

Antidiabetic Evaluations of Different Parts of *Vernonia Amygdalina*

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Abstract: Aqueous leaves extract of *Vernonia amygdalina* are used in different parts of Africa for treatment of certain uncomplicated ailments, of which hyperglycaemia is one of the ailments. This study was carried out to evaluate the antidiabetic effect of leaf, stem-bark and root methanolic extracts of *Vernonia amygdalina* for antidiabetic activity in streptozotocin induced diabetic rat model. Extraction was done by Soxhlet method. The rats were divided into groups and a dose of 2000 mg/kg body weight of extracts administered for acute toxicity. The rats were observed for toxic effects for 14 days. Diabetes was induced in the rats by intraperitoneal administration of 60 mg/kg body weight of streptozotocin. All the rats except the vehicle control and toxic control groups were treated for 21 days with a standard drug and different extracts at 250 and 500 mg/kg body weight. On the 7th, 12th, 16th and 21st days of treatment, bloods were withdrawn from the caudal vein of the rats and glucose levels determined. On the 21st day, the bloods were tested for liver enzymes, total cholesterol and triglyceride. The rats were sacrificed and their livers, kidneys and pancreases histopathologically studied. The rats showed no acute toxic effects. The extracts reduced the glucose levels to a certain extent, with the stem-bark and root extracts given a statistically significant result of $P < 0.05$, while the leaf gave $P > 0.05$. The root extract gave a correlation coefficient of 0.94 in comparison to the standard against the stem-bark and the leaf which gave 0.95 and 0.84 respectively. The stem-bark and root extracts reduced the liver enzymes, total cholesterol and triglycerides to a statistically significant level of $P < 0.05$, while the leaf gave $P > 0.05$. The histopathology showed an improvement in comparison to the normal and diabetic group. The methanolic extracts showed a positive activity toward a diabetic animal model. Extensive studies should be done especially on the stem-bark; since the root part though gave the best result could not be used because of the environmental impact.

Keywords: *Vernonia amygdalina*; Acute toxicity; Antidiabetic.

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I. Introduction

World Health Organisation in its 2016 Global Report on Diabetes estimates that, globally, 422 million adults aged over 18 years were living with diabetes in 2014 [1]. This figure has overtaken a figure obtained from a previous study which estimated that the total number of world diabetes patient to increase up to about 300 million by the year 2025 [2]. In African region, according to World Health Organisation Diabetes programme on Country and Regional Data on Diabetes 2017, estimated that there will be 18,234,000 diabetes patients by 2030 out of which around 4,835,000 coming from Nigeria alone. This makes Nigeria the most vulnerable for diabetes in African region.

There are many medicinal plants that have been implicated in the management of diabetes in different parts of the world [3][4] in West Africa [5], and in Nigeria as well [6][7][8][9].

Vernonia amygdalina is one of the plants in Nigeria which have been confirmed to have antidiabetic activity [10]. *Vernonia amygdalina* is an edible plant of the Asteraceae family. It is commonly known as bitter leaf due to its bitter taste, in Nigeria and in tropical African regions as well as in South Africa [11]. Some of its species; *Vernonia cinerea* and *Vernonia anthelmintica* are available in India. *Vernonia amygdalina* is known with different names in African which include Grawa (Amharic), Ewuro (Yoruba), Etidot (Ibibio), Onugbu (Igbo), Ityuna (Tiv), Oriwo (Edo), Chusar-doki (Hausa), Mululuza (Luganda), Labwori (Achoi), Olusia (Luo), and Ndoleh (Cameroon) [12][13]

Traditionally, according to Yeapet *et al.*, 2010 different parts of *Vernonia amygdalina* are used for stomachache, gastrointestinal troubles, oral hygiene, itches, parasitic infection, ringworm, fever, headache, diabetes, tinea, cough, constipation and piles (haemorrhoids).

Based on these ethnomedicinal uses and scientifically proven advantages, this present study was undertaken to evaluate and compare the antidiabetic activity of methanolic extracts of different parts (Leaf, stem-bark and root) of *Vernonia amygdalina* using diabetic rat model.

II. Materials And Methods

2.1 Plant collection

The different parts of the plant, *Vernonia amygdalina*, were collected during the month of August, 2015, from umuono community, Ngodo village, Nise, Anambra state, Southeast, Nigeria. The plant was identified and authenticated by a taxonomist, of International Centre for Ethnomedicine and Drug Development, Nsukka, Enugu State, Nigeria. The Voucher number is INTERCEDO/041.

III. Chemicals And Drugs

The Chemicals used were purchased from B.M Pharma, Dibrugarh. The chemicals include; Hexane (HIMEDIA), Methanol (HIMEDIA), Streptozotocin (SRL), Metformin and Carboxymethylcellulose (MERCK)

IV. Preparation Of Plant Extract

The leaves, stems and roots of *Vernonia amygdalina* were collected, dried under shade and powdered using a manual grinder. The parts were each subjected to successive Soxhlet extraction with Hexane and Methanol, concentrated and dried. The methanolic extracts were used for the study.

V. Animals

The Wister rats weighing 150-250 gram of either sex were used for the study and were housed in cages at temperature of $25 \pm 2^{\circ}\text{C}$, humidity of 50 to 60 and 12 hours' light/dark cycles. Free access to water and rat feed were maintained. Approval was obtained from the Institutional Animal Ethics Committee (Approval no. IAEC/DU/114, dated 18/02/2016)

VI. Acute Toxicity Study

The acute toxicity study was performed according to the Organisation for Economic Co-operation and Development (OECD) guideline 423 (OECD Guidelines for the Testing of Chemicals (No. 423) [14]

VII. Experimental Design

The Wister rats were grouped into nine, of five rats per group

Group I: Normal control rats,

Group II: Diabetic control rats,

Group III: Diabetic rats which received metformin hydrochloride (10 mg/kg body weight), Group IV A and IV B: Diabetic rats administered high dose (500 mg/kg body weight) and low dose (250 mg/kg body weight) of methanolic leaf extract (MLEV) respectively.

Group V A and V B: Diabetic rats administered high dose (500 mg/kg body weight) and low dose (250 mg/kg body weight) of methanolic stem-bark extract (MSEV) respectively

Group VI A and VI B: Diabetic rats administered high dose (500 mg/kg body weight) and low dose (250 mg/kg body weight) of methanolic root extract (MREV) respectively.

Carboxymethylcellulose (CMC) of 0.5 % was used as vehicle.

VIII. Study Protocol

Diabetes was induced in the rats by injecting Streptozotocin in normal saline at a dose of 60 mg/kg body weight intraperitoneally after overnight fasting. Diabetes was confirmed in the streptozotocin injected rats by measuring the fasting blood glucose concentration three days after the injection. Rats with blood glucose level above 250 mg/dl were considered diabetic and were used for the study. All the rats except the vehicle control and toxic control groups were treated for 21 days with a standard drug and different extracts (MLEV, MSEV and MREV) at 250 and 500 mg/kg body weight doses. On the 7th, 12th, 16th and 21st days of treatment, bloods were withdrawn from the caudal vein of the rats and glucose levels determined. On the 21st day, the bloods were tested for liver enzymes, total cholesterol and triglyceride. The rats were sacrificed and their livers, kidneys and pancreases histopathologically studied.

IX. Biochemical Estimation

After 21 days of treatment, animals were sacrificed by cervical dislocation. Blood samples were collected by cardiac puncture. The serum was separated by centrifugation and used for the determination of Alkaline Phosphatase (ALP) using AUTOSPAN ALP test kit, Aspartate Transaminase (AST) using Liquid Gold AST test kit, Alanine Transaminase (ALT) using Liquid Gold ALT test kit, Total Cholesterol (TC) using Liquid Gold Cholesterol test kit and Triglycerides (T) levels using Synergy Bio Triglycerides test kits.

X. Histopathological Analysis

At the end of the 21 days treatment, the rats were sacrificed and their livers, kidneys and pancreases were removed and fixed in neutral buffered 10% formalin. The tissues were processed further by dehydration, clearing and wax infiltration. Sectioning of the tissues was done with a rotary microtome, stained using eosin and haematoxylin stains, and observed under a light microscope for histopathological changes.

XI. Statistical Analysis

Statistical analysis was carried out using Correlation Coefficient, one-way ANOVA and Tukey's Honest Significant Difference Post Hoc Test on VassarStats platform. The results of the statistics analysis were considered based on statistical significance of $P < 0.05$.

XII. Results

12.1 Acute toxicity study

The mice showed no signs or symptoms of toxicity and no death occurred after orally administering a dose of 2000 mg/kg body weight of the methanolic extracts each of the leaf (MLEV), stem-bark (MSEV) and root (MREV) of *Vernonia amygdalina*, and observed according to the Organisation for Economic Co-operation and Development (OECD) guideline.

12.2 Effect of *Vernonia amygdalina* on blood glucose level of streptozocin-induced diabetic rats

Figs. 1A, 1B, 1C and 1D represent the effects of the methanolic extracts of the different parts of *Vernonia amygdalina* on streptozocin-induced diabetic rats in the course of treatment, in comparison with the control groups and a standard drug (metformin). On the horizontal axis, the point 0 represents the glucose level before induction of diabetes, point 1 represents the third day after induction, points 2, 3, 4 and 5 represent days seven, twelve, sixteen and twenty-one of treatment respectively. It can be observed that on the administration of the plant extracts, the glucose levels were reduced significantly ($P < 0.05$) in comparison with the diabetic control, except for the leaf part with $P > 0.05$ in comparison with the diabetic control. The correlation coefficient of the leaf, stem-bark and root extracts in comparison to the standard gave 0.87, 0.95, 0.94, which shows that the activity of stem-bark (MSEV) and the root (MREV) are more related to the standard.

12.3 Biochemical Estimation

12.3.1 Effects of the extracts on Alkaline Phosphatase (ALP), Aspartate Transaminase (AST), Alanine Transaminase (ALT)

Fig. 2 represents the levels of Alkaline Phosphatase (ALP), Aspartate Transaminase (AST), Alanine Transaminase (ALT) in normal rats, diabetic control, standard treated and extract treated rats. The results presented shows that treatment with the extracts causes reduction in the levels of ALP, AST and ALT in comparison with the normal control, diabetic control and standard treated. The decreases in the serum enzymes levels were statistically significant ($P < 0.05$) in comparison with the diabetic control except for the leaf extract ($P > 0.05$). The results also showed that among the extracts, the root extract (MREV) gave the best reduction of all the enzymes.

12.3.2 Effects of the extracts on Total Cholesterol and Triglycerides

Fig. 3 shows the levels of total cholesterol and triglycerides in normal rats, diabetic control, standard treated and extracts treated rats. The results presented shows that treatment with the extracts cause reduction in the levels of the total cholesterol and triglycerides in comparison with the normal control, diabetic control and standard treated. The decreases in the levels were statistically significant ($P < 0.05$) in comparison with the diabetic control except for the leaf extract ($P > 0.05$). The results also showed that among the extracts treated, the root extract (MREV) had the best total cholesterol and triglyceride reduction.

12.4 Effects of the extracts on histopathology of the liver, kidney and pancreas

Fig 4 represents the histological sections of livers, kidneys and pancreases of the normal control rats, diabetic control rats, standard drug treated rats and the extracts treated rats. The normal control liver, kidney and pancreas are represented by NCL, NCK and NCP respectively; diabetic control represented by DCL, DCK and DCP; standard drug treated represented by STL, STK and STP; leaf extract treated represented by LTL, LTK and LTP; stem-bark extract treated represented by SBTL, SBTK and SBTP and the root treated represented by RTL, RTK and RTP.

In the normal control, the liver section shows the portal vein (C), kupffer cells (KC), the sinusoids (S), hepatocyte (H) and the central vein (CV). The kidney section of the normal control shows the urinary space (US), the glomerulus (G) and the bowman's capsule (BC). The pancreas section of the normal control shows the Islet of Langerhans (IL) or pancreatic islet (PI).

XIII. Discussion

Diabetes Mellitus is a state of metabolic disorder which causes a loss of glucose homeostasis with the disturbance of carbohydrates, fat, protein metabolism resulting from defects in insulin [15].

In this experiment, streptozotocin was used for induction of diabetes. Alloxan can also be used for induction, but streptozotocin was preferred due to some advantages over alloxan, like relative long half-life, sustained hyperglycaemia and development of well characterized diabetic complications with fewer incidences of ketosis as well as mortality [16]. It exerts its hyperglycaemic effect through destruction of pancreatic b-cells [17][18].

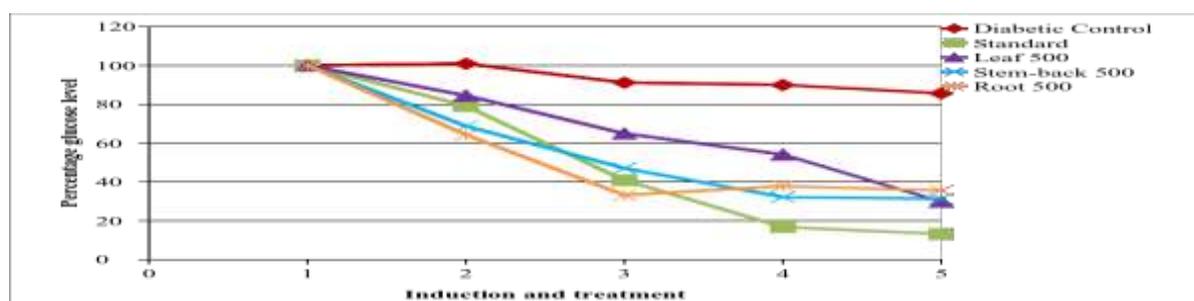
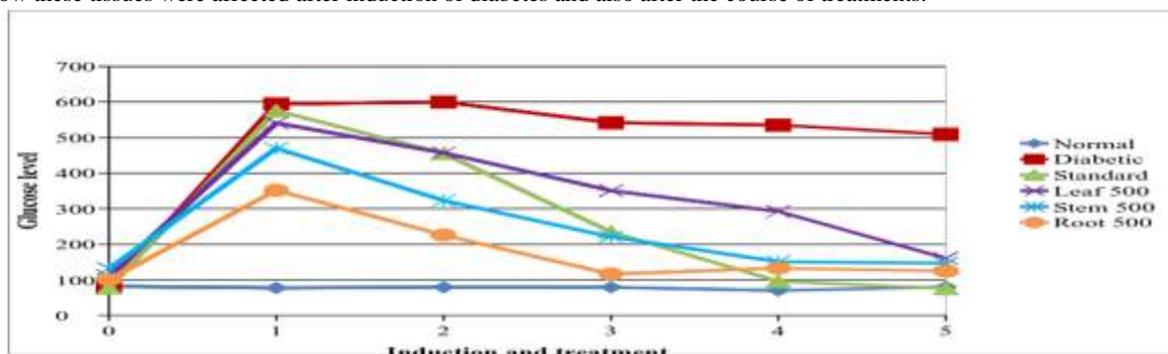
The Organization for Economic Cooperation and Development guideline 423 used for the acute toxicity study estimates the safety dose for the plant extract. In the study a maximum dose of 2000 mg/kg body weight was used. Since no toxic signs were observed, it means that any dose below or a dose of 2000 mg/kg body weight is safe for the animals. This necessitated the use of 250 mg and 500 mg/kg body weight.

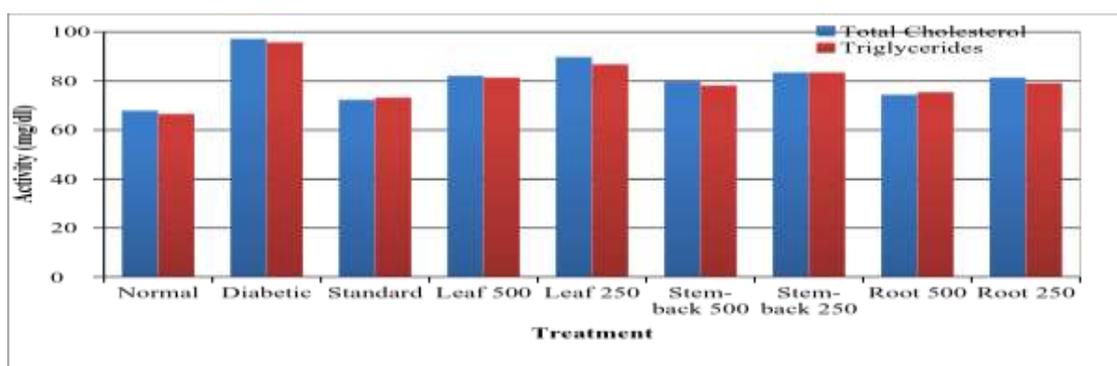
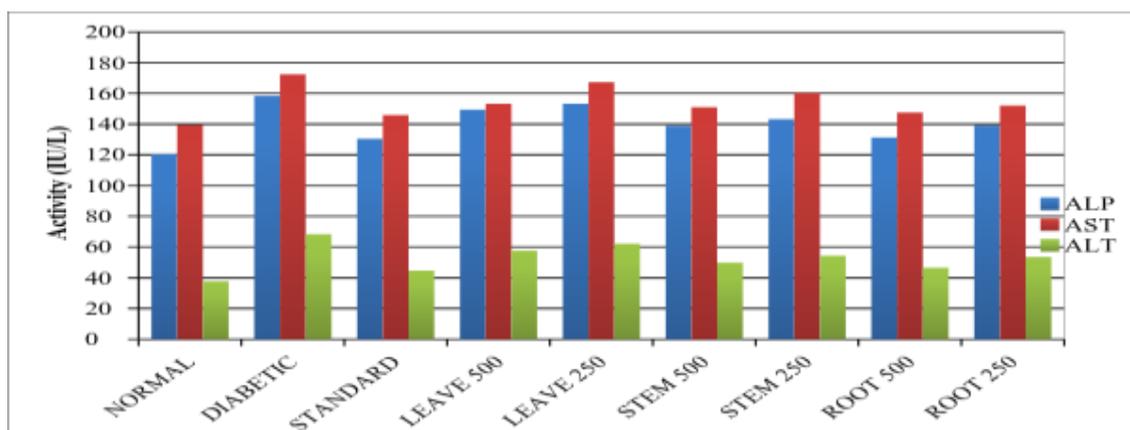
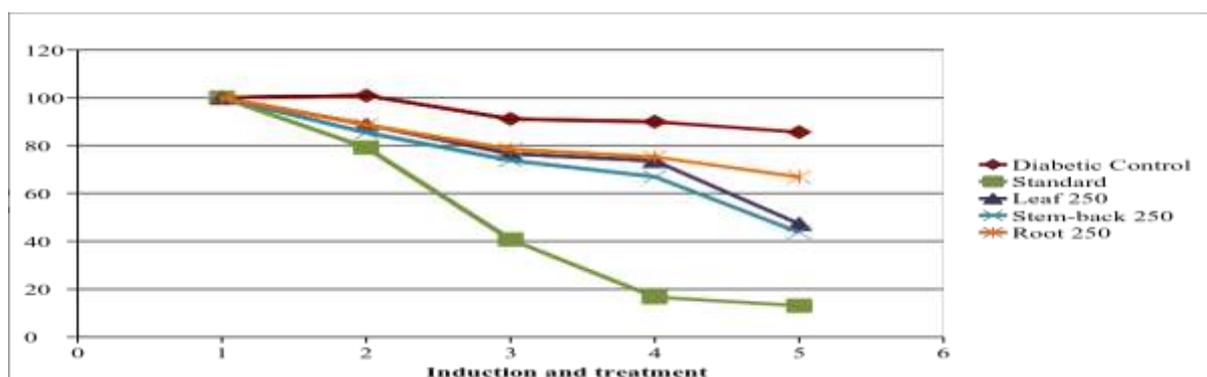
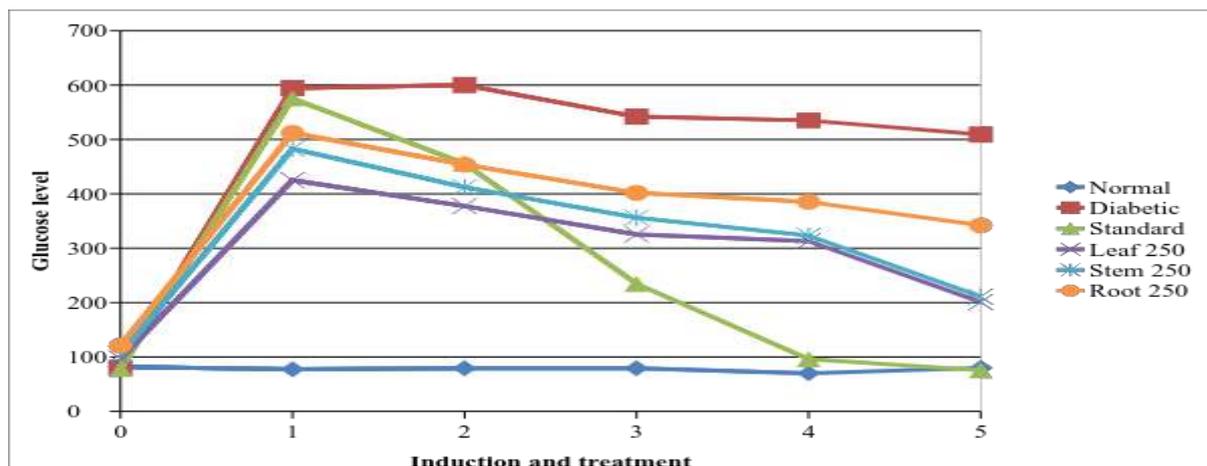
This study showed that the methanolic extracts of all the parts of *Vernonia amygdalina* reduces the glucose level of a diabetic rat in different amounts. This suggests the presence of an antidiabetic compound(s) in the plant. The antidiabetic activity graphically depicted in figs. 1B and 1D, represents the percentage reduction for each of the plant extracts which was obtained from figs. 1A and 1C respectively. The rats after induction of diabetes had increased varying levels of glucose which makes it difficult for comparison. Figs. 1B and 1D becomes a significant tool, since it represents the percentage reduction from the maximum level of glucose obtained after induction for each rat on each day of the glucose check.

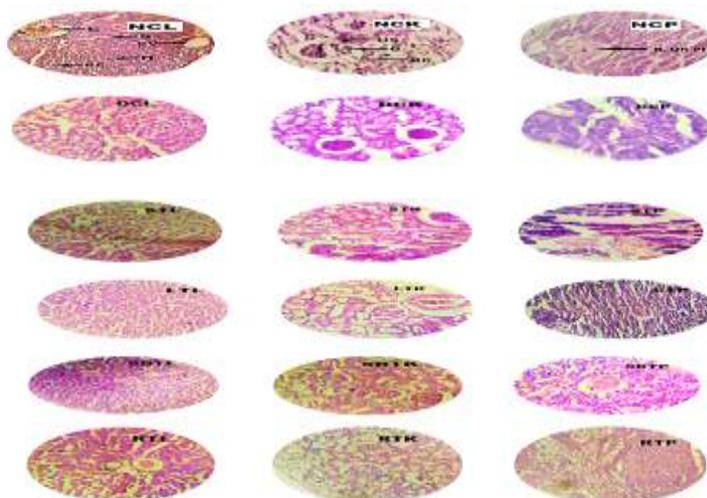
The root extract (MREV) as shown in the graphical representation and statistical values of 'P' showed the best antidiabetic activity, followed by the stem-bark (MSEV) and then the leaf (MLEV). This could be attributed to the presence of relatively large amount of suspected individual antidiabetic compound in the root in comparison to other parts or presence of more than one antidiabetic compound with synergistic or additive effect.

The data obtained from the study also indicated that the methanol extracts of different parts of *Vernonia amygdalina* significantly decreased the levels of Alkaline Phosphatase (ALP), Aspartate Transaminase (AST), Alanine Transaminase (ALT), Total Cholesterol and Triglycerides which were increased above normal due to induced hyperglycaemia in comparison with the control group. Increased activity of transaminases, which are active in the absence of insulin because of increased availability of amino acids in diabetes, are believed to be responsible for the increased gluconeogenesis and ketogenesis observed in the disease [19]. Alanine and aspartate transaminase activities are used as an indicator of hepatocyte damage [20]. Elevation of serum alkaline phosphatase concentration in patients with diabetes mellitus has been observed for several years, but the source and reasons are unknown [21]

The total cholesterol and triglycerides levels which were increased in comparison to the normal control due to the induced diabetes were decreased to significant levels with the administrations of the stem-bark and root extracts. The leaf extract also exhibited the same effect but statistically insignificant. There is a direct relationship between the liver function and cholesterol levels. A produce of the liver called bile helps to digest fats and process cholesterol. As observed in the result liver function test, and Total cholesterol and triglycerides, the increase in liver function test also resulted in the increased cholesterol and triglycerides which signifies liver malfunction. The reduction of glucose levels to significant level and also reduction of the liver enzymes, total cholesterol and triglyceride to significant level could suggest regeneration of the damaged parts of the organs or tissues involved and also could suggest protective activity of *Vernonia amygdalina* on the liver, kidney and/or pancreas. Histopathological studies of liver, kidney and pancreas in diabetic extracts treated groups in comparison with the normal group, diabetic control group and standard treated groups were done to microscopically study how these tissues were affected after induction of diabetes and also after the course of treatments.







XIV. Conclusion

The methanolic extract of the leaf, stem-bark and root part of *Vernonia amygdalina* reduced the glucose level, liver enzymes, total cholesterol and triglycerides levels of diabetic rats, with the stem-bark and root part giving statistically significant result. Though the root part showed best activity, but due to conservation and environmental impact, further investigation of the stem-bark part is on-going, including chronic toxicity study, phytochemical isolation of pure compounds and antidiabetic studies of the pure compounds.

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