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Antidiabetic effects of aqueous leaf extract of *Vernonia amygdalina* on serum liver markers in streptozotocin-induced diabetic albino Rats: a new data to support its Anti-diabetic effect

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Abstract

Background Numerous plants have been explored for their potential antidiabetic properties, and *Vernonia amygdalina* (VA) stands among them. This study aims to investigate the antidiabetic activities of VA and validate its efficacy.

Methods An aqueous extract of *Vernonia amygdalina* leaves was obtained through maceration. The antidiabetic effects of this plant extract were evaluated in vivo using diabetic model rats. Albino Wistar rats were induced into a diabetic state through intraperitoneal injection of streptozotocin and subsequently treated with an optimal dose of 250 mg/kg aqueous extract of VA over a 21-day period. Parameters such as body weight, blood glucose levels, and serum marker enzymes were measured.

Results The results demonstrated a significant reduction ($p < 0.05$) in the glucose levels of streptozotocin-induced diabetic rats following treatment with VA extract, highlighting its potential as an antidiabetic agent that performed comparably to the reference drug, glimepiride. Additionally, a significant increase ($p < 0.05$) in the body weight of the treated diabetic rats was observed. Aqueous extracts also significantly ($p < 0.05$) altered the serum concentrations of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in a manner similar to the glimepiride-treated group.

Conclusion This study affirms the anti-diabetic effects of the aqueous extract of *Vernonia amygdalina* in streptozotocin-induced diabetic rats and suggests that the extract holds promise as an important phytomedicine for the development of more effective treatments for diabetes.

Keywords Anti-diabetic, Streptozotocin, Glimepiride, *Vernonia amygdalina*

Introduction

Diabetes Mellitus (DM) is a severe disorder of sugar, protein, and fat metabolism primarily described by high blood glucose (Nelyanti [76]). DM is characterized by

an impaired release of insulin by the pancreatic β cells, production of inactive insulin or absolute insulin deficiency, and inadequate or defective insulin receptors (Li et al. [71]). The two types of Diabetes are type 1 (T1DM) and type 2 (T2DM) (Cooke and Plotnick [33]). T1DM is characterized by insufficient production of insulin from the body and T2DM is a condition of high blood glucose termed hyperglycemia that occurs due to an abnormal or inadequate peripheral tissue response to functional levels of insulin (Landon et al. [70]). DM has become a global health threat worldwide as the number of cases increases

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dramatically every year in all age groups (Ganten [41]). The global report released by the International Diabetes Federation on her 10th IDF [56] diabetes Atlas report estimated 537 million adults (> 20 years) living with diabetes (Saeedi et al. [96]). The total number of people with diabetes has been predicted to rise to 643 million (1 in 9 adults) by 2030 and 784 million (1 in 8 adults) by 2045. It has also been reported that 81% (4 in 5) of people with diabetes live in low-income and middle-income countries. Undiagnosed adults living with diabetes are estimated to be 44%. 90% of these people live in low-income and middle-income countries (Patel [89]). In a country like Nigeria, 3.6 million cases were recorded in 2021 with about 48,375 deaths reported. (IDF, Available from: <http://www.idf.org/diabetesatlas>. [Last accessed on 2023 Jan 23]). The surge in DM has necessitated research into different approaches to the treatment of the disease one of the approaches is the use of anti-diabetic drugs (Harries et al. [51]). Nevertheless, these drugs have their inadequacies which result in detrimental health effects, and aside from this, most of these drugs are not readily available and affordable in developing countries like Nigeria and also in some developed countries. (Adeneye and Agbaje [4]). Because of these shortcomings, research is now been made on medicinal plants with hypoglycaemic activities which are less toxic, affordable, readily available, and accessible as a suitable substitute (Ajayi et al. [11]; , Eluehike et al. [37]). Medicinal plants are plants that contain bioactive natural products which include flavonoids, carotenoids, terpenoids, glycosides, and alkaloids (Afrisham et al., 2015; (Akoko et al. [12])) that are capable of curing certain human diseases. Some of these plants have been proven scientifically to have abilities to wrestle against diseases acting as; Antioxidant (Ho et al., [53]; Iwalewa et al., [58]; (Adesanoye and Farombi [6]; , Iwalokun et al. [59])), Hepatoprotective (Johnson et al. [62]), Antimalarial (Omeregbe and Pal, [84] (Odeh and Usman [79])), Cathartic effect (Ganapathy [43]; , Wavinya et al. [105]), Anti-inflammatory (Georgewill and Georgewill [45]) and Antimicrobial (Adetunji et al., [7]; (Anibijuwon et al. [19])). They also perform, Antipyretic activity (Adiukwu et al. [8]), Analgesic activity (Adedapo and Aremu [3]), Antileishmanial (Alawa et al., [17]), Sedative and Anxiolytic activity (Oloruntobi et al., [83]), Chemopreventive (Izevbogie [60]; , Izevbogie et al. [61]; , Yedjou et al. [109]), Sweeny et al., [101]; (Blanco et al. [28]; , Colditz et al. [32]; , Song et al. [100]), Hypolipidaemic activity (Adaramoye et al. [1]), Anti-allergic (Ngatu, et al., [75]), Hemolytic activity (Oyedejo et al., [87]), Spermatogenic (Saalu et al. [94]; , Saalu et al. [94]; , Saalu et al. [95]; , Saalu et al. [95]), Antimutagenicity (Wu et al. [107]).

Medicinal plants have over time shown considerable scientific records to prove their non-toxicity and

efficiency in the treatment of diabetes. (Asase and Yohonu [24]; , Attama et al. [25]; , Oguanobi et al. [80]; , Soliman [99]). Considering studies from Nigeria, several plants have been tested and established to be effective in lowering blood glucose in induced diabetes. (Ezurike and Prieto [39]). One of the most studied plants is *Vernonia amygdalina* Delile (VA) among about 185 plant species from 75 families that have been studied for antidiabetic potential in Africa (Mohammed et al. [72]; , Toyang and Verpoorte [102]). *Vernonia amygdalina* is a shrub common in West Africa (Aikpokpodion et al. [10]; , Olaniyan et al. [82]) and in Asia. It is a member of the Asteraceae (Composite) family and a genus of *Vernonia* having green-colored leaves of about 200 mm long and 6 mm wide (Ijeh and Ejike., [57]). Its bitter taste supposedly earned it the name bitter leaf in English. It is a plant with continuous growth (Nwosu et al. [78]). Due to its various uses in Nigeria, it is known as Ewuro (Yoruba), Oriwo (Edo), Chusar Doki (Hausa), Ityuna, Fatefata, Onugbu (Igbo), Etido (Ibibio, Ijaw, and Efik) (Farombi et al., [40]), in Ghana as Awonwono (Komlaga et al. [67]), Togo as Aluma, Cameroon as Ndoleh, Ying (Simbo et al., [98]) in Uganda as Omubirizi, Mululuza (Njan et al., [77], Kigbua et al., [65]), in Tanzania as Omubilizi (Moshi et al., [73]), in Guinea as Kossa fina, Bantara bururé (Oyeyemi et al. [88]), Ethiopia as Ebichaa (Yineger and Yewhalaw, [108]), Gbondutsi (Agbodeka et al. [9]), Rwanda as Umubilizi (Mukazayire et al. [74]), Sierra Leone as Nje nyani, An-gbenth (Oyeyemi et al. [88]). *Vernonia amygdalina* is a medicinal plant rich in fibres, fats, minerals, vitamins, amino acids, protein, and carbohydrates, (Alara et al. [15]; , Eyang et al. [38]). Vernodalol (Erasto et al. [37]), (z,z,z)-methyl ester-9,12,15-octadecatrienoic acid (Alara et al., [16]), Epivernodalol (Owoeye et al. [86]) and Phytol, 4-methyl-vinyl butyrate, are some of the phytocompounds that have been isolated from *Vernonia amygdalina*.

This research is undertaken with the primary objective of exploring the antidiabetic potential of the aqueous extract obtained from *Vernonia amygdalina* leaves. The investigation is conducted with a focus on contributing valuable insights into the medicinal applications of this plant extract in the context of managing diabetes. The specific aims include evaluating the impact of the extract on relevant biochemical markers associated with diabetes and assessing its potential as an antidiabetic agent. This study endeavors to advance our understanding of the therapeutic properties of *Vernonia amygdalina*, providing a foundation for its potential use in medical interventions targeting diabetes (Fig. 1).

Reports from Njam et al., 2014 confirmed the effectiveness of VA as an antimalarial phytomedicine with a 67% reduction in glucose level. A report was also given

on its antioxidant properties at 75–99.3% DPPH and 96.2–100% of DBTS (Ayoola et al. [27]; Farombi and Owoeye [40]). Akah et al. [13] described its antidiabetic effectiveness to have significantly reduced blood glucose levels (Akah et al., [13]), higher level of anti-leukemia activity was reported by Khalafalla et al., [64]. There was a report by (Erasto et al. [37]) about its effectiveness against penicillin notatum and *Aspergillus falvus*. VA has also been reported to inhibit and prevent atopic or zema dermatitis syndrome in mice (Ngatu et al., 2012). It causes a significant reduction in inflammation (Geogemill and Geogemill 2010), it inhibits the proliferation of MCF-7 and MDA-MB-231, a significant reduction in CYP, A2 expression (anticancer) by Gershan et al., [46]; Wong et al., [106]; Opota and Izebigile, [85]; (Owoeye et al. [86]). A higher value of Minimum inhibitory concentrations (MIC) inhibitors in streptococcus mutants at 25 ng/ng (ethanolic extracts) and 55 ng/ng (aqueous extracts) (Anbijuwon et al., [19]). Significant reduction in the mean number of implantation sites (Egharevba et al., [36]). Strong potency against clinical bacteria; *pseudomona*, *aeruginosa*, *staphylococcus aureus* and *Escherichia coli* (Alo et al., [18], Adetunji et al., [7]).

Materials and methods

Experimental plant materials

Vernonia amygdalina leaves were systematically gathered from a suburban village located within the Ile-Ife metropolis of Osun State, Nigeria. The botanical identification of the plant was carried out by a qualified taxonomist affiliated with the Department of Botany at Obafemi Awolowo University, situated in Ile-Ife. The taxonomist employed rigorous botanical classification methods to accurately identify and categorize the *Vernonia amygdalina* plant, ensuring the precision and reliability of the botanical information obtained for subsequent research.

Experimental animals (Rats)

We employed thirty healthy Wistar rats (*Rattus norvegicus*), all of whom were 10 weeks old, as the subjects for our research. These rats were specifically bred within the Department of Anatomy and Cell Biology at Obafemi Awolowo University, situated in Ile-Ife, Osun State. The selection of rats, encompassing both male and female individuals, was carried out through a random process, and they were subsequently housed in separate cages. Their individual weights fell within the range of 150 g to 250 g (Asante [21]; , Asante et al. [23]; , Koubé et al. [68]).

The rats were nourished with standard rat pellets from Ladokun Foods, Ibadan, Nigeria, and had unrestricted access to tap water (Akoko et al. [12]). They were accommodated in well-ventilated cages with a regular replacement of bedding, maintaining a room temperature of

approximately 27 °C and following a 12-h light/dark cycle. To ensure their adaptation to the environment, the animals were allowed a period of 2 weeks for acclimatization before the initiation of the study (Hamaza et al., [50]).

The entire study protocol underwent approval from the Institutional Animal Research Ethics Committee, and utmost adherence was maintained to both national and international laws and guidelines governing the care and utilization of laboratory animals in biomedical research.

Chemicals and reagents

For the execution of our study, we procured a selection of chemicals and reagents from reputable sources, ensuring the reliability and quality of our experimental materials. The specifics of our acquisitions are detailed below:

Streptozotocin: Obtained from Sigma Co, USA.

Alkaline Phosphate (ALP) Assay Kit: Sourced from RX MONZA, Northern Ireland.

Reitman and Frankel ALT/AST Level 2 Control for Aspartate Aminotransferase: Acquired from Randox Laboratories, Northern Ireland.

Alanine Aminotransferase: Attained through the customer technical support service of Randox Laboratories, Northern Ireland.

By choosing reputable suppliers such as Sigma Co, RX MONZA, and Randox Laboratories, we aimed to ensure the precision and consistency of our experimental results. The meticulous selection of these chemicals and reagents is pivotal in maintaining the integrity and reliability of our study outcomes. Furthermore, any technical guidance or support provided by the customer service of Randox Laboratories contributes to the meticulous execution of our experimental protocols.

Preparation of plant extract

Fresh leaves of *Vernonia amygdalina* were meticulously chosen, and a thorough washing process was employed using distilled water. Great care was taken to prevent any squeezing during washing to avoid potential contamination with debris. Following the washing stage, these meticulously cleaned leaves underwent a gentle air-drying process at room temperature (Alara et al., [16]). Subsequently, the dried leaves were transformed into a fine powder using a warming blender.

To prepare the aqueous extract, a 200 g portion of the powdered *Vernonia amygdalina* leaves was immersed in 500 ml of distilled water and allowed to soak for an extended period of 72 h (Attama et al., [25]). The resultant mixture underwent a meticulous filtration process, and the obtained filtrate was subjected to concentration under vacuum conditions at a temperature of 35 °C, facilitated by a vacuum rotary evaporator. The

concentrated extract was then subjected to an oven-drying procedure, ensuring the removal of moisture content while preserving the bioactive components of interest (Ajayi et al. [11]). This meticulous process was employed to obtain a potent and concentrated extract for subsequent analysis or application in research and experimentation.

Chemical characterization of *vernonia amygdalina*

The chemical characterization of the leaf extract of *Vernonia amygdalina*, also known as bitter leaf, involves identifying and quantifying various chemical compounds present in the extract (Dumas et al., [35]). These compounds contribute to the medicinal properties and biological activities associated with *Vernonia amygdalina* (Asante [21]; , Asante et al. [23]), Adeoye et al., [5]). *Vernonia amygdalina* leaf extract has been reported to contain a variety of phytochemicals, including alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds. These phytochemicals contribute to the antioxidant, anti-inflammatory, and antimicrobial properties of the extract (Farouq et al., [42], Gbadeyan et al., [44]). Polyphenols are abundant in *Vernonia amygdalina* leaf extract and are responsible for its bitter taste (Bora et al., 2019). These include compounds such as flavonoids (e.g., quercetin, kaempferol) and phenolic acids (e.g., caffeic acid, chlorogenic acid), which exhibit antioxidant and anti-inflammatory activities (Halilu et al., 2012). Alkaloids are nitrogen-containing compounds found in *Vernonia amygdalina* leaf extract, including vernoniosides and vernodaline (Dumas et al., 2020). These alkaloids possess various biological activities, including antimalarial, antidiabetic, and anticancer properties (Olaniyan et al. [82]). Terpenoids are secondary metabolites present in *Vernonia amygdalina* leaf extract, such as sesquiterpene lactones (e.g., vernolide) (Akoko et al. [12]). These compounds have been shown to possess anti-inflammatory, antimalarial, and cytotoxic activities (Anibijuwon et al. [19]). Saponins are glycosides found in *Vernonia amygdalina* leaf extract, which contribute to its foaming properties (Ajayi et al. [11]). They have been reported to exhibit anti-inflammatory, antimicrobial, and anticancer activities. *Vernonia amygdalina* leaf extract has also been observed to contain various vitamins (e.g., vitamin C) and minerals (e.g., calcium, and iron) that contribute to its nutritional value and potential health benefits. In general, the chemical characterization of *Vernonia amygdalina* leaf extract involves identifying and quantifying these chemical constituents, which collectively contribute to its pharmacological properties and therapeutic potential.

Phytochemical qualitative analysis of *vernonia amygdalina*

Alkaloids, flavonoids, phenols, and tannins were detected by chemical analysis utilizing the usual protocols previously mentioned (Halilu et al., 2012, Dumas et al., 2020). Dragendroff's reagent (potassium bismuth iodide) was added to the filtrate after ethanolic leaf extracts were dissolved in diluted hydrochloric acid to identify alkaloids. The presence of alkaloids was indicated by a reddish-colored precipitate. The sodium hydroxide test was used to find flavonoids in leaf extracts by mixing two or three drops of sodium hydroxide with each distillates water. A bright yellow tint that became colorless when diluted acid was added indicated the presence of flavonoids. One milliliter of leaf extract was diluted with water, and two or three drops of a ferric chloride solution were added to detect phenolic components. Tannins were recognized by a blue-black tint.

Biological evaluation of *vernonia amygdalina*

The biological evaluation of *Vernonia amygdalina* leaf extract involves assessing its potential pharmacological and therapeutic properties through various in vitro and in vivo studies. Several investigations have investigated the promising potential of *Vernonia amygdalina* leaf extract as a therapeutic agent for managing diabetes mellitus (Anywar et al. [20]; , Attama et al. [25])(Asante [22]; , Djeujo et al. [34])). Through meticulously designed in vivo experiments utilizing diabetic animal models, researchers have sought to comprehensively elucidate the extract's impact on various facets of diabetic pathology (Alara et al., [16]). These include efficacy in modulating critical parameters including blood glucose levels, insulin secretion dynamics, glucose tolerance mechanisms, and markers indicative of insulin resistance (Opota and Izeibigle, [85]). By scrutinizing these essential metabolic pathways and physiological responses, researchers endeavor to uncover the intricate mechanisms underlying *Vernonia amygdalina* leaf extract's therapeutic potential in ameliorating diabetes mellitus and its associated complications (Ajayi et al. [11]; , Asante [21]; , Asante et al. [23]; , Herz et al. [52]; , Koubé et al. [68]; , Uqaili [104]). These helps contribute to the understanding of the extract's pharmacological properties but also hold promise for the development of novel therapeutic strategies aimed at combating prevalent metabolic disorders. Aside from these, the biological evaluation of *Vernonia amygdalina* leaf extract encompasses a wide range of pharmacological activities, highlighting its potential as a valuable source of bioactive compounds for the development of novel therapeutic agents for various forms of in vitro and in vivo studies such as Antimalarial Activity, Anti-inflammatory Activity, Anticancer Activity, Hepatoprotective

Activity among others (Rajpal et al., [91], Ejiofor et al., 2020, (Asante [22])).

Experimental animals (Grouping)

The initial pool of thirty Wistar albino rats underwent a random division into five distinct groups, with each group comprising six rats. The delineation of these groups is as follows:

NC (Control Group): This group consisted of non-diabetic rats, serving as the baseline or control for comparison.

DC (Experimentally Induced Diabetic Group): Rats in this group were intentionally induced with diabetes through the experimental protocol.

DV (Experimentally Induced Diabetic + Vernonia amygdalina Extract Group): Rats in this group were both experimentally induced with diabetes and concurrently administered with the aqueous extract of Vernonia amygdalina.

DG (Experimentally Induced Diabetic + Standard Antidiabetic Drug Group): Rats in this group, like Group DC, were experimentally induced with diabetes, but in addition, they received treatment with the standard antidiabetic drug, glimepiride.

NA (Non-Induced Diabetic + Vernonia amygdalina Extract Group): This group comprised rats that were not experimentally induced with diabetes but received the aqueous extract of Vernonia amygdalina.

This meticulous grouping allowed for a comprehensive exploration of the effects of diabetes induction, the potential therapeutic impact of Vernonia amygdalina extract, and a comparative assessment with the standard antidiabetic drug (Olaniyan et al. [82]). Each group served a specific role in the research design, contributing to a nuanced understanding of the variables under investigation.

Induction of diabetes

Following a two-week acclimatization period, diabetes mellitus was intentionally induced in rats from groups DC, DV and DG (Uqaili [104]). This induction was achieved through a singular intraperitoneal injection of 65 mg/kg streptozotocin, which was dissolved in a 100 g sodium citrate buffer (Hamza [50]). As a point of reference, rats in Group A (control) were administered an equivalent volume of citrate buffer intraperitoneally. The confirmation of diabetes occurred three days post the streptozotocin treatment, employing an Accu-CHEK glucometer. To assess the diabetic status, a single blood sample was obtained by pricking the tail vein using a tail clip. Rats exhibiting fasting glucose levels equal to or exceeding 250 mg/dl were deemed diabetic and subsequently included in the study. The overall duration of the

experiment spanned 21 days, encompassing the induction period, confirmation of diabetes, and the subsequent investigative phases (Oyeyemi et al. [88]). This timeframe was carefully chosen to allow for comprehensive observations and analyses throughout the course of the study.

Administration of plant extract

After the successful induction and confirmation of diabetic mellitus in groups DC, DV and DG, aqueous extracts derived from Vernonia amygdalina leaves were administered orally to rats in groups DV and NA, with each rat receiving a dose of 250 mg/kg body weight (Ben, et al., [29]). The administration process was conducted carefully and precisely using an oral cannula. To facilitate this, the rats' ears were gently pulled up, and their limbs were held to maintain a stable and upright position, ensuring accurate and consistent delivery of the extract (Al-Maula [14]). This methodical approach aimed to optimize the absorption and assimilation of the administered dose for subsequent observation and analysis in the study.

Biochemical analyses

The biological assays conducted in the study involved the utilization of a high-quality Alkaline Phosphatase (ALP) assay kit obtained from RX MONZA, Northern Ireland. This ensured the acquisition of reliable testing reagents, crucial for obtaining accurate results. Additionally, the assessment of Alanine Transaminase (ALT) and Aspartate Aminotransferase (AST) levels followed the methodology outlined by Reitman and Frankel (1956). This established method is well-recognized in the field and provides a standardized approach for measuring ALT and AST activities with precision. By adhering to these established protocols, the biochemical analyses in the research study were conducted credibly and reproducibly, enhancing the reliability of the obtained results.

Statistical analysis

Parametric data differences between two groups for one variable were evaluated using the student's t-test. Additionally, differences among four groups for two variables were assessed through a one-way ANOVA to determine the significance of differences, with significance considered at the level of $P < 0.05$. Data are presented as means $\pm$ standard deviation (SD), providing a comprehensive statistical approach to investigate variations and relationships within the dataset (Rajpal et al., [91]). This analytical approach ensures a rigorous examination of the data, enhancing the reliability and robustness of the findings (Tables 1, 2 and 3).

Table 1 Effects of aqueous VA on the average weight of animals

GROUPS	Day 1	Day 4	Day 6	Day 8	Day 11	Day14
NC	115.83 ± 7.00 ^a	115.00 ± 6.58 ^a	125.83 ± 6.33 ^a	129.17 ± 4.90 ^{ab}	129.17 ± 4.90 ^{ab}	130.00 ± 5.32 ^{ab}
DC	113.33 ± 7.27 ^a	120.00 ± 4.66 ^{ab}	105.00 ± 3.42 ^a	112.50 ± 3.82 ^a	105.00 ± 2.24 ^a	120.83 ± 3.00 ^{ab}
DV	227.50 ± 10.23 ^d	215.00 ± 9.13 ^d	205.00 ± 10.33 ^d	217.50 ± 10.06 ^d	209.17 ± 11.72 ^d	204.17 ± 14.94 ^d
DG	248.33 ± 13.27 ^a	240.00 ± 14.26 ^a	225.00 ± 14.32 ^d	235.00 ± 14.32 ^d	217.50 ± 14.32 ^d	217.50 ± 14.99 ^a
NA	124.17 ± 13.44 ^{ab}	125.83 ± 13.00 ^{ab}	136.67 ± 13.20 ^{abc}	143.33 ± 14.59 ^{abc}	146.67 ± 13.08 ^{abc}	148.33 ± 14.70 ^{abc}

Values of the average weights are given as mean ± SEM coded as 1,4,6,8,11 and 14 in each group a,b,c,d, ab, cd,abc within the columns signifies that means with different letters differ significantly at $P < 0.05$ while means with the same letters does not differ significantly at $P < 0.05$ (using one way ANOVA with Duncan multiple range test)

Table 2 Blood glucose level (g/dl) of Animals administered with *Vernonia amygdalina*

GROUPS	Day 3	Day 7	Day 14
NC	108.50 ± 2.59 ^a	103.83 ± 1.96 ^a	124.67 ± 5.15 ^a
DC	549.50 ± 31.94 ^a	514.83 ± 22.30 ^b	595.17 ± 4.09 ^b
DV	426.33 ± 21.39 ^a	547.67 ± 25.31 ^b	495.17 ± 30.16 ^{bc}
DG	464.50 ± 39.45 ^{cd}	526.00 ± 49.89 ^b	456.50 ± 75.18 ^b
NA	113.33 ± 6.80 ^a	104.00 ± 4.00 ^a	132.33 ± 16.50 ^b

Values of the blood glucose level are given as Mean ± SEM coded as 3,7 and 14 days in each group. a,b,c,d, bc, and cd within the column signifies that means with different letters differ significantly at $P < 0.05$ while means with the same letter do not differ significantly at $P < 0.05$ (using one-way ANOVA with Duncan multiple range test). For the blood glucose level for 3 days in each group NC, DC, DV, DG, and NA, there is no significant difference between group NC (Control) and group NA (*Vernonia amygdalina* only). Also no significant difference between group DV (Diabetic + *Vernonia amygdalina*) and group DG (Diabetic + glimepiride) at $P < 0.05$. there are no significant differences between groups A, B, and C

For the blood glucose level for 7 days in each group, there is no significant difference between groups DC, DV, and DG

For the blood glucose level for 14 days in each group, there is no significant difference between the NC group and NA group, also with the DV and DG group while the DC group differs significantly with other groups at $P < 0.05$

Result

Body weight decreases significantly in STZ-induced diabetic rats. After treatment with aqueous extract of VA, body weight increases when compared with the control. There is also a significant difference between STZ diabetic rats and the rats treated with glimepiride (insulin). Rats treated with only *Vernonia amygdalina* were not expressively different with respect to control rats, there is a slight increase in weight compared to the control rats (Figure 2).

Discussion

Scientists have recently dedicated time to research more on herbs in the treatment of diabetics, due to the fact that herbal drugs may have multiple therapeutic targets that can be connected to a number of active components, such as polyphenols, sesquiterpene lactones terpenoids, flavonoids, phenolics, alkaloids, tannins, terpenes, saponins, steroidal glycosides,

Table 3 Effects of *Vernonia amygdalina* on Serum liver function test enzymes in Streptozotocin induce diabetics rats

GROUPS	AST(U/L)	ALT(U/L)	ALP(U/L)
NC	106.97 ± 18.37	51.34 ± 9.89	50.71 ± 8.64
DC	152.20 ± 19.68	87.94 ± 5.21	66.95 ± 17.57
DV	125.57 ± 4.19	64.92 ± 6.13	52.17 ± 10.86
DG	126.57 ± 17.82	53.13 ± 11.64	66.54 ± 12.54
NA	116.90 ± 7.11	70.13 ± 19.99	62.96 ± 16.06 ^a

Values are given as Means ± SEM for 3 biochemical parameters coded as AST (Aspartate aminotransferase), ALT (Alanine aminotransferase), and ALP (Alkaline phosphatase) in each group. From the result obtained on the effect of the extract on the liver function test activities, it was observed that there is no significant difference in AST activity in the groups except for the DV group which shows a significant increase at $P < 0.05$. This group is the diabetics treated with the extract (*vernonia amygdalina*). ALT activity in the non-induced rats (NC group) and in the induced diabetics' rats in the DG group does not differ significantly. Likewise, the group of induced diabetic rats treated with extract and non-induced diabetic rats treated with extract does not differ significantly while the diabetic rats differ significantly with an increase. From the result obtained, ALP activity does not differ significantly in all the groups

Note: The normal range of Aspartate transaminase in the serum of the body should be between 10 and 34 (U/L), therefore any increase or decrease in AST means there is a liver disease

The normal range of Alanine transaminase in the serum of the body ranges according to gender. The normal range in males should be between 10 and 40 U/L or 0.017 and 0.68 microkats/L while in females is between 7–35 U/L or 0.12 and 0.06 microkats/L

triterpenoids (Erasto et al. [37]), Farombi and Owioye, [40]; Kiplimo et al., [66]; (Adedapo et al. [2]; , Toyang and Verpoorte [102]), Quasie et al., [90]). Some of the research conducted on these local herbs are from; Gupta et.al, [49]; Krishnakumari et.al, [69]; Andpierre et. al; 2006, Atangwho et.al, [26]; Preethi 2013; (Anywar et al. [20]; , Salehi et al. [97]). Of all the plants researched, *V. amygdalin* is a well-known species. This plant is popularly known among tropical Africans and some parts of Asia (Cienfuegos et al. [31]). In the preparation of *V. amygdalina*, the local users either use water or ethanol for their extraction. This current study appraised the antidiabetic effect of aqueous *V. amygdalina* on STZ-treated rats. Blood glucose concentrations were measured in this study and results showed a significant reduction ($p < 0.05$) in blood



Fig. 1 Vernonia amygdalina leaf (Field Photography by Falae Esther Adekemi)

glucose of diabetic rats treated with aqueous extracts of *V. amygdalina*. These observations correlate with previous research reports (Asante [21]; , Asante et al. [23]; , Goje et al. [48]; , Ojeh et al. [81]) where antidiabetic medicinal plants significantly reduced the high blood glucose level in STZ-induced diabetic rats.

Reduction in body weight is one of the indications of DM occurring as a deterioration in glucose control (Berger et al. [30]). It has been established through diverse studies that significant weight reduction is a symptom of untreated diabetes in rats (Ahmed et al.,

2005) which could effectually lead to the death of the animal if not properly treated or managed. The present study observed a significant reduction in food intake and an upsurge in water intake a week after confirmation of diabetes, before the commencement of treatment. Body weight decreases significantly in STZ-induced diabetic rats. There are some factors responsible for the reduction in body weight in diabetic animals/humans and these include; acute fluid loss, lipolysis, and proteolysis (Herz et al. [52]). Hyperosmolarity combined with obligatory renal water loss in diabetes tends to deplete intracellular water, activating the osmoreceptor of the thirst center of the brain and polydipsia occurrence, which leads to water intake (Rajpal et al., [91]). At a level of high-water intake, there is decreased appetite and hereafter catabolic effect prevails resulting in weight loss which was observed in the DC group in this study. The Aqueous extracts from VA reverse the loss in weight observed in the diabetic rats as it relatively improved the body weight and when compared with the insulin-treated there is a significant difference. Rats treated with only vernonia amygdalina were not expressively different with respect to control rats, there is a slight increase in weight compared to the control rats and this also confirms the efficiency of VA in the treatment of diabetics, this agrees with the report of (Russell et al. [93]) and Ejiofor et al., 2020. The administration of aqueous VA to diabetic rats for 21 days maintained the near the body weight of the rats and this can be associated with the preservation of the intake of food or through the increased availability of insulin that promotes the anabolic processes and prevent catabolism (Timmerman et al. [103]). Decreased body weight in diabetes may also be caused by increased muscle degradation (Hu et al. [54]) and this can be reversed by insulin for the stimulation of protein and lipid synthesis in

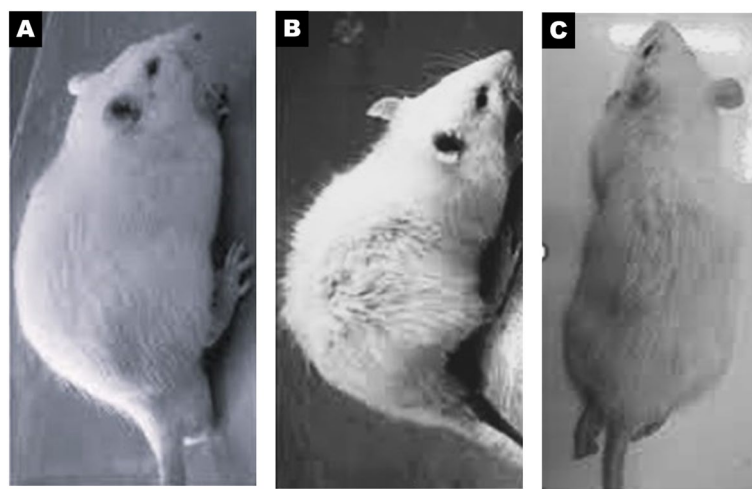


Fig. 2 a Control rat (b) Diabetic rat (c) Diabetic-treated rat

conjunction with glycogen storage (Timmerman et al. [103]) and (Asante [22])).

In this investigation, a noteworthy elevation in the serum levels of liver biomarkers (AST, ALT, and ALP) was observed in the treated DC group compared to the negative Control group. This increase indicated compromised liver function, attributed to hepatocellular necrosis. Upon treatment of the diabetic rat model with aqueous VA, a noticeable reduction in the activity of the enzymes (AST, ALT, and ALP) was discerned. This outcome aligns with previous findings by Soliman et al. and (Akah et al. [13]) which proposed that elevated activities of serum transferases commonly manifest in liver diseases prevalent in diabetic conditions. The heightened levels of AST, ALT, and ALP in the blood serum suggested hepatocellular damage resulting from STZ toxicity, leading to the leakage of these enzymes from the liver cytosol into the bloodstream. Additionally, Ghosh & Suryawanshi [47] and (Joseph et al. [63]) suggested that diabetic complications, such as increased ketogenesis and gluconeogenesis, may be linked to heightened transaminase activity. The restoration of these biomarker enzymes towards normal levels following treatment with aqueous VA indicates a reduction in diabetic conditions. This observation is particularly evident in animals treated with aqueous VA. Therefore, the decrease in AST levels in the VA treatment group, especially when compared with other treatment groups, notably the DC group, signifies a positive impact on liver health and function associated with the therapeutic effects of aqueous VA (Djeu et al. [34]). The ameliorative effects of VA on liver biomarkers underscore its potential as a therapeutic agent in mitigating liver damage associated with diabetes.

Conclusion

The present study strongly confirms the anti-diabetic effects of the aqueous extract of VA in streptozotocin-induced diabetic rats, highlighting its potential as a significant phytomedicine in the pursuit of more effective treatments for diabetes. These findings underscore the promising role of VA extract as a natural remedy for managing diabetes and pave the way for further research and development toward harnessing its therapeutic benefits for diabetic patients. In the future, it is essential to delve deeper into understanding the specific bioactive compounds within VA extract responsible for its anti-diabetic properties. Further investigations can explore optimal dosages, delivery methods, and potential synergistic effects when combined with existing diabetes treatments. Additionally, clinical trials involving human subjects can provide valuable insights into its safety and efficacy, ultimately moving us closer to practical applications for 1.

Abbreviations

VA	Vernonia amygdalina
ALT	Alanine aminotransferase
AST	Aspartate-aminotransferase
DM	Diabetes Mellitus
T1DM	Type 1 Diabetes Mellitus (T1DM)
T2DM	Type 2 Diabetes Mellitus (T2DM)
IDF	International Diabetes Federation
DPPH	Diphenyl-1-picrylhydrazyl
DBTS	Diblock Brush Terpolymers
MCF-7	MDA-MB-231
MIC	Minimum inhibitory concentrations
ALP	Alkaline Phosphate
NC	Control (non-diabetic)
DC	Experimentally induced diabetic
DV	Experimentally induced diabetic + aqueous extract of VA
DG	Experimentally induced diabetic + standard antidiabetic drug (<i>glimepiride</i>)
NA	Non-induced diabetic rats + aqueous extract of VA
STZ	Streptozotocin

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Authors' contributions

FEA collected, extracted the plant material, and carried out the experimentation while FPO analysed the data and drafted the manuscript. JK performed the literature review and edited the manuscript. All authors read and approved the manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

All applicable international, national, and or institutional guidelines for the care and use of animals were followed. Consent for publication is not applicable.

Competing interests

Authors declare no competing interest.

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