



# **Combined Anti-diabetic Effects of Extracts of *Artemisia annua* var. *chiknensis* (CBGE/CHNA/09/LTNGS/G) and Each of Three Other Plants (*Momordica charantia* Linn. *Vernonia amygdalina* Del. and *Aegle marmelos* Correa) Traditionally Used in Nigeria for the Treatment of Diabetes**

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## **Authors' contributions**

*This work was carried out in collaboration between all authors. Authors CICO and NO designed the study, authors IAO, JUI and AFU performed the statistical analysis, authors CICO, AIO and MFI wrote the protocol and wrote the first draft of the manuscript. Authors CICO, IAO, JUI and AIO managed the analyses of the study. Authors CICO, AIO and JUI managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

The combined anti-diabetic effects of Extracts of *Artemisia annua* var. *chiknensis* with Laboratory code number (CBGE/CHINA/09/LTNGS/G), *Momordica charantia* Linn, *Vernonia amygdalina* Del. and *Aegle marmelos* Correa traditionally employed in Nigeria for the treatment of diabetes were studied. Fifty male albino rats which had been subjected to overnight fasting were rendered diabetic through single intraperitoneal alloxan injections (120 mg/kg body weight). They were then divided into 5 batches of ten rats each. The first batch was treated with *A. annua* leaf extract only. The second batch was treated with a combination of *A. annua* and *M. charantia* extracts. The third batch was treated with a combination of *A. annua* and *V. amygdalina* extracts while the fourth batch was treated with a combination of *A. annua* and *Aegle marmelos* extracts. The extracts were used in the ratio of 1:1 and treatment was done twice daily for a period of 30 days. The fifth batch of diabetic rats was not treated and thus served as control. The sixth batch of non-diabetic rats (10) was set up for comparison. Both the *A. annua* extract and its various combinations with the other experimental plant extracts resulted in insulin level enhancements and fasting blood glucose level reductions of the diabetic rats. There were significant differences ( $P \leq 0.05$ ) in the insulin level of diabetic rats treated with *A. annua* leaf extract alone and those treated with different combinations of the plant extracts. The mean effects of the extracts on insulin and fasting blood glucose levels were most significant in *A. annua* + *M. charantia* (38.65  $\mu$ U/ml and 87.55 mg/dl) and *A. annua* + *A. marmelos* (38.55  $\mu$ U/ml and 87.92 mg/dl) treatments at  $P \leq 0.05$ . The non-treated diabetic rats had an average body weight of 94.90 g as compared to the original average body weight of 100 g at the commencement of the study. The treated diabetic rats had average body weight increments from 108.83g to 109.29 g. The non-diabetic rats had an average body weight of 114.10 g as compared to their initial average body weight of 100 g. The experimental plants were found to contain various biochemical constituents which were probably responsible for the blood serum insulin level enhancements and fasting blood glucose level reductions. The results obtained have shown that the *A. annua* leaf extract and its combinations with the other plant extracts could be employed in the management of hyperglycemia.

**Keywords:** Diabetes mellitus; albino rats; alloxan; *A. annua*; insulin; plant extracts.

## 1. INTRODUCTION

Diabetes is widely recognized as the most common metabolic and endocrine disorder worldwide. It is linked to disturbances in carbohydrate, fat and protein metabolism. It is highly very significant because the global incidence of diabetes has been reported to be on the rise. At least 250 million individuals worldwide suffer from diabetes and it has been estimated that this number will double by 2030 [1]. Surely, increases in complications will also be on the upward trend. More than 80% of deaths resulting from diabetes take place in low - and middle - income countries [2-3]. Nigeria is the most populous African Nation. It is one of the 32 countries of the "International Diabetes Federation (IDF), Africa region. More than 14 million people have been reported to have diabetes in Africa. It has been reported that Nigeria has the highest mortality rate of diabetes in Africa. As at 2014, over 3.9 million Nigerians were living with the disease [4].

The body cells absorb glucose, the final product of carbohydrate digestion and use it for energy production. Glucose utilization in the body is regulated by the hormone, insulin which is produced by the pancreas. Diabetes develops when the body is unable to produce insulin or is unable to use insulin or both. Insulin is made in the pancreas. The pancreas contains cells known as islets of Langerhans. Pancreatic islets house three major cell types, each of which produces a different endocrine product:

- i. The Alpha cells or "A" cells secrete the hormone, glucagon.
- ii. The Beta cells or "B" cells produce insulin. They are the most abundant of the islet cells.
- iii. The Delta cells or "D" cells secrete the hormone, somatostatin which is also produced by a number of other endocrine cells in the body.

Beta cells within the islets make insulin and release it into the blood. The insulin circulates, enabling glucose to enter the cells of the body.

Insulin lowers the amount of glucose in the body stream. As the blood glucose drops, the secretion of insulin from the pancreas drops.

If the beta cells do not produce enough insulin, or the body does not respond to the insulin that is present, glucose builds up in the blood instead of being absorbed by cells in the body thereby leading to prediabetic or diabetic condition. Prediabetes is a condition in which blood glucose levels are higher than normal but not high enough to be diagnosed as diabetes. In diabetes, the body cells are starved of energy despite high blood glucose level. Overtime, blood glucose damages nerves and blood vessels, thereby leading to complications, such as heart disease, stroke, kidney disease, blindness, dental disease and subsequently, amputation. Other complications of diabetes may include:

- i. Increased susceptibility to other diseases.
- ii. Loss of mobility with aging.
- iii. Depression
- iv. Pregnancy problems.

No one is very certain what induces the process of diabetes but it is believed that genes and environmental factors interact to trigger the metabolic problem.

There are two main types of diabetes. These include:

- a. Type 1 diabetes.
- b. Type 2 diabetes.

The other types include:

- i. Gestational diabetes which could develop during pregnancy.
- ii. Defective gene diabetes which results from defects in specific genes of the pancreas, certain drugs or chemicals, infections and other conditions. Some people exhibit signs of both types 1 and 2 diabetes.

Diabetes insipidus is an uncommon disease characterized by an increase in thirst and the passage of large quantities of urine of a low specific gravity [5]. The urine is otherwise normal. The disease may occur acutely, probably after a head trauma or surgical procedures near the pituitary region or may be chronic and insidious in onset. It could be due to insufficiency of the posterior pituitary or impaired function of the supraoptic pathways that regulate water metabolism. Partial forms of the disease exist.

More rarely, it could be due to unresponsiveness of the kidney to vasopressin (nephrogenic diabetes insipidus).

Clinical diabetes mellitus represents a syndrome with disordered metabolism and inappropriate hyperglycemia due to either an absolute deficiency of insulin secretion or a reduction in its biologic effectiveness or both. Therapeutic method of classification of diabetes has separated mellitus into 2 major types in which age of onset is no longer a criterion Karam and Nwosu, 2017 cited by [6].

The severe form of diabetes which is known as type 1 is an Insulin – Dependent Diabetes Mellitus (IDDM) and it is associated with ketosis in the untreated state. It occurs most commonly in juveniles but occasionally in adults, especially the non-obese and those who are elderly when hyperglycemia first appears. It is a catabolic disorder in which circulating insulin is virtually absent, plasma glucagon is elevated and the pancreatic B cells fail to respond to all insulinogenic stimuli. Exogenous insulin is therefore required to reverse the catabolic state, prevent ketosis, reduce the hyperglucagonemia and bring the elevated blood glucose level down [7-8].

Karam [9] reported that certain HLA antigens – B8, B15, DR3 and DR4 are strongly associated with the development of type 1 diabetes. The genetic determinants of all of these antigens located on the sixth human chromosome adjacent to immune response genes show increased linkage to the genetic determinants of type 1 diabetes. A polymorphic region of DNA flanking the 5' end of insulin gene on chromosome 11 was shown to have an association with type 1 diabetes in a white population. In addition, circulating islet cell antibodies have been detected in as many as 85% of patients tested in the first few weeks of their diabetes [10].

Karam [9] reported that Type II diabetes which is a Non-Insulin-Dependent Diabetes Mellitus (NIDDM), represents a heterogeneous group that comprises milder forms of diabetes that occur predominantly in adults but occasionally in juveniles. In this type of diabetes, circulating endogenous insulin is sufficient to prevent ketoacidosis but is often either sub-normal or relatively inadequate in the face of increased needs due to tissue insensitivity. Type II diabetes is defined in essentially negative terms as a non-

ketotic form of diabetes that is not linked to HLA markers on the sixth chromosome. It has no islet cell antibodies and it is not dependent on exogenous insulin therapy to sustain life, thereby being termed “non-insulin-dependent diabetes mellitus” (NIDDM). Two sub-groups of patients with type 2 diabetes are currently distinguished by the absence or presence of obesity.

The principles of treatment of diabetes otherwise known as rational therapy of diabetes, requires the application of principles derived from current knowledge concerning the nature of the disease and the mechanism of action together with the efficacy and safety of available treatment regimens (diet, oral hypoglycemic drugs and insulin).

A well-balanced nutritious diet remains a fundamental element of therapy [11]. However, in more than half of cases, diabetic patients fail to follow their diet. This stems from unnecessary complexity of the prescription as well as lack of understanding of the goals by both the patient and the physician. In prescribing a diet, it is important to relate dietary objectives to the type of diabetes.

For oral hypoglycemic drugs, there are two major types. These include sulfonylureas and biguanides. Their modes of action are quite different and considerable controversies exist over their mechanisms of action, therapeutic indications and especially their safety in long-term use. In some cases, the over-refined natures of such chemical drugs create new problems. In view of this it has become imperative to search for biological solutions from vegetable plant extracts which have double barrel effects in terms of stimulation of insulin secretion by  $\beta$  cells or the enhancement of residual insulin activity in the blood or inhibition of  $\alpha$  cell of the pancreatic islets. It has been estimated that around four billion people worldwide use herbal medicine which involve about 7,500 plant species [12]. The said plants include *Artemisia annua* which has a high content of essential oil and flavonoids. The extract of the plant has been reported to have anti-inflammatory, antioxidative, antihypertensive, ant-hyperlipidemia and anti-tumoral effects [13-15].

In view of the magnitude of the level of diabetic cases in Nigeria at the moment, which continues to increase annually like spreading wild fire, a detailed study was designed to find out the anti-

diabetic effects of the extract of Nigerian variety of *A. annua* singly and in combination with each of three other plants: *Momordica charantia*, *Vernonia amygdalina* and *Aegle marmelos*. These plants are used locally in Nigeria at the moment for the management of diabetes.

*Artemisia* is one of the diverse genera of *Asteraceae*. *Artemisia annua* var. *chiknesis* was developed from *A. annua* variety that was originally brought from China through genetic improvement on the artemisinin production. The original plant (sweet wormwood) is an annual plant and a native of Eurasia. The variety *chiknesis* which has high artemisinin content has naturalized in Nigeria. The essential oil extracted from it, is being put to so many medicinal uses and many compounds have been identified in it. The plant extracts (leaf, seed and root) have antimicrobial and antioxidant activities [16].

The aerial parts of *Artemisia annua* contain 0.01 to 0.8% of artemisinin per dry weight. Other constituents of *Artemisia annua* include deoxyartemisinin, artemisinic acid, arteannuin- B, stigmasterol, friedelin, friedelan – 3 beta – ol, artemetin and quercetagetin – tetramethyl ether.

Several effects such as anti-inflammatory, antioxidative, antihypertensive, anti-hyperlipidemia and antitumoral have been reported for the plant. It is also used for the management of gastrointestinal disorders and there have been reports on its effects on urinary tract disorders such as antispasmodic. In the Middle East, people use *A. annua* extract in the treatment of diabetic conditions.

*Momordica charantia* L. also known as Balsam pear, African cucumber, is a climbing herb with yellow flowers and orange fruits with carmine red seeds. The juice of the leaves and fruits is used as an antihelmintic and the pulverized plant is applied externally against malignant ulcers in Nigerian traditional medicine. The seeds could be used as an appetizer and in the treatment of biliousness and jaundice. The leaf and fruit extracts are used traditionally in Nigeria for the treatment of sugar health problems. The leaf extract has hypoglycaemic action.

*Vernonia amygdalina* Del. otherwise known as bitter leaf (Olugbu or Olubiri in Igbo Language) is a shrub with pubescent young branchlets and white sweet-scented flower heads. It is very common in villages in Eastern Nigeria. The twigs

are used as tooth cleaners (chewing stick) and are chewed as a stomachic, tonic and appetizer. The root is taken as a tonic and as an appetizer after the bark has been removed by scorching. It has been described as a substitute for ipecacuanha. The leaves are used in soups and are applied in local medicine for itching and parasitic skin diseases. A decoction of the leaves is taken as an antipyretic and laxative and for coughs (expectorant). The bark of the root or stem is astringent and finds use as a febrifuge and in diarrhoea. The leaves extract is believed to be effective in lowering blood glucose level and those who eat the leaves in form of bitter leaf soup are believed to be less prone to diabetes.

*Aegle marmelos* Correa is also known as Bael fruit tree. It is a spiny tree with small trifoliate leaves and a round smooth green fruit of the size of an orange, with woody shell. The unripe fruit is used as a mild astringent in the treatment of diarrhoea and dysentery in Nigeria.

In order to confirm that our own variety of *A. annua* (*A. annua* var. *chiknensis* – “*Nkpochapuiba*”) has antidiabetic effects, an investigation was carried out on its aqueous leaves extract employing the method described by Helal *et al.* [1]. The study was carried out on the said variety of *A. annua* singly and in combination with the fruit extract of *M. charantia*, leaf extract of *V. amygdalina* and the fruit extract of *Aegle marmelos*. This was done in order to simulate what the traditional users do in high blood glucose level ailment management. The extracts of the plants were administered on alloxan – induced diabetic male albino rats. The study was also carried out in order to find other sources of diabetic drugs since many Nigerians are suffering from diabetes and the drugs are expensive and not easy to come by. Some of the diabetic cases cannot even be treated with known conventional drugs.

## 2. MATERIALS AND METHODS

The experimental plants, *Artemisia annua* var. *chiknensis* with Laboratory code number (CBGE/CHNA/09/LTNGS/G), *Momordica charantia* Linn, *Vernonia amygdalina* Del. and *Aegle marmelos* Correa were locally sourced. A total of 60 male albino rats (*Ratus norvegicus*) each weighing 100g were also obtained from Veterinary Research Institute, Vom, Nigeria. The study was carried out in line with the International guidelines on the use of animals for experimentation. The method adopted for the

study was that described by Helal *et al.* [1]. The said rats were housed in clean plastic cages with wood chippings serving as the bedding. The animals were fed on a standard pellet rodent diet. The rats were maintained under favourable laboratory conditions, at 25°C (room temperature), 50% relative humidity and at a normal photo-period (12 hr light/dark cycle).

In order to make sure that all the experimental animals were well acclimatized, one of them that showed a sign of fungal infection was subjected to fungal isolation experiment prior to the diabetes inducement. The method adopted in this preliminary experiment was that described by Ogbonna *et al.* [17]. The diseased area of the animal's skin was thoroughly cleaned with alcohol. Scrapings were then taken from active lesion with the aid of sterilized sharp blade. Such scrapings were then collected with sterile Petri dish. Hairs from the diseased spot were examined with the aid of wood's lamp. The production of fluorescing green light acted as a preliminary indication of a dermatophytic infection. With a peak of 3650Å, the lamp demonstrates a characteristic fluorescence as a result of presence of some microbial agents. Such fluorescing hairs were then cut into very small pieces using a sterile blade. The pieced hairs were also stored in a sterile Petri dish. Both the scrapings and pieces of hair were separately plated out on Sabouraud Agar and Cycloheximide medium (Mycosel or Dermatophyte test medium). Cycloheximide was employed because saprophytic fungi and yeasts normally present as contaminants are inhibited by cycloheximide. Also media with antibacterial antibiotics greatly facilitate the isolation of fungi from non-sterile specimens. The confirmation of dermatophytic infection of the animal was then immediately followed by antifungal topical treatment to enable the experimental rat be in an excellent condition like the rest of the animals for the main experiment.

### 2.1 Inducement of Diabetes in the Experimental Rats

A total of 50 male albino rats which had been subjected to over-night fasting were induced to diabetic states through single intraperitoneal alloxan injections with freshly prepared physiological saline in dosages of 120 mg/kg body weight. The diabetic status of each of the experimental animals was closely monitored and the stability of each of the animals was also closely assessed. The diabetic animals were

sustained for seven successive days after the alloxan treatment as described by Helal et al. [1]. On the 8<sup>th</sup> day after the alloxan injections, only the rats with fasting blood glucose levels  $\geq 300$  mg/dl were selected and confirmed as diabetic and were subsequently employed for the rest of the experiments. Ten (10) rats were only injected with the physiological saline and these served as the control.

## 2.2 Extraction of the Experimental Plants

A weight of 2g of fresh leaves of *A. annua* var. *chiknensis* was aseptically ground and then put in a 500ml conical flask containing 200ml of distilled water. The flask was then heated on a bunsen flame and allowed to boil for a period of 10 minutes and the ground leaves were then extracted like tea. The flask was then cooled to room temperature, aseptically filtered and then stored in a refrigerator for further uses.

The other experimental plants fruits (*A. marmelos* and *M. charantia*) and leaves of *V. amygdalina* were also similarly extracted. The experiment was repeated using ethanol as the extraction solvent in order to obtain wider constituents of the experimental plants and employing the method described by Ogbonna et al. [18].

Portions of the extracts (both aqueous and ethanol) were biochemically analyzed using standard analytical procedures. References were also made to [19-23,18].

## 2.3 Artemisinin Determination

Artemisinin is a secondary or natural plant metabolite identified as a sesquiterpene lactone endoperoxide. Artemisinin determination in crude extracts of *A. annua* was carried out with the aid of high pressure liquid chromatography (HPLC) with reductive mode electrochemical detection which was first developed by Acton et al. [24] and later modified by Charles et al. [25]. This later method was found to be highly sensitive, rapid and of great value in the analysis of a sample needed for crop improvement programme. Using this later method, the artemisinin content of our plant was assessed. This was done in order to determine whether there was any genetic trait that could be exploited for increased artemisinin production. After the harvest of the initial batch of seeds from the first generation planting, our research team worked on the first generation seeds. The

resultant plants from these seeds yielded leaves which were found to have 4.8% of artemisinin. This final analysis was conducted by the Nigerian Institute for Pharmaceutical Research and Development (NIPRD). This result was obtained from repeated tests on the leaves. The plant we have now which is known as *A. annua* var. *chiknensis* (CBGE/09/LTNGS/G) and which contains 4.8% artemisinin is one of the best in the world.

## 2.4 The Essential Oil Component of Plant

The essential oil component of the experimental *A. annua* was extracted by steam distillation. The essential oil was extracted through hydrodistillation with a modified Clevenger trap and chemically characterized by gas chromatography (GC) analysis using a fused silica capillary column (12M x 0.2MM id) with a OV 101 (Varian, Polydimethylsiloxane) bonded phase. The direct injection of 0.5 ml of essential oil samples with He as a carrier gas (100:1 split vent ratio) and oven temperatures held isothermal at 80°C for 2 min and then programmed to increase at 3°C/min to 210°C, gave complete elution of all peaks (sensitivity 10 – 10). The injector and detector temperatures were 210°C and 300°C respectively. Confirmation of essential oil constituents was based upon comparison of retention time with standards and via GC/Mass Spectroscopy analysis.

## 2.5 The Treatment of the Experimental Diabetic Rats with the Experimental Plant Extracts

Prior to these particular treatments, preliminary administration of the extracts of the experimental plants on the male albino rats at the required dosages did not produce any noticeable acute or sub-acute toxic effects. Ten (10) of the Diabetic Rats (A) were treated intragastrically with the aqueous *A. annua* extract (28.5 mg/kg) as reported by Helal et al. [1], twice daily (at 8am and 8 pm) for a period of 30 days. Another set of 10 alloxan – induced diabetic rats (B) was treated with combined aqueous extracts of *A. annua* and *Momordica charantia* (28.5 mg/kg) in a ratio of 1:1 (*A. annua* extract to *M. charantia* extract), twice daily at same time (8 am and 8 pm) and for a period of 30 days. The 3<sup>rd</sup> set (10) of diabetic animals (C) was treated with aqueous extracts of *A. annua* and *Vernonia amygdalina* in the same dosage, 28.5 mg/kg and in the same ratio of 1:1, twice daily, same time and for a

period of 30 days. The 4<sup>th</sup> set (10) of diabetic rats (D) was treated with combined aqueous extracts of *A. annua* and *Aegle marmelos* in the same dosage of 28.5 mg/kg in same ratio of 1:1, twice daily, same time and for a period of 30 days. The 5<sup>th</sup> set of 10 diabetic rats (E) was not treated with experimental extracts and they thus remained diabetic for comparisons. The 6<sup>th</sup> set (10) of rats (F) which was not diabetic was treated with only fresh physiological saline and therefore acted as controls. The experimental emphasis was actually on *A. annua* var. *chiknensis* (CBGE/CHNA/09/LTNGS/G).

### 2.6 Determination of Both Fasting Blood Insulin and Glucose Levels of the Experimental Animals

One month after treatment with various plant extract, the animals were subjected to overnight fasting. Blood samples were collected from them early in the morning by 7am with the aid of sterile centrifuge tubes by means of cardiac puncture after mild anesthesia. Each fasting blood sample was subjected to centrifugation at 4000 rpm for a period of 10 minutes at 4°C in order to obtain the blood serum. The resultant serum was then stored at – 20°C for fasting blood glucose level determination. The sera insulin levels of both treated and non-treated rats were measured with the aid of enzyme immune-assay kit, while the fasting glucose level of each resultant serum of each set of the experimental animals (A – D) was determined using the method of Trinder [26]. The fasting blood glucose levels of the control animals were also determined.

### 2.7 Histopathological Examination and the Effects of the Experimental Extracts on the Body Weight of Rats

The animals were then subjected to pancreatic histopathological examinations for evidence of cellular regeneration. The effects of diabetic states and the subsequent diabetes treatments with the experimental extracts on the body weights of the animals were also determined.

### 2.8 Statistical Analysis

Data sets were examined by one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) to compare the means of the different variables. P-value of less than .05 was considered significant.

## 3. RESULTS

*Trichophyton mentagrophytes* was isolated from the diseased spot of one of the experimental animals. However, the animal was freed from the infection after the antifungal topical treatment prior to the commencement of the antidiabetic studies. The same fungus was isolated from the pulverized hairs that were plated out.

Both the extract of the *A. annua* and its various combinations with the other experimental extracts had reducing effects on sera glucose levels. All the extracts treatments had enhancement effects on insulin production and most likely its action. From the results obtained, it appears the greatest action on both glucose level reduction and insulin production or action came from *A. annua* extract (A). Its singular effect resulted in insulin production enhancement of 49.86% when the insulin level of the non-treated diabetic rats (24.65 µU/ml) is used as a baseline. The same treatment resulted in a glucose level reduction of 68.74% from a baseline of 288.50 mg/dl in non-treated diabetic rats. For the combination treatments, the best result was obtained from *A. annua* and *M. charantia* combination (B) which had a serum insulin level of 38.65 µU/ml which amounted to insulin production enhancement level of 56.80 % and a corresponding serum glucose level reduction of 69.65% using the same diabetic serum insulin level and glucose level as baselines. The treatment of diabetic rats with a combination of extracts of *A. annua* and *V. amygdalina* (C) produced a serum insulin level enhancement of 52.98% and a corresponding glucose level reduction of 69.53% for same serum baselines. The *A. annua* and *A. marmelos* (D) treatment combination produced a serum insulin level enhancement of 56.39% with a corresponding glucose level reduction of 69.32%. All figures recorded were mean figures. The mean figures for the serum insulin and serum glucose levels of non-treated diabetic rats (E) were 24.65 µU/ml and 288.50 mg/dl respectively, while the non-diabetic rats (F) had a mean value of 40.94 µU/ml and 86.55 mg/dl serum insulin level and serum glucose level respectively. There were significant differences ( $P \leq 0.05$ ) in the insulin level of the diabetic rats treated with *A. annua* leaf extract alone and those diabetic rats treated with different combinations of the plant extracts. The effects of the plant extracts on insulin level were most significant on *A. annua* + *M. charantia* and *A. annua* + *A. marmelos* treatments. As for the

fasting blood glucose level reductions, the most significant result was obtained from *A. annua* + *M. charantia* and *A. annua* + *A. marmelos* treatments at ( $P \leq 0.05$ ). The details are presented in Table 1. The histopathological examinations of the pancreatic cells of the treated rats showed signs of regeneration of the cells. The non-treated diabetic rats had an average body weight of 94.90 g as compared to the original average body weight of 100 g at the commencement of the experiment indicating that there was weight loss as a result of the diabetes while the treated

diabetic rats had average body weight increments from 108.83 g to 109.29 g. The non-diabetic rats had an average body weight of 114.10 as compared to their initial average body weight of 100 g. The details are presented in Table 2.

The biochemical analyses conducted on the extracts of the experimental plants revealed that they had varied biochemical constituents. The details are given in Table 3.

**Table 1. The effects of the experimental plants extracts on the alloxan – induced diabetic rats sets of experiments**

Treatments	Status of blood sera			
	Insulin $\mu\text{U/ml}$	Percentage Insulin enhancement	Glucose level mg/dL	Percentage glucose level reduction
Diabetic rats treated with <i>A. annua</i> (A)	36.94 $\pm$ 0.10 <sup>d*</sup>	49.86	90.19 $\pm$ 0.66 <sup>b</sup>	68.74
Diabetic Rats treated with <i>A. annua</i> + <i>M. charantia</i> (B)	38.65 $\pm$ 0.27 <sup>b</sup>	56.80	87.55 $\pm$ 0.35 <sup>cd</sup>	69.65
Diabetic Rats treated with <i>A. annua</i> + <i>V. amygdalina</i> (C)	37.81 $\pm$ 0.31 <sup>c</sup>	52.98	88.78 $\pm$ 0.72 <sup>bc</sup>	69.23
Diabetic rats treated with <i>A. annua</i> + <i>A. marmelos</i> (D)	38.55 $\pm$ 0.09 <sup>b</sup>	56.39	87.92 $\pm$ 0.40 <sup>cd</sup>	69.53
Non-Treated Diabetic rats (E)	24.65 $\pm$ 0.24 <sup>e</sup>	Nil	288.5 $\pm$ 0.63 <sup>a</sup>	Nil
Non-Diabetic Rats (F)	40.94 $\pm$ 0.34 <sup>a</sup>	Not applicable	86.55	Not applicable

\*mean in the same column having the same superscripts are not significantly different ( $p \leq 0.05$ )

**Table 2. The effects of the plants extracts administration on the experimental rats body weights**

Sets of experimental rats treatments	*Changes From Original 100g Body Weights
Diabetic Rats Treated with <i>A. annua</i> (A)	108.83 $\pm$ 0.45 <sup>b</sup>
Diabetic Rats Treated with <i>A. annua</i> + <i>M. charantia</i> (B)	109.29 $\pm$ 0.53 <sup>b</sup>
Diabetic Rats Treated with <i>A. annua</i> + <i>V. amygdalina</i> (C)	109.04 $\pm$ 0.47 <sup>b</sup>
Diabetic Rats Treated with <i>A. annua</i> + <i>A. marmelos</i> (D)	109.24 $\pm$ 0.43 <sup>b</sup>
Non-Treated Diabetic Rats (E)	94.9 $\pm$ 0.51 <sup>c</sup>
Non-Diabetic Rats (F)	141.1 $\pm$ 2.49 <sup>a</sup>

\* Mean values

**Table 3. Biochemical constituents of the experimental plants extracts**

Experimental plants extracts	Identified biochemical constituents
<i>A. annua</i> var. <i>chiknensis</i>	Alkaloids, Cardiac Glycosides, Flavonoids, Phenols Saponins, Steroids, Tannins, Terpenes Artemisinin, deoxyartemisinin, artemisinic acid, arteannuin – B, stigmaterol, friedelin, friedelam – 3 beta-ol, artemetin, quercetgetin. The essential oil including volatile compounds and several non-volatile sesquiterpenes (Some of the detected essential oil components included: alpha-pinene, camphene, $\beta$ – pinene, myrcene, 1, 8-cineole, Artemisia ketone, linalool, camphor, borne of and $\beta$ – caryophyllene).
<i>M. charantia</i>	Purgative oil, a bitter principle, momordicin, a coloured oil, resin, alkaloid.
<i>V. amygdalina</i>	A bitter glycoside, Vernonin
<i>A. marmelos</i>	Essential oil: $\alpha$ – and $\beta$ – phellandrine. $\beta$ – fagarin, a methoxyisodictamine (quinoline derivative), dihydrofurocoumarine, marmesine and umbelliferone (extracted from the mature bark).



#### 4. DISCUSSION

The results obtained from the study have shown that the extracts of the experimental plants have both the abilities of increasing the plasma insulin level and also the abilities to decrease the glucose levels of hyperglycemic rats. These said abilities were most pronounced in *A. annua* var. *chiknensis* than the rest of the extracts of the experimental plants. The *A. annua* extract enhanced insulin production by 49.86% after period of 30 days of administration at a dose of 28.5mg/kg, twice daily. Similar effects in terms of enhancement of insulin production were observed for the different combinations of *A. annua* extract with each of the extracts of the other experimental plants. There were corresponding levels in fasting blood glucose level reductions as a result of administration of various combinations of the plant extracts to the diabetic rats. *A. annua* alone reduced the plasma glucose level by 68.74%. The combined treatment of *A. annua* and *M. charantia* resulted in 69.65% plasma glucose level reduction while the combination of *A. annua* and *V. amygdalina* extracts treatment led to 69.23% plasma glucose level reduction. The treatment of the diabetic rats with a combination of the extracts of *A. annua* and *A. marmelos* resulted in a plasma glucose level reduction of 69.53% in the experimental diabetic rats after a period of 30 days. Thus there were significant differences ( $P \leq 0.05$ ) in the insulin level of the diabetic rats treated with *A. annua* leaf extract alone and those diabetic rats treated with different combinations of the plant extracts. Such effects were most significant in *A. annua* + *M. charantia* and *A. annua* + *A. marmelos* treatments. As for the fasting blood glucose level reductions, the most significant result was obtained from *A. annua* + *M. charantia* and *A. annua* + *A. marmelos* treatments at  $P \leq 0.05$ . They were followed by *A. annua* + *V. amygdalina* treatment and then by the singular *A. annua* extract treatment at  $P \leq 0.05$  (Table 1). The findings of this research work were in agreement with that of Helal et al. [1]. Mandour (2001) cited by Helal et al. [1], reported that there was a significant decrease in blood glucose level in animals that were treated with 28.5 mg/kg *A. annua* extract twice per day. He said that this could be attributable to the stimulation of the secretion of insulin by  $\beta$  cells, inhibition of  $\alpha$  cells of the pancreatic islets or the enhancement of insulin activity as reported by Winkelman [27]. *A. annua* extract contains flavonoids such as afroside, cirsimartin, chrysoplenol and cirsiolol [1].

There is evidence that hyperglycemia results in the generation of reactive oxygen which could lead to oxidative stress in various tissues including the vascular system. The linkage between oxidative stress, inflammatory response and insulin activity is now well known. The ability of antioxidants to protect against the deleterious effects of hyperglycemia and also to improve glucose metabolism and intake must be considered as educative in terms of choice of method in diabetes treatment as reported by Helal et al. [1].

The experimental rats with fasting blood glucose levels  $\geq 300$  mg/dL were selected and confirmed as diabetic prior to the plant extracts treatments. The normal glucose tolerance is considered to be prevailing when 2 – hour plasma glucose is less than 140 mg/dL, with no value between zero time and 2 hours exceeding 200 mg/dL. A diagnosis of diabetes mellitus requires plasma glucose levels to be above 200 mg/dL both at 2 hour and at least one other time between zero time and 2 hours. Values above the normal standard that do not meet the criteria for diabetes are considered non – diagnostic. Certain medications that could impair glucose tolerance include diuretics, contraceptive drugs, glucocorticoids, nicotinic acid and phenytoin.

In view of the difficulties in the interpretation of oral glucose tolerance tests and the lack of standards related to aging, they are now generally being replaced by documentation of fasting hyperglycemia as a means of diagnosing diabetes mellitus.

When fasting plasma glucose exceeds 120 mg/dL, pancreatic  $\beta$  cells generally do not respond well to added glucose in the face of chronic hyperglycemia, regardless of whether the glycemia results from insulin resistance predominantly or from sluggish early insulin release in cases of primary pancreatic B cell dysfunction. Also when fasting hyperglycemia is milder (110 – 120 mg/dL), a late hyperinsulinism may be present as a consequence of persistent hyperglycemic stimulation of pancreatic B cells, regardless of whether the later hyperglycemia results from primary insulin resistance (as in cases of obesity) or from sluggish early insulin release to a glucose load.

Atuchukwu (2017) cited by Ekezie [28], reported that the use of oral hypoglycaemic drugs has been on the rise in the prescription of many type II diabetes and that some of such drugs in the

process of stimulating the pancreatic beta cells to produce more insulin, affect their proper function. He reported that a good approach to avoid such was to adopt a combination of conventional and traditional methods of diabetes management under very careful watch of a qualified physician. He reported that one Nigerian herb that has shown such promise is bitter melon, *Momordica charantia* (African cucumber). He reported that the acetone extract of whole fruit powder of this plant in doses of 25, 50 and 75 mg/100 g of body weight lowered the blood glucose from 13.30 to 50% after 8 – 30 days treatment. He thus confirmed the hypoglycaemic effect of this plant.

Biochemical analyses of the extracts of the experimental plants revealed that they contained various biochemicals including flavonoid which was identified in the *A. annua* extract (Table 3). Many flavonoids have been shown to act as antioxidants. They also act on biological targets involved in type 2 diabetes mellitus such as  $\beta$  – glycosidase, glucose co-transporter or aldose reductase. Flavonoids in this context act as antioxidants by acting as biological targets involved in diabetes mellitus. The flavonoid component of the *A. annua* extract has been shown to have a potent antioxidant action, attenuating the oxidative stress induced by free radicals [29]. The *A. annua* extract may have played major roles in the combined effects with the other experimental extracts in inhibiting hyperglycemia and also ameliorating metabolic abnormalities induced by diabetes through its antioxidant actions. Ahameethunisa and Hopper [30] isolated alkaloids, amino acids, carbohydrates, flavonoids, phenol, phlobatannins, quinines, saponin, tannins, terpenoids and volatile oils from *Artemisia nilagirica* leaf extracts. This is further proof of the potentials of *Artemisia* species extracts in terms of diabetes management. The diabetic actions of *A. annua* could be enhanced if used in combination with other plants extracts like *M. charantia*, *V. amygdalina* and *A. marmelos* as indicated in the present results.

There was a significant decrease in the body weight of the diabetes – induced experimental animals (Table 2). Helal et al. [1] also reported that there was a significant decrease in body weights of their diabetic animals after one month of diabetes induction as compared to those of their control rats. Insulin – dependent diabetes mellitus (IDDM) cases could be associated with weight losses especially in advanced insulin deficiency situations. Such weight losses could

stem from a combination of dehydration, loss of subcutaneous fat and muscle wasting as reported by Karam [9]. There were upward body weight increases in diabetic animals treated with the extracts of the experimental plants. Such body weight gains were in fact tending towards normalcy (Table 2). It was necessary to have subjected one of the experimental rats to dermatophytic fungal isolation experiment and the eventual antifungal topical treatment. If this had not been done, the disease could have affected the physical well-being of the animal and which could have in turn affected its final body weight.

## 5. CONCLUSION

The results have shown how effective *A. annua* var. *chiknensis* extract is in terms of lowering the blood glucose level or in the prevention of hyperglycemia. The results have also revealed that such blood glucose level reduction could be enhanced when the said extract is used in combination with other plant extracts that have similar effects in terms of management of hyperglycemia. It is hoped that new diabetes drugs that stem from the findings can be developed. However, in order to minimize the risk of diabetes cases in Nigeria, both conventional and traditional preventive measures should be considered.

## ETHICAL APPROVAL

Approval from the University of Jos Institutional Animal Ethical Committee was taken prior to the experimental work.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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