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Glucose Enzymatic Modulation by *Vernonia amygdalina* in Streptozotocin-Diabetic Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AEO and ACO designed the study, wrote the protocol and interpreted the data. Authors LOE and IPN anchored the field study. Author LOE gathered the initial data and performed preliminary data analysis. Authors AEO and IPN managed the literature searches and produced the initial draft. Authors JCI and PCA supervised the field work, the laboratory analysis and the literature development. All authors read and approved the final manuscript.

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ABSTRACT

The present study examines the effect of some bioactive constituents of *Vernonia amygdalina* crude extract and fractions on enzymes involved in glucose metabolism and treatment of Diabetes Mellitus. Fresh *Vernonia amygdalina* leaves were extracted using ethanol. Four fractions from this extract were obtained by liquid-liquid fractionation technique using solvents of varying polarity and the bioactive compounds were identified by Gas Chromatography–Mass Spectrum (GC-MS) analysis. Resultant fractions were administered at 300 mg/kgBW to streptozotocin (60 mg/kg) induced diabetic wistar rats. The results shows that various fractions produced a fall in the fasting blood glucose level in diabetic rats; Chloroform (65.85%), ethyl acetate (69.65%) Benzene

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(45.59%) and Butanol (37.31%) and all fractions showed increase in the activity of glucose metabolic enzymes (hexokinase, pyruvate kinase, Glucose-6-phosphate dehydrogenase (G6PDH) and Diaphorase (NADPH) between 20–81%, significantly higher than that of the metformin (reference drug at 50 mg/kg). Administration of Crude ethanolic leaf extracts of *Vernonia amygdalina* at dose of 300 mg/kg produced a fall (81.45%) in the fasting blood glucose level and subsequent increase in glucose metabolic enzyme activity (hexokinase, pyruvate kinase, Glucose-6-phosphate dehydrogenase (G6PDH) and Diaphorase (NADPH) (20–35%) in the diabetic Wistar rats after 28 days of treatment. GC-MS analysis showed that the plant and its fractions possess hypoglycaemic bioactive constituents such as Phytol, Palmitic acid, stearic acid and oxirane. The findings suggest that *Vernonia amygdalina* extract reduced the hyperglycaemic effect of streptozotocin-induced diabetes through its effect on glucose metabolism by promoting enzymes of the glycolytic and the pentose phosphate pathway, a property attributable to its active chemical constituents.

Keywords: Vernonia amygdalina; fractions; glucose metabolism; streptozotocin; diabetes.

1. INTRODUCTION

Diabetes mellitus comprises a group of chronic diseases characterized by hyperglycemia, due to diminished insulin secretion, diminished membrane response to insulin or both [1]. The World Health Organization, in 2004, released a report stating that more than 150 million people throughout the world suffered from diabetes. Present therapeutic measures for diabetes mellitus appear to be effective, yet the burden of disease continues to increase. According to Amos et al. [2], the disease incidence is projected to have an estimated increase from 171 million in 2000 to 366 million in 2030. Reasons for this projection include increase in sedentary life style, consumption of energy rich diet, obesity, among others [3]. Prior to 1922 diabetes therapy revolved around dietary measures including the use of traditional anti-hyperglycemic plants [4]. The World Health Organization has recommended that treatments for diabetes mellitus, using plants warrant greater attention [5]. Some authors have reviewed local plants of medicinal benefits [6] and many such herbs have been recommended for the treatment of diabetes [7], Some of which includes, *Acosmium panumensa, Alluim cepa, Aloe vera Annona squanosa, Brymonia alba* amongst others. Folks medicine for diabetic mellitus from rayalasema, reported 26 plants with anti-diabetics activity. *Vernonia amhelmintica* is one example of such plant. Plants have been suggested as a rich, and yet unexplored source of potentially useful anti diabetic drugs. However, only a few have been subjected to detailed scientific investigation to be able to clearly understand their mechanism of action *in-vitro* [8]. The present study examines one plant with purported anti-diabetic properties called *Vernonia amygdalina* (bitter leaf). Several studies [9,10] done on the safety of the plant suggests severe toxicities can only be observed with high doses above 1000 mg/kg. Raw chewing of *Vernonia amygdalina* leaf was found to control post prandial blood glucose without inducing severe hypoglyamia [11]. And hot water extract of *Vernonia amygdalina* leaf (500 mg/kg) reduced blood glucose concentration of both normoglycemic and hyperglycemic rats [12]. In the management of diabetes mellitus, *Vernonia amygdalina* has been used locally however, there is at present; no clinical evidence to support its use for clinical management of diabetes alongside other existing anti-diabetes drug due to poor understanding of its pharmacokinetics and pharmacodynamics. It is against this background that this research study was carried out; to investigate some of the mechanism by which the plant affects glucose metabolism in vitro. This study focuses on elucidating the bioactive compounds in *Vernonia amygdalina* and the effect of the plant on some glucose metabolic pathways. The result of this finding suggest possible mechanism of action similar to that observed in treatment of diabetes using herbs like *Biophytum sensitivum* [13] which increases henokinase activity and promote glucose oxidation via pentose phosphate pathway observed was similar with that seen in *lagerstroemia speciose* [14].

2. MATERIALS AND METHODS

2.1 Source of *Vernonia amygdalina*

Fresh leaves of *Vernonia amygdalina* were collected at the staff quarter, located at Site III, Delta State University Abraka, Delta State, Nigeria and was authenticated in Forestry

Research Institute of Nigeria, Ibadan, with herbarium number; FHI 110336. The plants were transported to Emma-Maria Scientific and Research Laboratory Abraka for extraction.

2.2 Extraction Procedure

The fresh *Vernonia* leaves were air-dried, crushed and soaked in ethanol for 48 hours after which the ethanolic extract was sieved out and allowed to dry. The resultant ethanol-free juice was subjected to liquid-liquid fractionation using solvents of varying polarity from non-polar to highly polar according to Ekam et al. [15]. Each fraction was further subjected to Gas Chromatography – Mass Spectrum (GCMS-QP2010 PLUS SHIMADZU, JAPAN) so as to identify the various chemical compounds in them.

2.3 Phytochemical Identification

The preliminary qualitative and quantitative phytochemical studies were conducted for the above crude and fractionated extracts of the plant to ascertain the presence of phytochemicals, same procedure as Ojieh et al. [16] was employed.

2.4 Handling of Animals

Having received approval from the Faculty bioethics committee for the use of laboratory animals for research, Sixty five (65) adult male Wistar rats, weighing between 100-180 gm were used in this research. The animals were bred and purchased from the Emma-Maria Laboratory Animal unit, Abraka, Delta State and transported in plastic basket to the College of Health Sciences Laboratory Animal Facility, Delta State University, Abraka.

They were housed in an environment of normal ambient temperature and the lighting period was about 12 hours daily. The relative humidity was between 40 and 60%, they were kept in matabolic cages, supplied with clean drinking water and fed *ad libitum* with standard commercial pelleted feed (Vital feed, UAC, Lagos).

2.5 Induction and Treatment

For the diabetic study, hyperglycaemia was induced with streptozotocin at 60 mg/kg b.w [17]. Prior to induction, their fasting blood glucose of the animals were checked after an overnight fast. Streptozotocin was prepared by dissolving 2 g of Sodium Citrate in 100 ml of water to yield 0.1 mole of citrate buffer; 0.6 g of Streptozotocin was dissolved in 10 ml of citrate buffer to yield 60 mg of Streptozotocin, 60 mg/kg bw was injected into the animals through the lateral tail vein and their fasting blood glucose was assessed using ACCUCHEK active blood glucometer, 72 hours after induction. A 50% increase in pre-induction fasting Blood glucose level was considered to be diabetic. Diabetes was not induced in animals for the normoglycaemic study.

2.6 Treatment Group

The research was divided into two phases; the normoglycaemic experimental study with 30 animals divided into 6 groups (n=5). Group 1a served as Control, Group 1b was treated with 300 mg/kgbw of Crude *Vernonia amygdalina*, Group 1c was treated with 300 mg/kgbw of Benzene fraction, Group 1d was treated with 300 mg/kgbw of Chloroform fraction, Group 1e was treated with 300 mg/kgbw of Ethyl acetate fraction and Group 1f was treated with 300 mg/kgbw of Butanol fraction. The diabetic study which comprised of 35 animals divided into seven (7) groups (n=5). Group 2a (negative control) induced diabetes but untreated, Group 2b was induced diabetes and treated with 50 mg/kgbw of Metformin, Group 2c was induced and treated with 300 mg/kgbw of Crude *Vernonia amygdalina*, Group 2d was induced diabetes and treated with 300 mg/kgbw of Benzene fraction, Group 2e was induced diabetes and treated with 300 mg/kgbw of Chloroform fraction, Group 2f was induced diabetes and treated with 300 mg/kgbw of Ethyl acetate fraction, Group 2 g was induced diabetes and treated with 300 mg/kgbw of Butanol fraction. Body weight and fasting blood glucose level were measured using electronic weighing balance and the one touch glucometer weekly measurement [16].

2.7 Sacrificing of Animals and Sample Collection

The animals were sacrificed using cervical dislocation after an overnight fast in order to determine their final fasting blood glucose level prior to sacrificing. The animal was pinned on the board and a laparotomy was carried out to expose the internal organs; blood was culled by cardiac puncture using 5ml syringes into a blood sample container.

2.8 Determination of Body Weight and Organ Weight

Body weight of experimental animals was checked before and after induction and subsequently weekly and on the last day of experiment before termination. Percentage change was later calculated as follows.

$$\text{Percentage weight change } (\%) = \frac{final - intial\ body\ weight\ (g)}{intial\ body\ weight\ (g)} X \frac{100}{1}$$

$$\text{Percentage change } (\%) \text{in FBGL} = \frac{final - intial\ FBGL\ (mg/dL)}{intial\ \text{FBGL}\ (mg/dL)} X \frac{100}{1}$$

2.9 Analytical Procedures

Biochemical analysis was carried out on the samples collected to determine the level enzyme activity in glucose metabolic pathways as stated below.

2.10 Enzymes of Glucose Metabolism

2.10.1 Estimation of glycolytic enzymes

2.10.1.1 Determination of pyruvate kinase

Pyruvate kinase (PK) was measured by [18] methods. Reaction mixture was prepared by adding 22.71 ml Potassium phosphate buffer, 2.40 ml Phosphoenol pyruvate, 0.45 ml of NADH, 3.00 ml $MgSO_4$, 0.48 ml Adenosine DiPhosphate, 0.90 ml Lactate Dehydrogenase and 0.06 ml Pyruvate Kinase. 3.00 ml of reaction mixture into a cuvette was pipette incubate 30℃ for about 3 minutes. After which 0.01 ml of enzyme solution was pipetted into the respective cuvette and mixed. Absorbance change at 340 nm per minute was read in the linear portion curve. Results were calculated as Specific activity (µU/mg protein) [18].

2.10.1.2 Determination of hexokinase activity

Hexokinase activity was assay by the methods of Brandstrup et at. [19], reaction mixture was prepared by adding 2.28 ml Tris$MgCl_2$ buffer, 0.50 ml of 0.67 m Glucose, 0.10 ml of 16.5 mM ATP, 0.10 ml of 6.8 mM NAD and 0.01 ml of G-6-PDH in a cuvette and incubated in the spectrophotometer at 30℃ for 6-8 minutes to achieve temperature equilibration and establish blank rate, if any. 0.1 ml of diluted enzyme solution was added to the reaction mixture and mixed thoroughly. Change in absorbance at 340 nm per minute was recorded for 3-4 minutes; Absorbance/min was determined from initial linear portion of the curve. Units/mg protein.

2.10.1.3 Estimation of pentose phosphate pathway enzymes

2.10.1.3.1 Determination of glucose-6-phosphate dehydrogenase activity

Glucose-6-phosphate dehydrogenase (G6PD, D-glucose-6-phosphate: oxidoreductase, EC 1.1.1.49) was assayed by methods of Kornberg and Horecker and of Lohr and Waller [20,21]. 100 µl of serum was added to 3.0 ml working reaction (composed of 25.20 ml of Tris-HCl buffer, 1.20 ml of $MgCl_2$, 1.20 ml of $NADP^+$ and 2.4 ml of G6P) and incubated at 30℃ for about 3 minutes, dispensed into the cuvette and mix. The absorbance change at 340 nm per minutes (ΔAbs340) was read in a linear curve. The liberated pyruvate was read at 540 nm.

2.10.1.3.2 Estimation of diaphorase (NADPH) activity

Diaphorase was measured by Brower and Woodbridge [22]. The reaction mixture was prepared by adding 3.00 ml Tris-buffer, 2.28 ml NADH solution, 23.22 ml H_2O. 1.85 ml of the reaction mixture was pipette into the cuvette and incubated at 30℃ for about 3 min. After incubation, 1.5 ml of DCIP solution and 0.01 ml of enzyme solution were pipetted into the cuvette and mix. Absorbance change at 600 nm per minute was read in linear portion of cuvette. Distilled water was used in place of enzyme solution for blank. Diaphorase was calculated as the amount of Di-1 that reduces 1µmol of DCIP per minute at 30℃.

2.11 Statistical Analysis

All data were expressed as mean ± standard error of mean. Statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD). The SPSS software (version 20) was used in the statistical analysis using multiple comparison tests. A p-value of less than 0.05 was considered significant.

3. RESULTS

3.1 Analysis on the Plant Phytochemistry and Active Constituents

Table 1 shows the phytochemical screening of the extracts. It reveals that saponnins, flavonoids, Cardiac glycosides, steroids, were all abundantly present in the ethanol extract. Also alkaloids, tannins, Anthraquinones were found in the ethanol extracts of the leaves.

Table 1. Qualitative analysis of the ethanol extract of *Vernonia amygdalina*

Chemicals	*Vernonia amygdalina* Del
Alkaloids	+ + -
Saponins	+ + +
Tannins	+ - -
Flavonoids	+ + +
Phenols	+ + +
Anthraquinones	+ + -
Cardiac glycosides	+ + +
Steroids	+ + +

Keys: + + + (Abundantly present), + + - (Moderately present), + - - (trace amount)

Table 2. Quantitative analysis of the phytochemical extract of *Vernonia amygdalina* Del

Chemicals	Fresh juice extract
Alkaloids	3.9%
Saponins	8.3%
Tannins	3.2%
Flavonoids	7.6%
Phenol	6.9%

The result of the quantitative assessment of the ethanol extract of the plant leaves reveals that saponnins 8.3%, was highest followed by flavonoids 7.6%, phenol 6.9%, Glycosides (5.8%) and alkaloids 3.9% and tannins 3.2%, were the least.

3.2 Identification of Bioactive Constituents in *Vernonia amygdalina*

Respective fractions from *Vernonia amygdalina* ethanolic extract was subjected to Gas Chromatography and Mass Spectrum analysis for elucidation of bioactive constituents. Results from the assay were as follows;

Analysis on benzene fraction

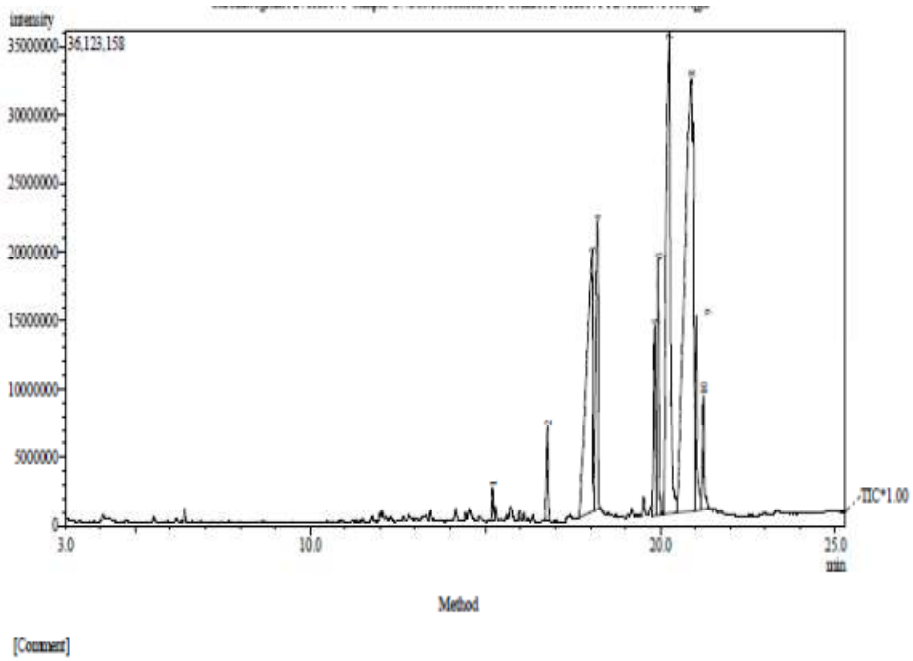

Fig. 1. GC-MS chromatogram of benzene fraction of *Vernonia amygdalina*
Ion fragmentation pattern for spectral peak at t_R20.264 mins and 20.879 mins was specific for 15-MethylHexadeconoic acid and Octadecatrienoic acid respectively

Analysis of chloroform fraction

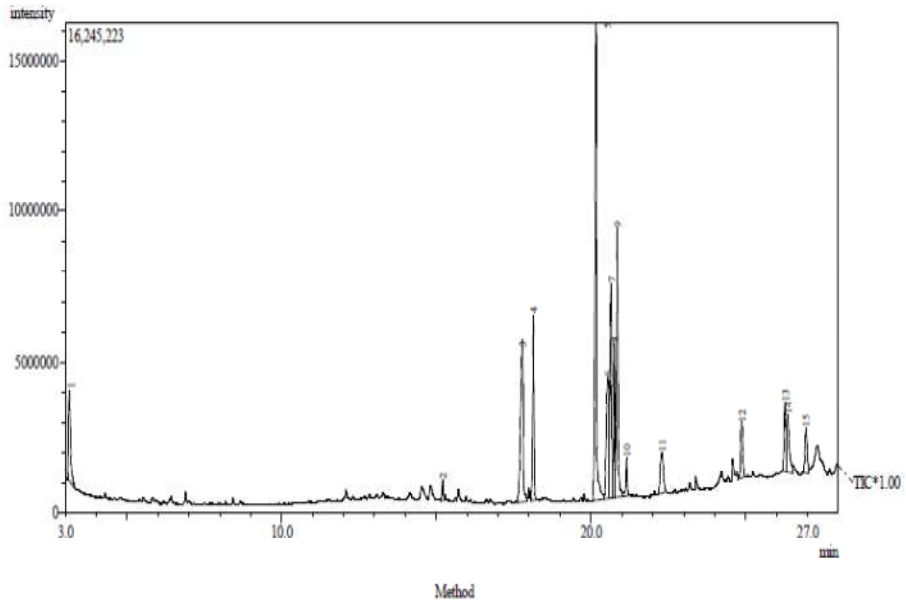

Fig. 2. GC-MS chromatogram of chloroform fraction of *Vernonia amygdalina*
Ion fragmentation pattern for spectral peak at t_R 20.161 mins was specific for 2-Hexadecen-1-ol

Analysis of ethyl acetate fraction

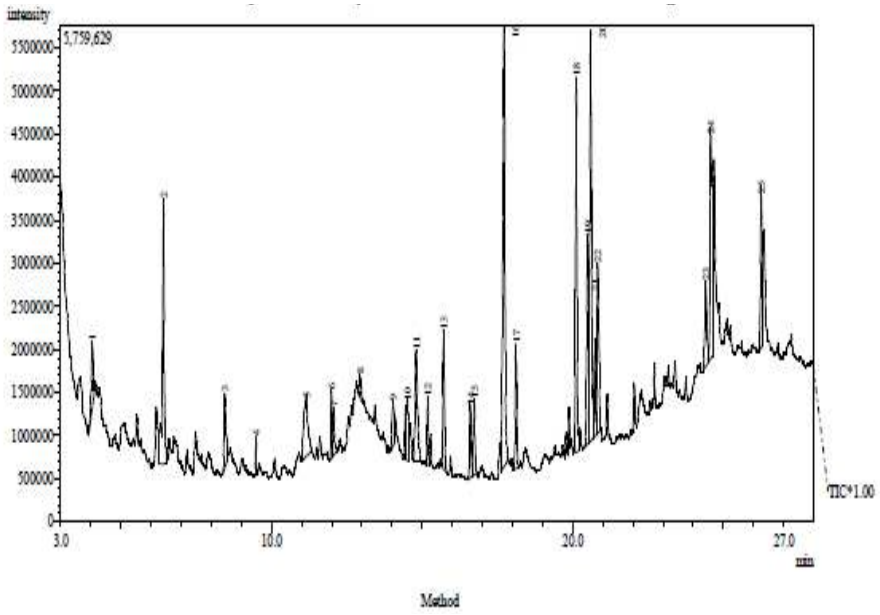

Fig. 3. GC-MS chromatogram of ethyl acetate fraction of *Vernonia amygdalina*
Ion fragmentation pattern for spectral peak at t_R 17.722 mins, 20.116 mins and 20.595 mins was specific for Hexadecanoic acid, Phytol (Hexadecen-1-ol) and 9,12,16-Octadecatrienal respectively

Analysis of butanol fraction

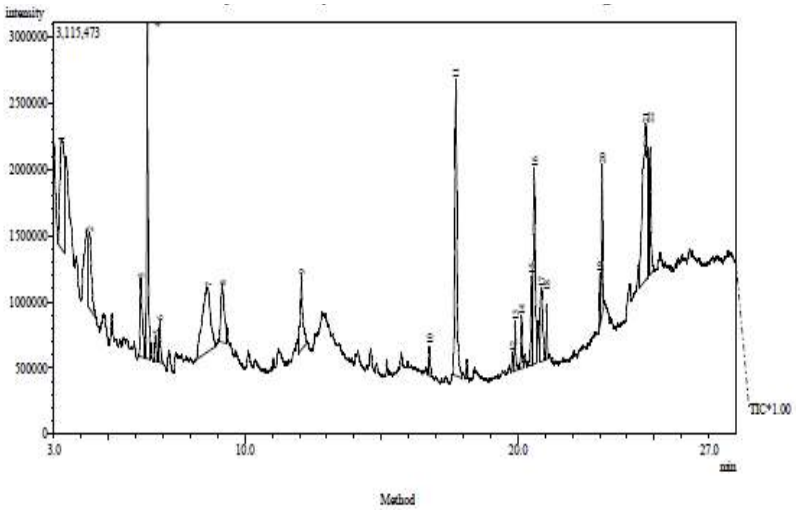

Fig. 4. GC-MS chromatogram of butanol fraction of *Vernonia amygdalina*
Ion fragmentation pattern for spectral peak at t_R 6.43 mins and 17.729 mins was specific for 2,3-Pentanedione and Hexadecanoic acid respectively

3.3 Effect of *Vernonia amygdalina* on Biochemical Parameters in Experimental Rats

Results from statistical analysis are presented in the figure below with the following designations;
All values designated (a) showed significant increase when compared to diabetic control
All values designated (b) showed significant decrease when compared with diabetic control
All values designated (*) showed significant increase when compared with control
All values designated (#) showed significant decrease when compared with control

Effect of *Vernonia amygdalina* crude and fraction on the body weight of experimental animals

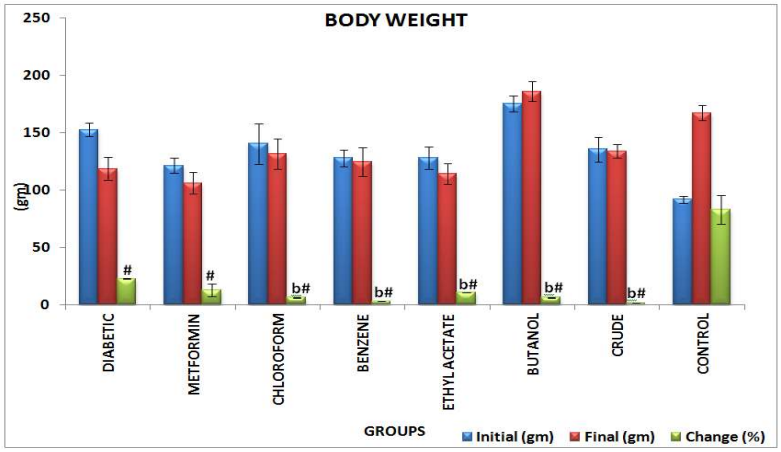

Fig. 5. Showing the effects of *Vernonia amygdalina* fractionates on the body weight of treated STZ induced diabetic and non-diabetic experimental rats expressed as Mean±S.E.M
Values designated (a) showed significant increase, (b) showed significant decrease when compared to diabetic control while values designated (*) showed significant increase, (#) showed decrease when compared with normal control rats

The level of body weight in diabetic rats at initial and final of administration was showed in Fig. 5. There was a significant improvement of body weight level in diabetic group treated with metformin (50 mg/kg), ethyl acetate and butanol fractions as compared to diabetic control rats after 28 days treatment.

Effect of *Vernonia amygdalina* crude and fraction on the fasting glucose level of experimental animals

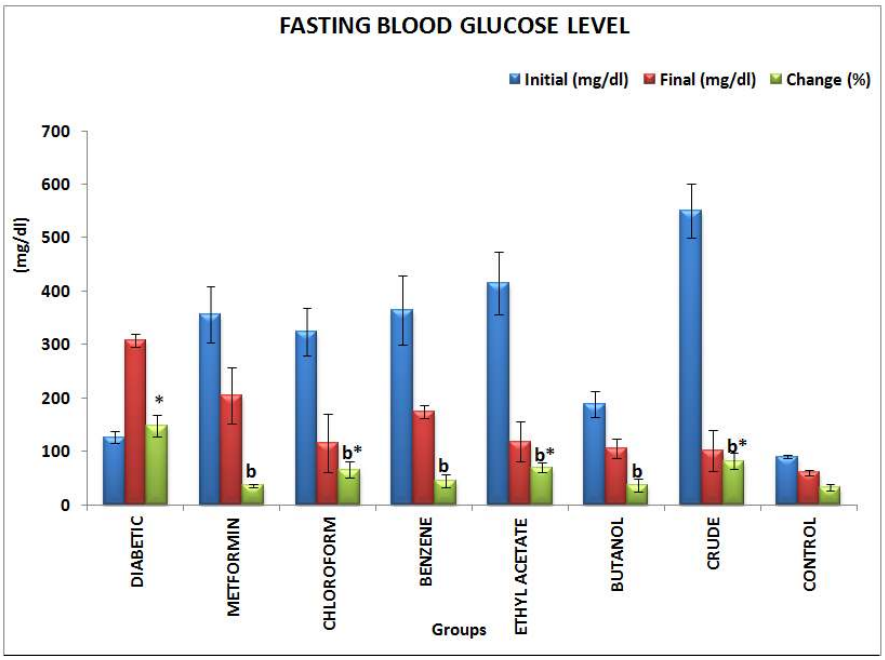

Fig. 6. Showing the effects of *Vernonia amygdalina* fractionates on the fasting blood glucose level of treated STZ induced diabetic and non-diabetic experimental rats expressed as Mean±S.E.M

Values designated (a) showed significant increase, (b) showed significant decrease when compared to diabetic control while values designated (*) showed significant increase, (#) showed decrease when compared with normal control rats

There was a significant elevation of blood glucose level in diabetic group as compared to normal control rats. The administration of metformin (50 mg/kg) and crude extract and fractions of *Vernonia amygdalina* (300 mg/kg), reduced the blood glucose in diabetic rats as compared to diabetic control rats.

Table 3. Effect of *Vernonia amygdalina* on the body weight and fasting blood glucose level of diabetic experimental animals

Group	Body weight (gm)			Fasting blood glucose (mg/dL)		
	Initial	Final	% Change	Initial	Final	% Change
Control	91.5±2.98	167.2±6.7	83.5412.79	90±3.21	60.33±4.41	-32.61±6.62
Diabetic	152.6±5.96	118.4±20.03	-22.41±0.33$^{\#}$	126.6±10.76	307.4±12.18	148.54±20.44*
Metformin	121.33±16.33	106.03±19.53	-12.56±5.77$^{\#}$	355.67±52.64	204.67±52.11	-36.53±2.77^{b}
Chloroform	140.2±17.98	131.63±13.30	-6.11±0.45 $^{b\#}$	323.4±44.40	115.6±54.77	-65.85±14.50^{b*}
Benzene	127.75±7.09	124.42±12.55	-2.61±0.06 $^{b\#}$	364.25±65.38	175.25±12.09	-45.59±11.86^{b}
Ethyl Acetate	128.03±9.628	114.26±8.97	-10.79±0.07$^{b\#}$	415±59.32	118.33±37.42	-69.65±8.88^{b*}
Butanol	175.22±7.191	186±8.95	6.15±0.41 $^{b\#}$	188.5±23.59	105.67±17.68	-37.31±12.36^{b}
Crude	135.4±10.86	133.85±5.65	-1.14±0.01 $^{b\#}$	550.00±50.05	102±38	-81.45±15.23^{b*}

Mean±S.E.M. Values designated (a) shows significant increase, (b) shows significant decrease when compared to diabetic control while values designated (*) shows significant increase, (#) shows decrease when compared with healthy control rats

Effect of *Vernonia amygdalina* crude and fraction on the hexokinase activity of experimental animals

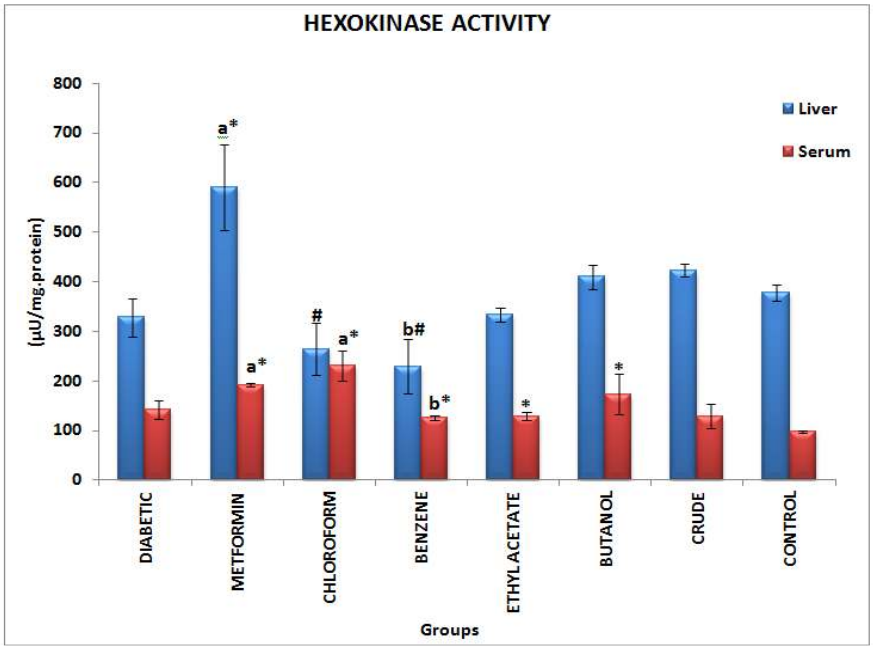

Fig. 7. Showing the effects of *Vernonia amygdalina* on the level of hexokinase activity in the liver and serum of treated STZ-induced diabetic and non-diabetic experimental rats expressed as Mean±S.E.M

Values designated (a) shows significant increase, (b) shows significant decrease when compared to diabetic control while values designated (*) shows significant increase, (#) shows decrease when compared with normal control rats

There was a significant reduction of Hexokinase activity in diabetic group as compared to normal control rats. The administration of metformin (50 mg/kg) and crude extract and fractions of *Vernonia amygdalina* (300 mg/kg), increased the activity of Hexokinase in diabetic rats as compared to diabetic untreated rats.

Table 4. Effect of *Vernonia amygdalina* on the activity of glycolytic enzymes in diabetic experimental animals

Group	Hexokinase (µU/mg.Protein)		Pyruvate kinase (U/mg.Protein)	
	Liver	Serum	Liver	Serum
Control	378.34±1.66	98.21±0.2	331.72±17.91	109.14±17.91
Diabetic	328.56±38.818	142.36±18.759	104.98±20.755	90.72±6.288
Metformin	589.87±86.236^{a*}	191.49±3.533 ^{a*}	407.92±36.30^{a*}	147.72±15.37^{a}
Chloroform	264.65±52.117$^{\#}$	230.17±30.63 ^{a*}	158.55±31.823$^{b\#}$	104.18±12.064
Benzene	229.66±54.215 $^{b\#}$	125.13±4.95 ^{b*}	311.36±27.477^{a}	65.78±4.656$^{b\#}$
Ethyl Acetate	333.73±14.307	128.44±8.083*	317.43±29.646^{a}	105.74±11.855
Butanol	410.45±24.692	173.56±41.735*	511.19±10.616^{a*}	23.65±5.508$^{b\#}$
Crude	422.92±13.42	129.23±24.55	668.3±11.95 ^{a*}	69.52±10.75^{b}

Mean±S.E.M. Values designated (a) shows significant increase, (b) shows significant decrease when compared to diabetic control while values designated (*) shows significant increase, (#) shows decrease when compared with healthy control rats

Effect of *Vernonia amygdalina* crude and fraction on the pyruvate kinase activity of experimental animals

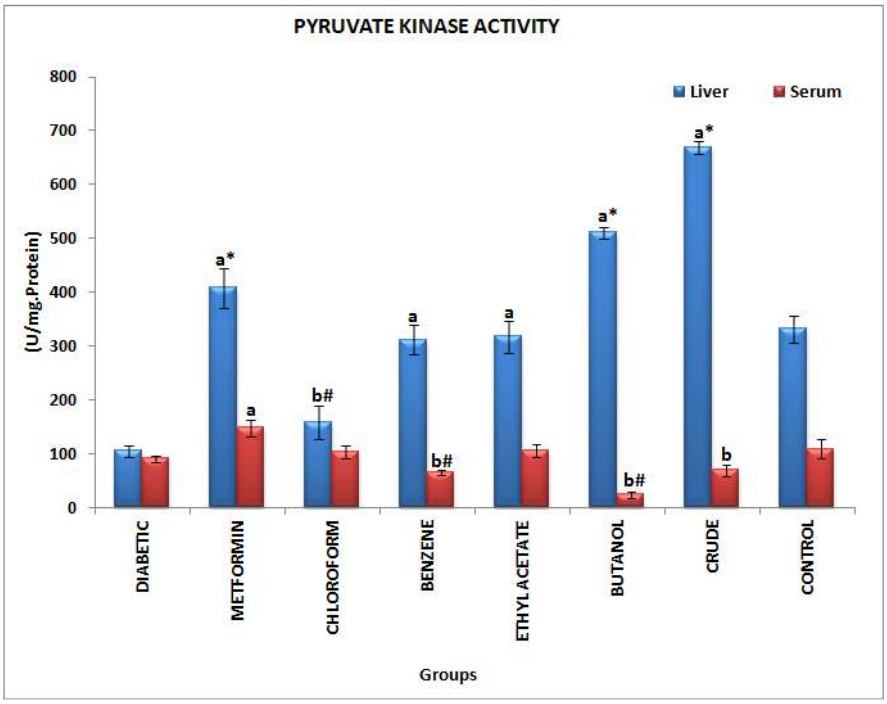

Fig. 8. Showing the effects of *Vernonia amygdalina* on the level of pyruvate kinase activity in the liver and serum of treated STZ-induced diabetic and non-diabetic experimental rats expressed as Mean±S.E.M

Values designated (a) shows significant increase, (b) shows significant decrease when compared to diabetic control while values designated (*) shows significant increase, (#) shows decrease when compared with normal control rats

There was a significant reduction of pyruvate kinase activity in diabetic group as compared to normal control rats. The administration of metformin (50 mg/kg) and crude extract and fractions of *Vernonia amygdalina* (300 mg/kg), increased the activity of pyruvate kinase in diabetic rats as compared to diabetic untreated rats.

Table 5. Effect of *Vernonia amygdalina* on the activity of pentose phosphate pathway enzymes in diabetic experimental animals

	Glucose 6-phosphate dehydrogenase	NADPH-diaphorase
Control	12.67±4.54	5.7±0.39
Diabetic	4.43±0.272$^{\#}$	14.96±1.52*****
Metformin	4.21±0.905$^{\#}$	26.55±7.09*****
Chloroform	6.84±1.448	16.2±1.071*****
Benzene	12.92±1.78^{a}	6.76±1.45^{b}
Ethyl Acetate	5.65±0.498	13.33±2.296*****
Butanol	8.15±2.772 ^{a}	6.07±0.393^{b}
Crude	10.95±4.1^{a}	39.76±8.1^{a}*****

Mean±S.E.M. Values designated, (a) shows significant increase, (b) shows significant decrease when compared to diabetic control while values designated, (*) shows significant increase, (#) shows decrease when compared with healthy control rats

Effect of *Vernonia amygdalina* crude and fraction on the pentose phosphate pathway enzymes of experimental animals

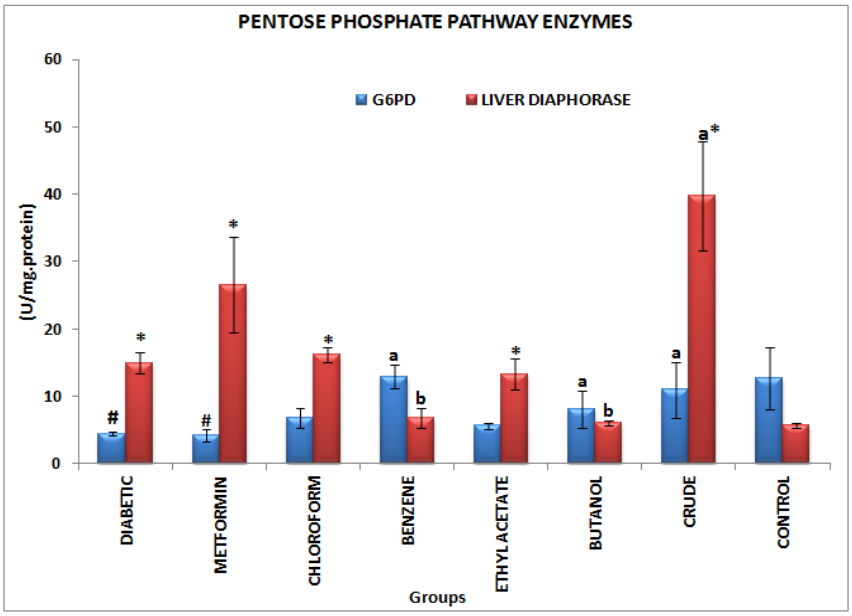

Fig. 9. Showing the effects of *Vernonia amygdalina* on the level of Glucose-6-phosphate dehydrogenase (G6PD) and Diaphorase (NADPH) activity in the liver of treated STZ-induced diabetic and non-diabetic experimental rats expressed as Mean±S.E.M

Values designated (a) shows significant increase, (b) shows significant decrease when compared to diabetic control while values designated (*) shows significant increase, (#) shows decrease when compared with normal control rats

There was a mild reduction of Diaphorase (NADPH) activity in diabetic group as compared to normal control rats. The administration of metformin (50 mg/kg) and crude extract and fractions of *Vernonia amygdalina* (300 mg/kg), increased the Glucose-6-phosphate dehydrogenase (G6PD) and Diaphorase (NADPH) activity in the liver of diabetic rats when compared to diabetic untreated rats.

4. DISCUSSION

Plasma glucose homeostasis and amelioration of metabolic derangement is the target of antidiabetic therapy. The result of this study shows that administration of *Vernonia amygdalina* crude ethanolic extract and fractions caused a significant decrease in fasting blood glucose level of diabetic rats, similar to the observation of Osinubi [12] and Ekam et al. [15] who also stated that fractions from *Vernonia amygdalina* significantly reduced blood glucose levels of diabetic rats. This confirms the hypoglycaemic property of the plant and its fractions.

Diabetes causes alteration in the metabolism of macromolecules resulting in changes in body composition and body weight. Significant weight loss is usually evident with progression of the disease. In this study, *Vernonia amygdalina* extract and fractions treatment lead to a significant improvement in body weight of diabetic rats compared to the control groups. This is in contrast with the finding of Ibrahim *et al* [23] who reported a significant reduction in body weight of Wistar rats on chronic feeding with leaves of *Vernonia amygdalina*, but consistent with the findings of Iwalokun et al. [24] who observed an increase in the body weight of acetaminophen induced toxicity in mice treated with *Vernonia amygdalina* extracts.

Derangement in glucose metabolic enzymes resulting in high levels of plasma glucose is a common feature in diabetes mellitus. In this study, the level of hexokinase activity in the serum and liver of diabetic rats treated with *Vernonia amygdalina* were significantly increased beyond the levels seen in the untreated diabetic rats. This is similar to the

findings of Shetti et al. [25] who purported that decreased glucose level in diabetic rats treated with *Phyllantus amaru* (a plant with recognised hypoglycemic property), was due to increased hexokinase and gluckokinase activity. The level of pyruvate kinase activity in this study showed that rats treated with respective fractions of the plant expressed higher level of pyruvate kinase activity and this observation was comparatively similar to the findings in the metformin group (Table 1). This suggests that *Vernonia amygdalina* promotes the activity of glycolytic enzymes hence promoting glucose ultilization within cells for energy production. However, Atangwho et al. [26] reported a decreased expression of hexokinase and increased glucokinase activity in hepatocytes of rats treated with *Vernonia amygdalina* suggesting the gluconate pathway as an alternate pathway of glucose utilization.

Glucose 6-phosphate dehydrogenase (G6PDH) an important enzyme in the pentose phosphate pathway and the level of NADPH-diaphorase; a product of the pathway were determined. The result showed that the expression of G6PDH was significantly decreased in untreated diabetic rats, similar to a previous report [27] but became highly expressed at the end of the plant extract and fractions administration, similar to what was reported by Atangwho et al. [26]. According to Ugochukwu and Babady, [28] the G6PDH gene or enzyme is a glucose modulatory target of several anti-diabetic agents and of some medicinal plants. Glucose oxidation via the G6PDH pathway primarily produces or generates reducing power –NADPH- needed in synthetic (anabolic) reaction and deactivation of reactive oxygen species (ROS) in the cell (antioxidant action).

Similarly, there was an increase in the expression of NADPH-diaphorase by this pathway in rats treated with the plant, suggesting a potentiating of the pentose phosphate pathway.

The hypoglycaemic potency of *Vernonia amygdalina* has been attributed to the basic phytochemical constituents of the plant. Some of which include sesquiterpene lactones, Saponins, Tannins, Flavonoids, Phenol, Glycosides and steroid glucosides [29,30]. It has been reported that a sesquiterpene lactone isolated from the extract of *Ambrosia maritima* is an effective hypoglycemic agent. Bioactive chemicals with known antidiabetic property such as; Phytol, Hexadecanoic Acid, Stearic acid, Benzoate salts, Butylphenol, Oxirane and Octadecatrienoic acid has been identified in the plant.

Other antidiabetic mechanism of action of the plant such as through the inhibition of intestinal glucose absorption, increase sensitivity of membrane receptors to insulin, insulinase inhibiting effect, and stimulation of increased peripheral tissues uptake of glucose cannot be ruled out.

5. CONCLUSION

This research study has confirmed the hypoglycaemic property of *Vernonia amygdalina* and demonstrated some possible means by which this is achieved. Several phytochemicals and bioactive compounds with antidiabetic properties contained in this plant has also being shown Thus, in the management of diabetes mellitus, *Vernonia amygdalina* can be considered as a good alternative or a compliment agent to other existing antidiabetic formulations. Also with more studies on the pharmacokinetics and pharmacodynamics of this plant, it could become a source for the production of the next generation of antidiabetic agents.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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