hypoglycemic and hypolipidemic potentials to effectively reduce the progression of oxidative stress in alloxan-induced diabetic rats.

Abbreviations

ABTS: 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); ALT: Alanine aminotransferase; AQ: Aqueous extract; AST: Aspartate aminotransferase; DM: Diabetes mellitus; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; EA: Ethylacetate fraction; EDTA: Ethylenediamine tetraacetate; FC: Folin–Ciocalteu; FPGC: Fasting plasma glucose concentration; GDM: Gestational diabetes mellitus; HDL-C: High density lipoprotein cholesterol; IDDM: Insulin dependent diabetes mellitus; LDL-C: Low density lipoprotein cholesterol; ME: Methanol fraction; NIDDM: Non-insulin dependent diabetes mellitus; PBS: Phosphate buffer saline; SOD: Superoxide dismutase; TG: Triglycerides.

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Author contributions

The study was conceptualized by AID and AK, and methodology was designed by AID, TYG, SOA, AMA and RT. Samples collections were carried out by SOA, AMA and RT and FES. The experiments were carried out by AID, AID, TYG, SOA, and A. Statistical analysis and validation of results were carried out by AID, MK, AOF and AK. Draft of the original manuscript was carried out by AID, TYG, SOA, RT, AMA, AOF, VM, MK and AK. All authors have read and approved the manuscript.

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Availability of data and materials

Data will be available by corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

Ethical approval for the use of animal was obtained from the Research and Ethical Committee of Federal University of Technology Minna, Niger State, Nigeria and assigned a number: 000018EAU.

Consent for publication

Not applicable.

Competing interests

There is no competing interest for this study.

Study involving plants

The plant sample was collected from the environment of Federal University of Technology Minna, Niger State. Plant identification was authenticated by Dr. Mohammed Dangana of Plant Biology Department, Federal University of Technology Minna. Permissions and/or licenses for the study were not required.

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