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In vivo antidiabetic and antioxidant activities of chloroform fraction of *Nelsonia canescens* Leaf in Alloxan-induced Diabetic Rats

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ABSTRACT

Background: Diabetes mellitus, a metabolic disorder is responsible for the death of millions of people across the globe. Poor efficacy, high cost and adverse side effects associated with available synthetic anti-diabetic drugs have necessitate the need to search for anti-diabetic drugs of natural origin. Therefore, in this study, *in vitro* anti-diabetic and antioxidant activities of chloroform fraction of *Nelsonia canescens* were evaluated.

Methodology: Phytochemical composition, *in vitro* antioxidant and *in vivo* antidiabetic activity of the fraction were evaluated following standard protocols. Hyperglycemia was induced via intraperitoneal injection of 90 mg/kgbwt of alloxan monohydrate. Male Wister rats weighing between 120.20±15.25 g were randomly distributed into five groups consisting of five rats each and administered 50, 150 and 300 mg/kgbwt of the fraction, 5 mg/kgbwt of glibenclamide, and 2 mL/kgbwt of normal saline respectively.

Results: The quantitative phytochemical screening revealed the presence of phytochemicals (mg/100 g) such as phenols (1624 ± 1.12), flavonoids (994.18 ± 1.26), tannins (75.71 ± 0.21), saponins (1038.40 ± 2.00) and alkaloids (88.59 ± 1.84). The fraction exhibited antioxidant activity in a concentration-dependent manner with percentage inhibition of 68.20% at 100 µg/mL against DPPH radicals compared with ascorbic acid (89.28%). At 300 mg/kgbwt the fraction reduced the blood glucose concentration of rats to 119.00±2.70 mg/dL compared with 70.50±5.50 for the glibenclamide treated group after 21st day. The fraction administration resulted in lowered levels of total cholesterol, LDL-cholesterol and triglycerides and increased level of HDL-cholesterol. There was a significant increase ($p<0.05$) at 300 mg/kgbwt in the levels of catalase (33.36±0.36 U/mL), superoxide dismutase (71.66±0.56 U/mL) and reduced glutathione (100.64±3.25 µg/mL) activities compared with 26.86±0.76 U/mL, 33.32±0.73 U/mL and 63.82±2.10 µg/mL respectively for the control groups.

Conclusion: The results of this study showed that chloroform fraction of *N. canescens* possess significant antioxidant and anti-diabetic activities.

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder of chronic hyperglycemia characterized by alteration in carbohydrate, protein and fat metabolism arising from total or partial insulin deficiency with dysfunction in organ systems [1]. Factors such as changes in social status, eating habit, smoking, alcohol intake among others may be attributed to the recent increase in the prevalence of this disease over the years [2]. Diabetes is a serious threat to global health that respects neither socioeconomic status nor national boundaries. The recent data reported by International Diabetes Foundation (IDF) shows that 463 million adults are currently living with diabetes [3]. Without sufficient action to address this pandemic, it has also been reported that 578 million people will have diabetes by 2030 and this figure may rise to about 700 million by 2045 with Africa and Asia contributing the largest proportion of this figure [3]. There is also a rising burden from the complications of DM alongside the ever-increasing prevalence of the disease [1]. Currently, there is high rates of DM-related amputation, cerebrovascular disease, heart-related issues, and kidney diseases in populaces that were not recently connected with these medical conditions [2]. In sub-Saharan Africa (SSA) including Nigeria, there is a growing concern over the rising prevalence of DM. Recent survey shows that approximately 5.8% (about 6 million) of adult Nigerians are living with DM [4]. This

Abbreviations: DM, Daibetes Millitus; IDF, International Diabetes Foundation; SSA, Sub-Saharan Africa; DPPH, 2, 2 -Diphenyl - 1 - picrylhydrazyl; PBS, Phosphate Buffer Saline; GSH, Reduced Glutathione; SOD, Superoxide Dismutase; CAT, Catalase; DTNB, 5, 5 - dithiobis - 2 -nitrobenzoic acid.

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figure is not a true picture of the current reality as it is estimated that two-thirds of diabetes cases in Nigeria are yet to be diagnosed [5].

Diabetic patients are at risk of coronary heart disease like atherosclerosis. Atherogenesis is a process which prompts the formation of atheromatous plaque on the inner walls of arteries in the vascular tissues which is enhanced by cellular and acellular elements. This atheromatous plaque causes the narrowing of blood vessels as well as hardening of the walls of arteries, a process known as atherosclerosis resulting in cardiovascular diseases [2]. Cardiovascular disease brought about by coronary and blood vessels injuries are responsible for the pathogenesis of groups of heart related disorder [6]. Moreover, it has been reported from observational examinations that the danger of atherogenic dyslipidemia was raised by a few folds in DM patients [2]. Atherogenic dyslipidemia in DM is relative to expanded danger of silent myocardial ischemia and coronary artery disease [6].

There is a connection between diabetes and oxidative stress which assume a vital part in the development of this disease both microvascular and cardiovascular. In many diseases characterized by tissue damage, oxidative stress is either a cause or outcome of this damage. In a diabetic condition, there is a rise in a steady-state oxidative damage as a result of an increase in oxidizable substrates, an increase in the rate of autooxidation of substrates, a decline in the antioxidant defense, or a combination of all of these processes [7]. A precise knowledge and understanding of oxidative stress in diabetes and the adaptive response to it may require the knowledge of the time of onset of manifestation of oxidative stress, their characterization in terms of oxidative damage to biomolecules like lipids, and the antioxidant enzymes affected and their regulation at the level of transcription or activity or both. Traditional plant remedies have been used for centuries in the treatment of diabetes, but only a few have been scientifically evaluated [7].

According to report by World Health Organization, medicinal plants have continued to be an alternative source to conventional chemotherapy and possibly the major source of daily medication for those in the developing countries who lack access to basic health amenities [8]. The use of medicinal plants for the treatment of ailments date back to the history of man as documented by the ancient Chinese and Babylonian archives on traditional herbal remedies [9]. For millennia, traditional Chinese medicine has been employed for pharmaceutical and dietary therapy [10]. Yellow Emperor's Canon of Medicine, compiled approximately 475–221 BC., uses a phrase meaning "tumor" in the earliest Chinese medical treatise presently existing [10]. Chinese medicine has its own unique idea of disease prevention and treatment, which includes prescriptions containing numerous therapeutic plants, rather than just one. During long-term folk practice, a variety of anticancer Chinese medicinal herbs and numerous related prescriptions have been tested and used for treating and preventing various malignancies [10]. Anticancer medicinal plant therapies are still commonly used today, notably in the countryside and remote mountainous regions, and even in urban areas [11]. Phytochemicals such as phenols, flavonoids, tannins, saponins and alkaloids have been documented to be used for the management of different ailments [12]. Phytochemicals of plant origin have emerged as novel therapeutic agents for treating chronic disorders [13]. Due to different classes of phytochemicals, they are used for treating different kinds of diseases such as cancer, inflammation, and neurodegenerative disorders, and have been a useful source of analog compounds for the formulation of novel chemotherapeutics [13]. Phenols and flavonoids are natural human antioxidant used for the management of degenerative diseases such as diabetes and cancer [13]. Tannins rich plant extracts are used as diuretics against stomach upset and antidiarrhoea [14]. They are also used as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals [15. Alkaloids are group of phytochemicals that contains nitrogen in their heterocyclic cyclic ring. They are synthesized naturally by a large number of organisms, including animals, plants, bacteria and fungi [16, 17]. The medicinal significance of alkaloids includes antibacterial, antifungal, antihypertensive (some indole alkaloids), antiarrhythmic (quinidine, Sparein) [18], antimalarial activity (quinine), and anticancer (dimeric indoles, vincristine, vinblastine) [12]. Saponins have also been reported to be used as membrane permeabilizing, immunostimulant, hypocholesterolaemic and anticarcinogenic agents and they have also been found to significantly affect growth, feed intake and reproduction in animals [15].

Nelsonia canescens (Lam). Spreng. of the family Acanthaceae commonly known as blue pussy plant is a wild medicinal plant which grow abundantly in various parts of the world particularly India and Africa [19]. In India, the root is reported for its anti-inflammatory and analgesic effects and whole plant for its hepatoprotective action. N. canescens is also reported to be used as a cover crop to suppress weeds in banana plantations [20]. However, as at the time of this report, there is no documented report on the phytochemical constituents as well as the use of this plant in the management of diabetes or any related complications hence, this study is aimed at evaluating the antioxidant and antidiabetic activities of chloroform fraction of *Nelsonia canescens* leaf in alloxan-induced diabetic rats.

2. Materials and methods

2.1. Chemicals and reagents

The chemicals and reagents used in this study were of analytical grades and product of BDH Laboratory Supplies, England. The chemicals and reagents used include Gallic acid, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Sodium acetate anhydrous and Alloxan Monohydrate, Folin–Ciocalteu phenol reagent and the standard drug; Glibenclamide.

2.2. Plant preparation and extraction

Fresh leaves of *Nelsonia canescens* were collected from the environment of Federal University of Technology Minna, Niger State, Nigeria. The leaves were rinsed with distilled water and air dried at room temperature (27±2.00 °C) at the Biochemistry Department Laboratory of Federal University of Technology Minna, Nigeria for 2 weeks. The dried leaves were pulverized into fine powder using electric blender. To extract the plant material, five hundred grams (500 g) of the powdered sample was extracted exhaustively with chloroform under reflux at 50 °C for 2 h. The chloroform fraction collected was filtered using muslin cloth and Whatmann No 1 filter paper. Chloroform was evaporated by placing the fraction in water bath until constant weight is obtained. The fraction was preserved in a refrigerator at 4 °C until required for use.

2.3. Quantitative phytochemical analysis of the extract

2.3.1. Determination of total phenol

Folin-Ciocalteau spectrophotometric method was used for the determination of total phenolic content of the extract. The reaction mixture contained 0.5 mL (1 mg/mL) of the extract, 2.5 mL of 10% Folin-Ciocalteau reagent (v/v) and 2 mL of 7.5% Na_2CO_3 (w/v). The reaction mixture was shaken properly and incubated for 40 mins at 45 °C and the absorbance was taken at 765 nm. The total phenolic content of the extract was extrapolated form Gallic acid standard curve using different concentrations (6.25 to 100 µg/mL) [21].

2.3.2. Determination of total flavonoid

Aluminum chloride ($AlCl_3$) Colorimetric method was adopted to determine the flavonoid content of the extract. The reaction mixture contained 0.5 mL (1 mg/mL) of the extract, 1.5 mL of methanol, 0.1 mL of 10% $AlCl_3$ (w/v), 0.1 mL of 1 M sodium acetate and 2.8 mL of distilled water. The mixture was shaken properly and allowed to stand at room temperature for 30 mins. The absorbance of the reaction mixture was taken at 415 nm. Quercetin solutions at concentrations of 12.5 to 100 g/mL in methanol was used for the calibration of the standard curve [21].

2.3.3. Determination of alkaloids

The molar extinction coefficient of Vincristine (ε=15,136 mol/cm) was used to estimate the alkaloids content of the extract. The powdered plant sample (0.5 g) was extracted with 5 mL of 96% ethanol-20% H_2SO_4 (1:1, v/v) and filtered. The reaction mixture consists of 1 mL of the filtrate and 5 mL of 60% H_2SO_4 (v/v), the mixture was shaken properly and allowed to stand for 5 mins before 5 mL of 0.5% of formaldehyde solution (v/v) was added and absorbance of the mixture was read at a wavelength of 565 nm after 3 hrs [22]. The concentration of alkaloids was determined using the equation:

$$\text{Concentration} = (\text{Absorbance of sample}/\text{molar extinction coefficient of vincristine}) \text{ x actual concentration of sample used}$$

2.3.4. Determination of saponins

The amount of saponins in the extract was extrapolated from saponin standard curve using 6.25 to 10 µg/mL of standard saponins. The plant extract (0.5 g) was dissolved with 20 mL of 1 M HCl, boiled for 4 hrs and allow to cool. This was filtered and the ether layer was obtained by adding 50 mL of petroleum ether to the filtrate and separated using a separating funnel. The ether layer was evaporated to dryness and 5 mL of acetone-ethanol (1:1, v/v) was added to the residue, 6 mL ferrous sulfate reagent and 2 mL of Conc. H_2SO_4. The mixture was homogenized and allowed to stand for 10 mins before reading the absorbance at 490 nm [23].

2.3.5. Determination of tannin

The tannic acid content of the extract was extrapolated from the tannic acid standard curve using 6.25 to 10 µg/mL of tannic acid. The plant powdered sample (0.2 g) was weighed into a 50 mL beaker and 20 mL of 50% methanol (v/v) was added, covered with para film and heated in a water bath at 80 °C for 1hr. The mixture was thoroughly shaken and the content was transferred into a 100 mL volumetric flask. To the mixture, 20 mL of water, 2.5 mL Folin-Ciocalteau reagent and 10 mL of 17% Na_2CO_3 (w/v) were added and mixed thoroughly. The mixture was allowed to stand for 20 mins. The absorbance of tannic acid standard solution as well as sample was taken after color development with spectrophotometer at a wave length of 760 nm [24].

2.4. Determination of free radical scavenging activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The free radical scavenging ability of the extract against DPPH radical was evaluated as described by Dhanasekaran [25]. Equal volume (1 mL) each of the plant extract of various concentration (12.5 – 100 µg/mL) and 0.4 mM methanolic solution of DPPH were mixed together in a test tube. The mixture was left in the dark for 30 mins and the absorbance was recorded at 516 nm. The percentage inhibition of DPPH radical was subsequently calculated with respect to the reference (which contains all the reagents without the test sample) using the equation below:

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

where A0 is the absorbance of control (without sample) and A1 is the absorbance of the extract and DPPH solution. The experiment was performed in three replicates and the mean of the values was calculated. Eqn. (2), 12

2.5. Experimental animals/ethics

A total of thirty (30) male Wistar rats weighing 116 ± 2.84 g were purchased from the animal farm of the University of Jos, Jos, Plateau State, Nigeria. The rats were kept in well-ventilated metal cages and maintained at room temperature of 27 ± 2 °C, 45–55% of relative humidity on a 12 hrs light/12 hrs dark cycle, with access to water and pelletized standard guinea feed *ad libitum*. The rats were kept for 2 weeks to acclimatize to environmental conditions [26]. The present study was approved by the Ethical Committee on the use of animals for research, Department of Biochemistry, Federal University Technology, Minna, Nigeria (000018EAU). Handling of the rats was in accordance with the standard principles of laboratory animal care of the United States National Institutes of Health.

2.6. Induction of diabetes mellitus

Diabetes mellitus (DM) was induced in the rats by a single intraperitoneal (i.p.) injection of 90 mg/kgbwt of alloxan monohydrate (Sigma, St. Louis, USA) in PBS (pH=7.4) [26]. Animals with fasting plasma glucose concentration (FPGC) > 111 mg/dL, measured using Fine test Autocoding Premium Blood Glucose Monitoring System for self-testing, for 5 consecutive days were considered diabetic and selected for the study [26]. A total of twenty-four (24) male Wistar rats were divided into 6 groups of 4 rats each. The animals were deprived of food and water for additional 16 h before commencement of treatment.

2.7. Animal grouping

Male Wister rats weighing between 120.20±15.25 g were randomly distributed into five groups as follows:

Group I: not induced and administered 2 mL/kgbwt of Phosphate buffer saline (PBS) designated control group

Group II: induced and administered 5 mg/kgbwt of glibenclamide

Group III: induced and not treated designated untreated group

Group IV: induced and administered 50 mg/kgbwt of the chloroform fraction of *N. canescens*

Group V: induced and administered 150 mg/kgbwt of the chloroform fraction of *N. canescens*

Group VI: induced and administered 300 mg/kgbwt of the chloroform fraction of *N. canescens*

The fraction was administered orally at the same time once daily throughout the period of treatment. The body weight of the experimental animals was monitored at the beginning and end of the experiment. However, the blood glucose level was monitored every five (5) days throughout the experimental period by puncturing the tail to collect blood from animals. There were five rats in each of the experimental groups.

2.8. Collection and preparation of blood and tissues

About 24 h after the last treatment on the 21st day, rats were sacrificed under chloroform anesthesia. Blood was collected from rats from each group by intra-cardiac puncture and dispensed into plain sterilized tubes. The blood was allowed to stand on the bench for at least 2 h, spun with a centrifuge at 3000 rpm for 10 mins, then serum was separated with clean Pasteur pipette and stored frozen until used for biochemical analyses. The liver from each rat in all groups was excised, rinsed in normal saline and preserved in 20% sucrose solution for liver enzyme analyses [27].

2.9. Biochemical assays

The lipid profile of the serum samples was determined using commercial Agappe biochemical kit according to the method of Nauck et al. [28].

2.10. In vivo *antioxidant enzyme activity*

Liver of rats from each group were immediately removed after sacrifice and washed using chilled saline solution and homogenized in ice-cold sucrose (20% w/v) using mortar and pestle. The homogenate was centrifuged at 10,000 × *g* for 20 mins at 4 °C [29]. The resultant supernatant of liver was used to determine reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) enzyme activities.

2.10.1. Catalase enzyme activity

Catalase (CAT) enzyme activity was determined according to the method of Awad et al. [29]. the reaction mixture consists of 2.5 mL of 30% H_2O_2 buffer, 50 µL of liver homogenate and vortex. The absorbance of the mixture was measured after 30 secs and then 90 secs at 240 nm. The blank contained 2.5 mL of 30% H_2O_2 buffer in place of the sample. The CAT activity was measured using the formula below:

$$CAT activity(U/minspermLof serum) = \frac{\Delta Abs}{0.0008} X1\ min$$

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

2.10.2. Superoxide dismutase enzyme activity

Superoxide dismutase (SOD) enzyme activity was assayed following the method reported by Misra and Fridovich [30]. The reaction mixture consists of 20 µL of liver homogenate, 960 µL of sodium carbonate buffer (50 mM, pH 10.2) and 0.1 mM EDTA. The reaction was initiated by adding 20 µL of 30 mM epinephrine (dissolved in 0.05% v/v acetic acid) to the mixture. The control contained 20 µL of distilled water instead of the sample while sodium carbonate buffer (0.05 M, pH 10.2) was used as the blank. The increase in absorbance was measured at 480 nm for 4 mins and activity calculated as follows:

$$\%\text{inhibition} = 100 - \left(\frac{\Delta\text{Abs control} - \Delta\text{Abs sample}}{\Delta\text{Abs control}} X100\right)$$

$$\text{SODActivity(U/mL)} = \%\text{inhibitionX3.75}$$

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

2.10.3. Reduced glutathione concentration

Reduced GSH content was assayed following the method of Jollow *et al.* [31]. The reaction mixture consists of 150 µL of the liver homogenate, 150 µL of sulfosalicylic acid (SSA) in a test tube and centrifuged at 5000 × *g* at 4 °C for 10 mins. The amount of GSH was determined by adding 66 µL of supernatant to 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 mins, the absorbance was measured against SSA as blank at 412 nm and the concentration calculated as follows:

$$\text{GSHconcentration(U/mL)} = \frac{Abs}{0.416} X2 \quad (12)$$

where Abs is the sample absorbance.

2.11. Statistical and data analyses

The values obtained from each analysis were subjected to statistical analysis using One-way ANOVA and the significant differences among groups were determined by Duncan multiple comparison *post hoc* test (Statistical Package for Social Sciences, version 22.0, SPSS Inc., Chicago, IL, USA). P-value less than 0.05 was considered significant. The data were expressed as mean ± standard error mean of five replicates.

3. Results

3.1. Quantitative phytochemical constituents of chloroform extract of N. canescens

As shown in Table 1, the quantitative phytochemical analysis confirmed the presence and amount of the screened secondary metabolites (mg/100 g) in descending order of phenols (1624.57±6.12) > saponins (1038.40 ± 2.00) > flavonoids (994.18 ± 1.26) > alkaloids (88.59 ± 1.84) > tannins (75.71 ± 0.21).

Table 1
Quantitative phytochemical constituents of chloroform extract of *N. canescens*.

Phytochemicals	Amounts (mg/100 g)
Phenols	1624.57 ± 1.12
Flavonoids	994.18 ± 1.26
Tannins	75.71 ± 0.21
Saponins	1038.40 ± 2.00
Alkaloids	88.59 ± 1.84

Values are expressed in mean ± standard error of mean of three replicates.

3.2. DPPH radical scavenging activity of the chloroform fraction of N. canescens leaf

The DPPH radical scavenging activity of the fraction was also found to be concentration dependent. However, the percentage radical scavenging ability of ascorbic acid was significantly higher ($p < 0.05$) than that of the fraction. The highest percentage DPPH radical scavenging activity (68.20%) was observed at the highest tested concentration of 100 µg/mL with an IC_{50} of 41.04±0.25 µg/mL while that of ascorbic acid (89.28%) was also observed at 100 µg/mL with an IC_{50} of 24.98±0.16 µg/mL (Table 2).

3.3. Effects of chloroform fraction of N. canescens leaf on fasting blood glucose level in alloxan-induced diabetic rats

As shown in Fig. 1 below, all tested doses of the fraction significantly reduced the glucose level compared to the untreated group. The lowest glucose level obtained on the 21st day of the experiment (119.00±2.70 mg/dL) was observed at 300 mg/kgbwt which was significantly higher ($p<0.05$) than the glibenclamide-treated group (70.50±2.90 mg/dL). The negative control group could not survive beyond 10 days due to their high blood glucose level (Fig. 1).

3.4. Effect of chloroform fraction of N. canescens leaf on the body weight of alloxan-induced diabetic rats

Table 3 shows the result of the effect of the chloroform fraction of *N. canescens* leaf on the body weight of alloxan-induced diabetic rats. It was observed that group treated with 300 mg/kgbwt of the fraction showed the highest bodyweight gain (3.77%) on the 21st day more than the group treated with glibenclamide (2.34%).

3.5. Effect of chloroform fraction of N. canescens leaf on lipid profile of alloxan-induced diabetic rats

At different doses of the fraction (50, 150 and 300 mg/kgbwt), the extract significantly lowered ($p<0.05$) the total cholesterol, LDL-cholesterol and triglycerides concentrations when compared to the untreated group. In addition to the reduced levels of the aforementioned parameters, the fraction at different doses increased the level of HDL-cholesterol which were significantly higher than glibenclamide-treated group (66.83±2.35 mg/dL) but lower than control group (187.32±1.69 mg/dL) (Table 4).

3.6. Effect of chloroform fraction of N. canescens leaf on liver antioxidant enzymes alloxan-induced diabetic

As shown in Table 5, the administration of 300 mg/kgbwt of chloroform fraction shows highest elevation in liver antioxidant enzymes; catalase (33.36±0.30 U/mL), superoxide dismutase (71.66±0.56 U/mL) and reduced glutathione (100.64±3.25 µg/mL) significant different with untreated, control and glibenclamide-treated groups.

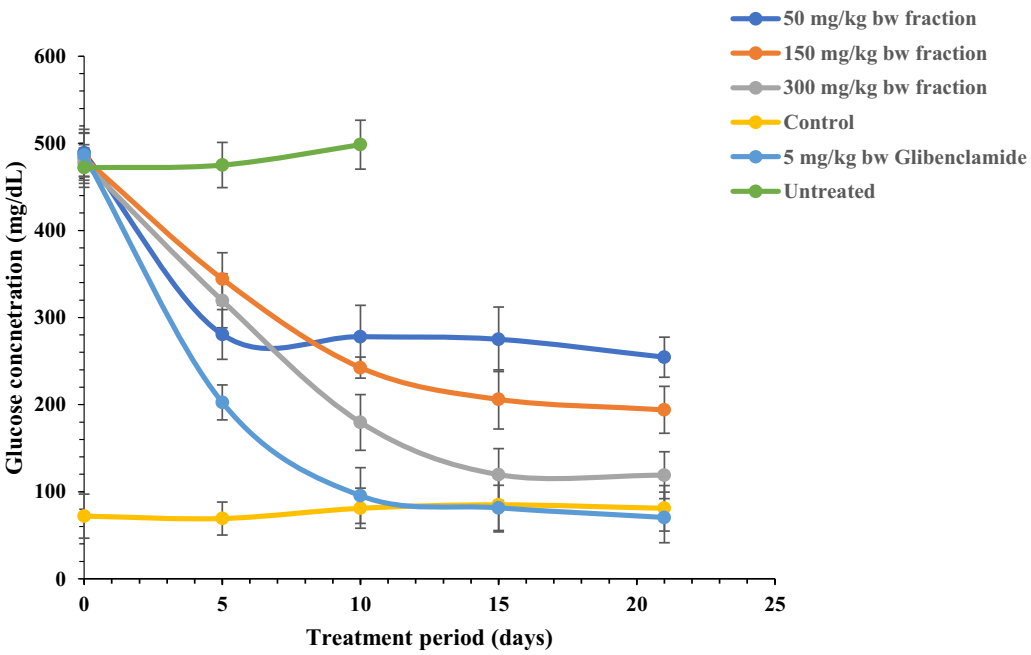

Fig 1. Effect of chloroform fraction of *N. canescens* leaf on the blood glucose concentration of alloxan-induced diabetic rats.

Table 2
DPPH radical scavenging activity of chloroform fraction of *N. canescens* leaf.

Concentration (µg/mL)	Chloroform fraction	Ascorbic acid
100.00	68.20 ± 0.12^{a}	89.28 ± 0.41^{b}
50.00	63.83 ± 0.01^{a}	68.89 ± 0.11^{b}
25.00	50.84 ± 0.20^{a}	51.91 ± 0.13^{a}
12.50	26.47 ± 0.13^{a}	38.59 ± 0.21^{b}
IC_{50}	41.04 ± 0.25^{b}	24.98 ± 0.16^{a}

Values are expressed in mean ± standard error of mean of three replicates.
Values with different superscripts in a row are significantly different at $p < 0.05$.

4. Discussion

The aim of this study was to evaluate the antioxidant and antidiabetic activities of chloroform fraction of *Nelsonia canescens* leaf in alloxan-induced diabetic rats. Quantitative phytochemical study of chloroform fraction of *N. canescens* revealed the presence of phenols, flavonoids, tannins, saponins and alkaloids at varying concentrations (Table 1). The pharmacological importance of any medicinal plants depends on the types and quantity of secondary metabolites contained in the plant. Hence, the presence of these secondary metabolites justifies the ethnomedicinal uses of this plant in treating various ailments [32]. The result support the findings of Haruna et al., [33] who reported the presence of phenols, flavonoids, tannins, saponins, alkaloids and steroids in aqueous extract of *N. canescens*. *In vitro* antioxidant assay using DPPH shows a significant dose-dependent increase in the antioxidant activity of the fraction with highest inhibition of 68.20±0.12% for DPPH radicals observed at 100 µg/mL compared with ascorbic acid with a percentage inhibition of 74.30±0.11% (Table 2). The antioxidant activity of the chloroform fraction could be attributed to the presence of secondary metabolites such as phenols and flavonoids present in this fraction. Phenols and flavonoids are natural antioxidants with documented reports of their high antioxidant capacities [34,35]. Furthermore, the dose-dependent increase in the antioxidant capacity of the fraction could be as a result of high amount of these secondary metabolites at the higher dose resulting in the observed level of the antioxidant activity [36,37].

Three different doses of the fraction (50, 150 and 300 mg/kgbwt) were used for the antidiabetic study based on the previous report by Haruna et al. [33] that the extract is safe at 2000 mg/kgbwt. Alloxan induces diabetes by destructing the pancreatic beta cells using reactive oxygen species (ROS) which result in hyperglycemia since the secretion of insulin is either partially or totally shut down by this damaged pancreas [38]. Hyperglycemia has been identified as the major hallmark of diabetes [38]. Hence, the substantial glucose-lowering power exhibited by the chloroform fraction at all test doses could be due to its ability to stimulate insulin secretion by the pancreas, and its antioxidant activity which results in the quenching of ROS generated from glucose auto-oxidation in the hyperglycemic condition [39]. Secondary metabolites such as phenols, flavonoids, saponins and alkaloids have been reported to possess antidiabetic activities [40–41]. Therefore, the antidiabetic activity exhibited by the chloroform fraction could be attributed to the presence of these secondary metabolites. Again, the higher glucose-lowering power exhibited by the fraction at dose of 300 mg/kgbwt than other test doses maybe due to the higher amount of these secondary metabolites at this dose (Fig. 1). Although, the fraction did not lower the glucose level than or up to the glibenclamide-treated group, it lowered the blood glucose significantly ($p<0.05$) compared to the untreated group, and this could be a beneficial property that could prevent hypoglycemia in a long-term use of this fraction, which in case of glibenclamide could result in hypoglycemia, a physiological condition more dangerous than hyperglycemia.

Alloxan-induced diabetes is accompanied by loss of body weight in experimental animals perhaps due to the cells inability to take up glucose from the blood stream for various physiological activities thus resulting in lipolysis in the adipose tissue and protein break down in the skeletal muscles [35,42]. Interestingly, the gain in body weight of the rats administered 150 and 300 mg/kgbwt of the fraction similar to the control and glibenclamide-treated groups could be due to its ability to stimulate glucose uptake by cells resulting in increased glucose utilization and thus prevented lipolysis and proteolysis in adipose tissue and skeletal muscle, respectively (Table 3). This also implies that the antidiabetic activity of the fraction is dose dependent and elicited the activity at higher doses.

Table 3
Effect of chloroform fraction of *N. canescens* leaf on the body weight of alloxan-induced diabetic rats.

Dosage	Initial	Final	% Change
50 mg/kgbwt.	118.67 ± 2.78[a]	110.75 ± 1.21[a]	−7.15
150 mg/kgbwt.	123.45 ± 3.12[a]	127.09 ± 1.89[b]	2.86
300 mg/kgbwt.	116.09 ± 2.89[a]	120.64 ± 2.10[b]	3.77
Normal	117.86 ± 4.02[a]	136.09 ± 1.02[c]	13.40
5-G	124.12 ± 1.74[a]	127.09 ± 0.50[c]	2.34
untreated	121.13 ± 3.90[a]	All animals died before the end of the study	

Values are expressed in mean ± standard error of mean of three replicates.
Values with different superscripts in a column are significantly different at $p < 0.05$.
5-*G* = 5 mg/kgbwt glibenclamide.

Table 4
Effect of chloroform fraction of *N. canescens* leaf on lipid profile of alloxan-induced diabetic rats.

	Lipid profile (mg/dL)			
Dosage	Total cholesterol	HDL-Cholesterol	LDL-Cholesterol	Triglycerides
50 mg/kgbwt	448.06 ± 2.64[d]	158.90 ± 3.12[d]	55.90 ± 1.09[a]	321.15 ± 2.34[e]
150 mg/kgbwt	317.22 ± 2.98[b]	92.50 ± 3.22[c]	108.40 ± 2.38[d]	230.32 ± 1.98[c]
300 mg/kgbwt	310.83 ± 1.87[b]	93.75 ± 4.10[c]	97.92 ± 1.11[c]	234.17 ± 3.10[c]
Control	371.39 ± 2.22[c]	187.32 ± 1.69[e]	148.39 ± 3.87[e]	222.85 ± 0.50[b]
5-G	257.87 ± 1.38[a]	66.83 ± 2.35[b]	78.70 ± 2.12[b]	213.80 ± 2.37[a]

Values are expressed in mean ± standard error of mean of three replicates.
Values with different superscripts in a column are significantly different at $p < 0.05$.
5-*G* = 5 mg/kgbwt. glibenclamide.

Table 5
Effect of chloroform fraction of *N. canescens* leaf on liver antioxidant enzymes alloxan-induced diabetic.

	Antioxidant enzymes		
Dosage	Catalase(U/mL)	Superoxide dismutase (U/mL)	Reduced glutathione (μg/mL)
50 mg/kgbwt	21.49±0.44[b]	40.05±0.54[c]	84.87±1.26[c]
150 mg/kgbwt	25.84±0.21[b]	62.49±0.39[e]	57.87±2.30[a]
300 mg/kgbwt	33.36±0.30[c]	71.66±0.56[f]	100.64±3.25[d]
Control	26.86±0.76[b]	33.32±0.73[b]	63.82±2.10[a]
5-G	23.54±0.42[b]	47.91±0.42[d]	76.41±3.38[b]

Values are expressed in mean ± standard error of mean of three replicates.
Values with different superscripts in a column significantly different at $p < 0.05$.
5-*G* = 5 mg/kgbwt. glibenclamide.

Diabetes mellitus has been reported to impair lipid metabolism [43], as the hyperglycemia is accompanied by significant increase in the levels of triglycerides, total cholesterol and LDL-cholesterol and decrease in the level of HDL-cholesterol which result in development of atherosclerosis and other cardiovascular diseases [44]. Thus, it is rational to infer that the ability of the fraction at higher doses to prevent hyperglycemia could be the major reason for the decreased triglycerides, total cholesterol and LDL-cholesterol levels with increased level of HDL-cholesterol in the rats administered 150 and 300 mg/kgbwt. of the fraction, respectively (Table 4).

Oxidative stress has been shown to be responsible for the development of vascular complications in diabetic patients, and this is the major factor causing death in diabetic individuals other than hyperglycemia [45]. This is as a result of destruction of vital organs initiated by free radicals generated in diabetes which result in the depletion of the antioxidants in the body. For example, elevated level of ROS in diabetes results in decreased levels of catalase, superoxide dismutase and glutathione making a number of cells susceptible to free radicals [46]. Elevated levels of these enzymes in the rats administered 150 and 300 mg/kgbwt contrary to the untreated group could be as a result of the antioxidant activity of the fraction as confirmed by *in vitro* antioxidant assays (Table 5). In addition to the antioxidant activity of the fraction, the increased levels of these antioxidants could be, in part, due to the ability of the bioactive principles in the fraction to upregulate the transcription of CAT, SOD and GPx genes that are translated to these antioxidants.

5. Conclusion

From the results obtained in this study, it can be concluded that the chloroform fraction of *N. canescens* possess antioxidant and antidiabetic activities at higher doses. Thus, the fraction could be employed in the management and/or treatment of diabetes.

Declaration of Competing Interest

The authors declared that there is no competing interest for this study.

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