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In vitro and *In vivo* Assessment of the Anti-diabetes Potentials of *Murraya koenigii, Hibiscus cannabinus, Vernonia amydalina* and *Telfairia occidentalis* Leave Extract

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Authors' contributions

This work was carried out in collaboration between all authors. Author SAJ designed the study, wrote the protocol and supervised the work. Authors JME and IS carried out all laboratories work under close supervision. Authors SAJ, IS and JME performed the statistical analysis. Authors EMO, IAJ and SAJ managed the analyses of the study. Author EMO prepared the manuscript for publication. Author IAJ was professional in counsel. Authors EMO, IAJ and SAJ managed the literature searches and edited the manuscript before publication. Author EMO fine-tuned the discussion of research findings and conclusion. All authors read and approved the final manuscript.

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ABSTRACT

Assessing the inhibitory activities of *Telfairia occidentalis*, *Murraya koenigii*, *Hibiscus cannabinus* and *Vernonia amygdalina* on α-amylase with the view of providing a sustainable remedies for the management of diabetes mellitus was conducted. Findings from *In vitro* studies showed that the ethanolic extracts (at a concentration of 10 – 100 µg/ml) of *Vernonia amygdalina*, *Telfairia*

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occidentalis, *Murraya koenigii* and *Hibiscus cannabinus* exerted a maximum percentage inhibition on α-amylase at 74.28, 64.87, 58.60 and 71.20 respectively; with IC_{50} of 10.70 µg/ml, 680 µg/ml, 62.62 µg/ml and 30.0 µg/ml when compared with the standard drug. *In vivo* studies also showed that hypoglycaemic activity of ethanolic extracts of *Vernonia amygdalina*, *Telfairia occidentalis*, *Murraya koenigii* and *Hibiscus cannabinus* on oral administration (400 mg/Kg b.w) on alloxan induced diabetes albino rats significantly ($P<0.05$) improved body weight and significantly decrease blood glucose levels ($P<0.05$). This suggest among others that these plants have the potential of being is a promising adjuncts for the management of diabetes in lowering post prandial hyperglycaemia. *In vivo* studies further shows that *Murraya koenigii*, and *Vernonia amygdalina* leaves could be important in the management of diabetes mellitus as results indicated a competing hypoglycaemic activity with the standard drug (Acarbose). The implication of adopting the extracts of these plants in the design of cost friendly drugs with minimal side effects for diabetes mellitus was discussed.

Keywords: Telfairia occidentalis; Murraya koenigii; Hibiscus cannabinus and Vernonia amygdalina, diabetes mellitus; hypoglycaemia; α-amylase; ethanolic extracts; Alloxan-induced diabetic albino rats.

1. INTRODUCTION

The prevalence of diabetes mellitus is on the increase. It was reported in 2000 that the global prevalence of this metabolic disorder affect 171 million and was projected that by 2030 the global prevalence would have risen to 366 million [1]. However, reports in 2014 issued by International Diabetes Federation indicates that those affected by the disease have drastically risen to 387 million people globally, while about 46.3% of these are yet to be diagnosed [2]. Presently, it is projected that by 2035 the prevalence would have increased to 592 million [2,3].

Chinenye and Young [4] noted that when diabetes is left untreated, there is the likelihood of degeneration from the primary level of being associated with multiple biochemical lesions characterized by chronic hyperglycaemia, with impaired carbohydrate, fat and protein metabolism as a result of defect in either insulin secretion or its response by cell receptors to a secondary adverse effects that give rise to microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular (ischaemic heart disease, stroke and peripheral vascular disease) damages.

Changes in lifestyles have been implicated in precipitating diabetes with subsequent complications. This is associated with deterioration in glycaemic control which could increase microvascular complications [5]. Furthermore, smoking and inadequate protein intake at early stage of life has been suggested as risk factors for diabetes. Moreover in sub-Saharan Africa, Nigeria is implicated to be housing the largest cases of diabetes mellitus where an exponential socioeconomic growth over the past few decades has led to a sedentary and affluent lifestyle of the people in the urban region [4,6].

People diagnosed with diabetes mellitus virtually developed socioeconomic challenges where their medical expenditure increases 2.3 times higher than what their expenditures would have been in the absence of diabetes. Apart from early mortality, there is the prevalence of increased absenteeism and reduced productivity at work that has been reported [7].

Currently available therapies for diabetes management include insulin and various oral anti-diabetic agents such as sulfonylureas, biguanides and glinides among others. Many of such agents have been reported to aggravate serious adverse drug reactions, hence the search for a more effective, safer and cost effective hypoglycemic agents [8]. For example, insulin therapy apart from being expensive, is associated with undesirable weight gain and hypoglycaemia. Also, Acarbose and Miglitol (a deoxy mijirimycin derivatives) which competitively and reversibly inhibit the activity of two glucosidases enzyme from the intestine as well as in the pancreas have been known to be associated with gastrointestinal side effect such as abdominal pain, flatulence and diarrhea [9].

A number of plants have been studied to have anti-diabetic remedy [10]. Some of these included aqueous roots extract of *Tinospora cordifolia* and banana flowers (*Musa sapientum*) used in Asia and Africa among others [11,12].

However, the quest for a new anti-diabetic drug from natural plant is still an attractive strategy and a great deal is yet to be achieved. In our previous study, the anti-diabetic potential of these plants have been established [13]. Our present study therefore focuses on the evaluation of their *In vitro* and *In vivo* alpha amylase inhibitory effects of the above plants (*Vernonia amygdalina* (bitter leaf), *Telfaira occidentalis* (also known as *Ugu* among the Ibo tribe of South Eastern Nigeria), *Hibiscus cannabinus* (also known as *Rama* among the Hausa tribe of Northern Nigeria) and *Murray koeniigii* (curry leaf) which are common vegetable consumed in Nigeria. This is with a view of providing sustainable remedies that is not only cost friendly but also has minimal side effects for the management and reduction in the incidences of complications arising from diabetes mellitus.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Plant materials

The plant Samples used for the experiment were purchased from the Central Market Kaduna, Nigeria. They were authenticated at the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. Voucher specimen was deposited at the Herbarium for reference. The leaves were further transported to the Biochemistry department Research Laboratory of Kaduna State University (KASU), Kaduna before being prepared for use according to standard procedure.

2.1.2 Experimental animals

Albino rats of both sexes of ages 8 to 10 weeks weighing between 85 to 194 g, obtained from the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria were used. The animals were acclamatized in the Animal house of Biochemistry department, Kaduna tate University under standard condition of temperature and illumination. They were also allowed free access to food and water under well ventilated conditions daily prior to the commencement of the experiment.

2.1.3 Chemicals and reagents

α – amylase (01260-Milehidistilling UK); 3,5 – dinitrosalicylic acids (22633-33-6 – Alfa earsar); Acarbose (28/12.2/042 – Bayer Health care AG Leverkusen, Germany); Starch (PU 5536 - BDH); Alloxan monohydrate (Burgoyne Burbidges and Co., India). All other chemicals and reagents used were of analytical grade and were purchased from reputable chemical suppliers.

2.2 Experimental Design and Methods

2.2.1 Plant Preparation and extraction

Plant preparation and extraction was carried out using standard method. Fresh leaves of *Murraya koenigii, Hibiscus cannabinus, Vernonia amydalina and Telfairia occidentalis* were dried at room temperature and grounded into powder with pestle and mortal. They were thereafter transferred into labelled air tight plastic containers and stored at 8℃ until further analysis.

2.2.2 Animal preparation

The Albino rats used for the experiment were divided into seven groups of four rats each, namely:- Group – 1: Treated with normal saline (Normal Control-NC); Group – 2: Normal saline treated diabetic rats (Diabetic Control - DC); Group – 3: *Telfairia occidentalis* leaf extract (400 mg/Kg body weight) treated diabetic rats (TL); Group – 4: *Murraya koenigii* leaf extract (400mg/Kg body weight) treated diabetic rats (ML); Group – 5: *Hibiscus cannabinus* leaf extract (400 mg/Kg body weight) treated diabetic rats (HL); Group – 6: *Vernonia amydalina* leaf extract (400 mg/Kg body weight) treated diabetic rats (VL); Group -7: Acarbose – a standard drug (400 mg/Kg body weight) treated diabetic rats (AT).

2.2.3 *In vitro* and *In vivo* studies

For *In vitro* inhibitory study of *Murraya koenigii, Hibiscus cannabinus, Vernonia amydalina and Telfairia occidentalis* on α-amylase activity, n-hexane, chloroform, ethanol (70%) and petroleum ether leave extract were prepared for use.

The leaves extract that exert the greatest inhibition were subjected to *In vivo* studies using experimental (albino) rats for a period of 15 days. The first day was for induction of diabetes in rats and the following 14 days were the investigative period with the leave extract of *Murraya koenigii, Hibiscus cannabinus, Vernonia amydalina* and *Telfairia occidentalis* separately.

Based on results from *In vitro* employed the for each leaf extract, 30 g of the previously prepared powdered plant was separately transferred into a beaker and 300 ml of 70% ethanol was added, mixed and the mixture seal with an aluminium foil and was allowed to stand for 24 hr at room temperature in the cupboard. After 24 hrs the mixture was filtered using muslin cloth and re-filtered with the aid of Whatman No. 1 filter paper. The final filtrate was then evaporated via rotatory evaporator and the solid crude extract obtained was transferred into a sample vial and stored at 8℃ in the refrigerator. The above procedure was repeated for various solvent which included; chloroform, n-hexane and petroleum ether. Each extract was then subjected to α – amylase inhibitory assay.

2.2.4 *In-vitro* α – amylase inhibitory assay by Miller (1959) method

The α – amylase inhibitory activity was determined using the modified Miller [14] method as described by Dinesshkumar et al. [15] and Imam et al. [16]. Into each separate test tubes 200 µl of 0.02 M sodium phosphate buffer, 20 µl of enzyme porcine pancreatic α – amylase, and the plant extracts in various concentration of 10 – 100 µg/ml was prepared by dissolving 1 mg of the solid crude extract in 1 ml of 0.1 % dimethyl sulphoxide out which the serial dilution was made. Each respective tubes were incubated for 10 minutes at room followed by addition of 200 µl of 1% starch in all the test tubes. The reaction was terminated with the addition of 400 µl of 3,5-dinitrosalycylic acid (DNSA) colour reagent and then was placed in a water bath for minutes at 37℃ for colour development, cooled to room temperature and diluted with 2 ml of distilled water and absorbance measured at 540 nm (Schimadzu UV-Vis Spectrophotometer). The control test was conducted without the crude extract; while the standard drug (Acarbose) was subjected accordingly without any plant extract except for the standard drug and were compared with the test sample. Results were expressed as % inhibition calculated using the formula:

$$\% \text{ inhibition} = \frac{A_c - A_e}{A_c} \times 100$$

Where: A_c and A_e are the absorbance of control and extract respectively.

From result obtained IC_{50} value were determined from plots of percent inhibition verses log inhibitor concentration.

2.2.5 *In-vivo* α – amylase inhibitory assay

2.2.5.1 Induction of diabetes

The albino rats were made to fast 12 hrs. before the induction of diabetes. Thereafter they were injected intra-peritoneally with freshly prepared alloxan monohydrate (150 mg/kg body weight). A day after injection, the rats with fasting blood glucose higher than 9.5 mmol/L were considered diabetic and used for the experiment.

2.2.5.2 Weighing of animals

The weight of the rats were determined at every stage of the experiment with the aid of a weighing balance.

2.3 Estimation of Blood Glucose Concentration by Glucose Meter Method

2.3.1 Principle

Fasting blood glucose determination using the strip of digital ACCU-CHEK glucose meter (Roche diagnostic, Mannheim Germany) uses glucose dehydrogenase chemistry. The glucose dehydrogenase in the strip converts the glucose in the blood sample to gluconolactone. This reaction liberates two electrons that react with a coenzyme electron acceptor where the oxidized form of the mediator hexacyanoferrate (III), forms the reduced form of the mediator, hexacyanoferrate (II). The test strip employs the electrochemical principle of biamperometry. The meter applies a voltage between two identical electrodes, which causes the reduced mediator formed during the incubation period to be reconverted to an oxidized mediator. This generates a small current that is read by the meter as glucose level.

2.3.2 Procedure

A drop of blood was obtained from the tip of conscious rat's tail and placed on the strip. The reading on the meter was noted and recorded as the blood glucose concentration.

2.4 Statistical Analysis

Statistical Package for Social Sciences (version 13.0) software (SPSS, Chicago, IL) was used to analyze data that was obtained. This analysis is to determine whether there are significant

differences between the means of two independent groups. Tukey's HSD (highest standard deviation) was used to compare between the groups while paired sample t-test was used to compare within the treatment periods.

3. RESULTS

For *In vitro* studies, results shows that ethanolic extracts of *V. amygdalina* and *T. occidentalis* shows more percentage inhibition from 49.21 to 74.28 and 25.38 to 64.87 with IC_{50} of 10.70 µg/ml and 68.00 µg/ml respectively when compared with the standard drug shows inhibition from 19.54 with IC_{50} of 80 µg/ml (see Fig. 1). Also, results from the chloroform extract shows that *T. occidentalis* and *H. cannabinus* have the significant inhibition from 30.38 to 71.20 and 50.94 to 63.29 with IC_{50} of 30 µg/ml and 10.02 µg/ml respectively when compared with the standard drug (see Fig. 2). The n-hexane extracts showed that *V. amygdalina* and *T. occidentalis* percentage inhibition form 2.42 to 14.49 and 6.10 to 33.60 with IC_{50} of 110 µg/ml and 120 µg/ml respectively when compared with standard drugs (see Fig. 3).

Also, the ethanolic extract of *Hibiscus cannabinus*, n-hexane extract of *Telfairia occidentalis*, and *Vernonia amygdalina* and chloroform extracts of *Murraya koenigii*, and *Vernonia amygdalina* exhibited a minimum alpha amylase inhibitory activity.

The ethanolic extract of the 100g powdered plants leaves yielded 6.28g, 7.62g, 6.95g, and 5.20g extracts of *V. amygdalina, T. occidentalis, H. cannabinus* and *M. koenigii* respectively.

Findings from *In vivo* study revealed that the mean effective doses or medial lethal doses of these plants has been investigated on test doses of 100-2000 mg/kg body weight with no mortality being recorded or any manifestation of toxicity observed after the period of 24 hrs as determined by Luke et al. [17].

After induction of diabetes mellitus the rats treated group shows a significant increase ($p<0.05$) in weight. This was also observed among the normal control group (see Fig. 4). Although increase in weight for HL treated group was not significant ($p<0.05$), while DC and AC treated groups shows a non-significant and significant decrease in weight respectively.

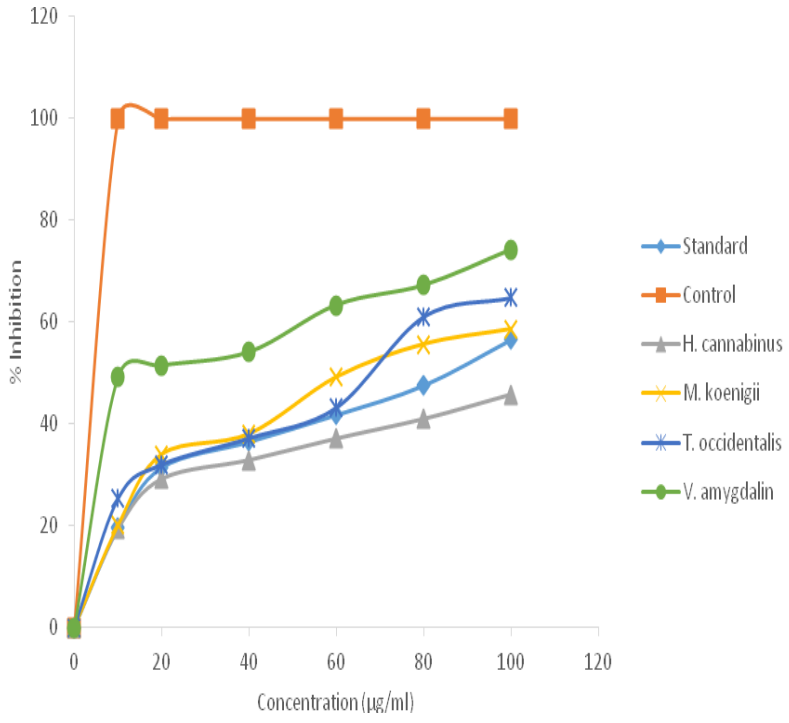

Fig. 1. Effect of ethanolic plant extract on α-amylase

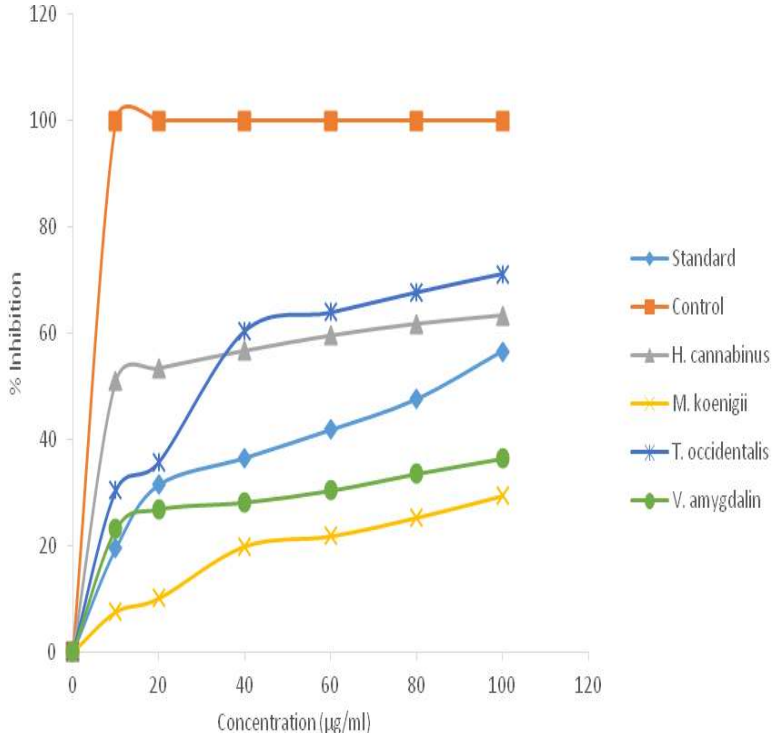

Fig. 2. Effect of chloroform plant extract on α-amylase

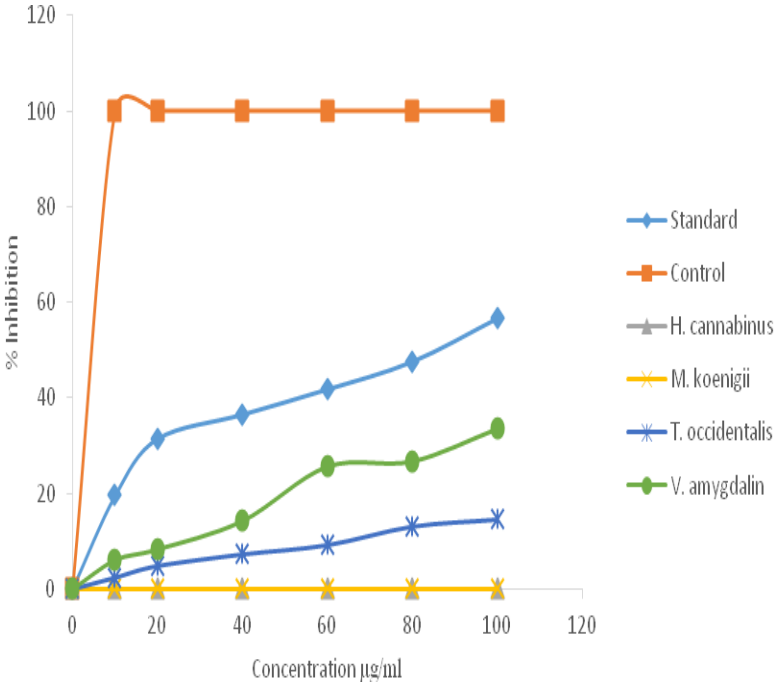

Fig. 3. Effect of n-hexane plant extract on α-amylase

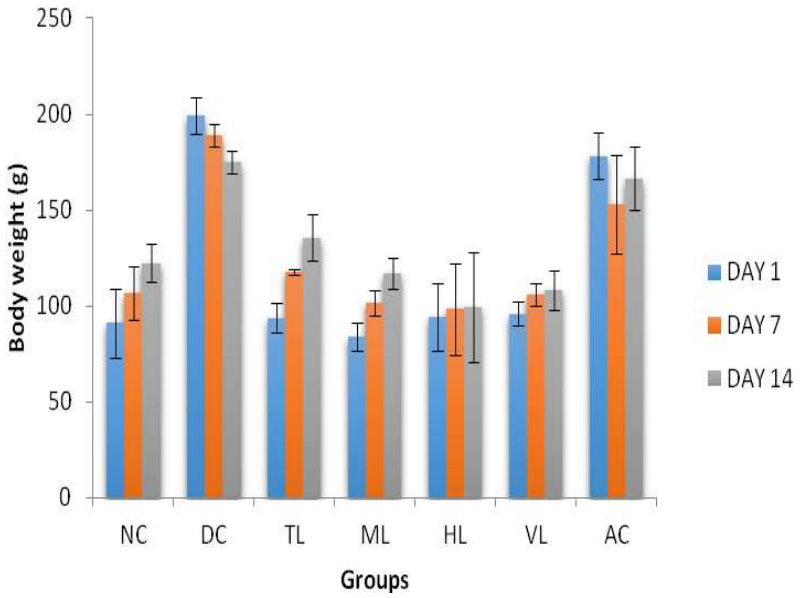

Fig. 4. The effect of ethanolic leaf extracts of *Telfairia occidentalis*, *Murraya koenigii*, *Hibiscus cannabinus* and *Vernonia amygdalina* on body weight on alloxan induced diabetic rats

[Key: NC-Normal Control, DC-Diabetic Control (Day 1), TL (T. occidentalis leaf extract (400 mg/kg)), and ML (M. koenigii leaf extract (400 mg/kg), and HL (H. cannabinus leaf extract (400 mg/kg), and VL (V. amygdalina leaf extract (400 mg/kg), and AC (Acarbose: Standard drug (400 mg/kg))]

The initial blood glucose level among the groups shows no significant difference as they are within the range of 4.9-6.20 mmol/L. But after induction the rats blood glucose levels effectively showed hyperglycaemia which was followed with the administration of the ethanolic leaf extracts of *V. amygdalina, T. occidentalis, H.cannabinus* and *M. koenigii* to study their anti-hyperglycaemic activities.

After 7 days of treatment, there was a significant decrease ($p<0.05$) in blood glucose levels in ML, VL and AC treated groups respectively; while the TL and HL treated group shows no significant ($p<0.05$) in blood glucose levels (see Fig. 5). Interestingly at end of 14 days of treatment with the plant extracts, the blood glucose levels in TL, ML, VL and AC treated groups dropped significantly, while that of HL treated group had no significant decrease as compared to the diabetic control groups (see Fig. 5).

4. DISCUSSION

The quest for a better means for the management of diabetes mellitus has assumed a monumental proportion globally. One of the management strategies adopted is by directing substances to inhibit key enzyme(s) that play a role in the digestion of starch and glycogen, thereby regulating the uptake of glucose. Moreover, it has been observed that biopharmaceuticals are currently drawing more attention in the management of diabetes mellitus as their side effects are minimal and the therapies well tolerated compared to other forms of oral hypoglycaemic agents currently available [18,19]. The ultimate goal of every administration of hypoglycaemic agents be it biopharmaceuticals or conventional is to maintain or bring blood glucose levels to normal levels as well as prevent the accompany effects of diabetes which include skin infection, diabetic nephropathy, cardiovascular disorder and the likes [20].

The rationale behind using the various solvents of varying polarity from the *In-vitro* studies is to search for a more potent inhibitory compound in the organic solvent. The *In-vitro* inhibitory activity on the α-amylase by the ethanolic, chloroform and n-hexane extracts of *Telfairia occidentalis*, *Murraya koenigii*, *Hibiscus cannabinus* and *Vernonia amygdalina* all exhibit significant correlation between the plants. Although the ethanolic extracts exhibited a maximum percentage inhibition on the α-amylase activity (at a concentration of 10–100 µg/ml) as

compared with the standard drug (Acarbose). The inhibition is as a result of phytochemicals (tannins, terpenoids, glycosides, flavonoids and alkaloids) that have been reported to be present in the plants [13,21,22]. These phytochemicals have been shown previously to possess the ability to precipitate proteins by chelating them [23].

The ethanolic hypoglycaemic activities of *V. amygdalina, T. occidentalis, H. cannabinus* and *M. koenigii* leaves extract evaluated in alloxan induced diabetic rats for a period of 14 days exerted a significant change in rats body weight. The loss in weight in DC group might be the result of protein wasting due to unavailability of carbohydrate as an energy source as noted by Chen and Ianuzzo [24]. Tissue wasting is a characteristic of poor glycaemic control in diabetes and this usually foster protein and fat mobilization [25]. Atangwho et al. [26] and Okokon et al. [27] also reported significant weight reduction in untreated diabetic rats. This was also observed in the present study with untreated diabetic rats. The sustained but gradual reduction in weight of untreated diabetic rats over 14 days clearly indicated the deterioration in glucose control mechanism. This observation shows that as the weight of rat decreases as the blood glucose levels increases over the experimental period, hence establishing inverse relationship between blood glucose and weight changes in untreated diabetic rats.

Treatment of diabetic rats with leaf extracts of *V. amygdalina, T. occidentalis, H. cannabinus* and *M. koenigii* improved the weight gain as compared to the untreated diabetic rats (Fig. 4). The gradual appreciation in weight on treatment given, suggest that the treatment would have allowed the tissues access to glucose, both to supply energy and build tissue material needed for growth [28].

The fasting blood glucose levels of the untreated diabetic control rats was significantly higher ($p<0.05$) than non-diabetic control group by 3 and 6 folds respectively. In our present study, the treatment caused significant decrease in blood glucose level with *V. amygdalina* and *M. koenigii* (400 mg/kg, body weight) having higher hypoglycaemic activity, while *T. occidentalis* and *H. cannabinus* leaves extracts (400 mg/kg, body weight) showing lower hypoglycaemic activity (Fig. 5).

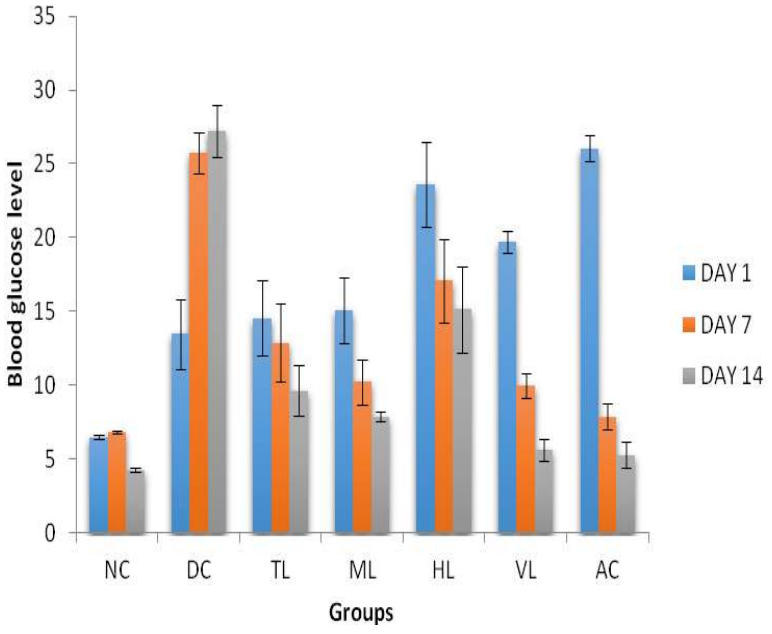

Fig. 5. The effect of ethanolic leaf extracts of *Telfairia occidentalis*, *Murraya koenigii*, *Hibiscus cannabinus* and *Vernonia amygdalina* on blood glucose levels on alloxan induced diabetic rats

[Key: NC-Normal Control, DC-Diabetic Control (Day 1), TL (T. occidentalis leaf extract (400 mg/kg)), and ML (M. koenigii leaf extract (400 mg/kg), and HL (H. cannabinus leaf extract (400 mg/kg), and VL (V. amygdalina leaf extract (400 mg/kg), and AC (Acarbose: Standard drug (400 mg/kg))]

Many plant extracts have been reported to exert hypoglycaemic action by potentiating the insulin effect by either increasing the pancreatic secretion of insulin from the cells of islets of Langerhans or its release from bound insulin [29] while others act through extra-pancreatic mechanisms by inhibition of hepatic glucose production [30] or corrections of insulin resistance [31]. There is the strong probability that these leaves extracts used in our present study may have acted through one of these mechanisms. This is however open to further investigation. The hypoglycaemic effect may also be as a result of the composition of the plant extracts that are known to contain phytochemicals (tannins, terpenoids or glycosides, flavonoids and alkaloids), which may have inhibited intestinal α-amylase. Also, these phytochemicals have been reported to have antioxidant effect by protecting cells (e.g. β-cells) against the damaging effect of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals and hydroxyl radicals which may result in oxidative stress leading to cellular damage [32].

The earlier *In-vitro* studies showed that these plant's leaves ethanolic extract has inhibitory effect on α-amylase. Alpha amylase catalyses the hydrolysis of α (1-4) glycosidic bond of starch, glycogen and various oligosaccharide into simple sugar which can be readily available for intestinal absorption, therefore inhibition of α-amylase in the digestive tract of human is being considered to be more effective in the management of diabetes mellitus by reducing the intestinal absorption of glucose thereby lowering postprandial hyperglycaemia. The continuous treatment of the leaf extracts for a period of 14 days produced a significant decrease in blood glucose levels in diabetic rats treated with ML and VL extracts which is comparable to that of standard drug, Acarbose (AC) that is used in the treatment of type 2 diabetes mellitus. The standard drug Acarbose stimulates insulin secretion from beta cells of islets of Langerhans. The findings from present study suggests that the likely possible mechanism by which the plant's extracts decreases the blood glucose level may be by potentiating of insulin effect either by increase in pancreatic secretion of insulin from beta cells of islets of Langerhans or by increase in peripheral glucose uptake; otherwise by the α-amylase or glycosidase inhibition.

5. CONCLUSION

A good glycaemic control is the cornerstone in diabetes mellitus management. In the present study, we have found that the leaf extracts of *Telfairia occidentalis*, *Murraya koenigii*, *Hibiscus cannabinus* and *Vernonia amygdalin* exhibited hypoglycaemic effects in alloxan-induced diabetic rats thus fuelling the potential use of these plants in the management of diabetes mellitus. However, the effect was more pronounced in *V. amygdalina* and *M. koenigii* which showed potent anti-diabetic effect relative to the standard drug. It is in the light of these findings that these plants extract (VL and ML) are seen to be more promising adjunct in the management of diabetes mellitus. These evaluation, no doubt, is also an encouragement in the management of post prandial hyperglycaemia in the control of type 2 diabetes mellitus. Future collaborative studies in this area intend to focus on the characterization and molecular transduction of these plant extracts (especially *Vernonia amygdalina*) to verify its inhibitory action on α-amylase. This in our view will lead to development of complementary and affordable drugs with minimal side effects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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