The Effects of Aqueous *Moringa oleifera* and *Gongronema latifolia* on the Defense System of Diabetic Rabbits

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ABSTRACT

This study was conducted to determine the effects of aqueous *Moringa oleifera* and *Gongronema latifolia* on the defense system of diabetic rabbits. This study was carried out in the Agriculture Laboratory of the Department of Animal Science, University of Uyo, Uyo. A total of twenty five (25) rabbits consisting of 10 males and 15 females were randomly from animal house of University of Uyo, Uyo. The experimental animals were allowed two weeks of stabilization period and feeding trials lasted for twelve weeks. Five rabbits were randomly allocated to each treatment. The floor of the pens were cleaned daily, feed and water were provided ad libitum. Leaves were air dried under shade for ten days, all leaves were threshed carefully to separate leaves from twigs before blending. Twenty percent (20%) of *M. oleifera*, (20%) *G. latifolia* (20%) leaf meals were added to each supplemented diet. The proximate composition, nutritional factors and vitamin content of air dried *G. latifolia*, and *M. oleifera* leaves were separately analyzed using standard methods to have 26.92% crude protein, 13.60% crude fibre, 10.25% Ash, 11.90% moisture and 2129IU vitamin A, 6.05% vitamin C, 805.5mg/100g of *G. latifolia*, and *M. oleifera* had 26.96% crude protein, 9.60% crude fibre, 7.13% Ash, 14.23% moisture and 1806IU vitamin A, 7.43% vitamin C, and 666.6mg/100g vitamin E. The results of the study showed significant differences (P<0.05) among the treatment in final body weight, average daily weight gain, average daily feed intake and total body weight gain. The effect of treatment on feed conversion ratio were significantly different (P<0.05) in favour of rabbits on *M. oleifera* leaf meal T1 and *G. latifolia* leaf meal T2. Also, other growth performance parameters – daily weight gain and final body weight were better (P<0.05) and higher for rabbits on *M. oleifera* leaf meal T1 and *G. latifolia* leaf meal T2 diets. Average daily weight gain, final body weight and feed conversion ratio values of 10.20 ± 0.54g, 1.52 ± 0.18kg and 33.00 ± 2.89 was reported for rabbits on T1, respectively, while the respective values recorded for rabbits on T2 were 10.23 ± 0.81g, 1.44 ± 0.61kg and 31.85 ± 3.42. Average feed intake was higher for rabbits in T1 (1024g/day) and lowest for those in T2 (830g/day). The feed cost/kg gain was lowest for rabbits in T1 (40.34). Graded doses of the leaves extract (100, 200 and 300 mg/kg oral) were separately administered to groups of fasted normal and alloxan induced diabetic rabbits. Following treatment, *Moringa oleifera* (100, 200 and 300 mg/kg oral) produced highly significant (p<0.001) reduction in blood glucose levels at 2nd hour in fasted normal and alloxan induced diabetic rabbits. But, maximum percentage reduction in blood glucose was seen with 200mg/kg dose when compared to control. The same thing was applicable with aqueous extract gongronema latifolium leaves. It was therefore concluded that the leaves of *moringa oleifera* and *gongronema* administered at the dosages used and for the duration of the experiment had significant treatment effects the defense system of diabetic rabbits and finally that the use of both herbs simultaneously will have higher effects in treatment of diabetics than when used differently. Implications and recommendations were made from the findings of the study.

Keywords: Aqueous *Moringa oleifera*, Defense System, Diabetic Rabbits, *Gongronema latifolia*.

I. INTRODUCTION

Over the years, despite the presence of many methods, approaches and medicines, the management of type 2 diabetes mellitus remains unsatisfactory (Singh, Vats, Suri, Shyam, Kumria, Ranganathan & Sridharan, 2001). The increasing prevalence of diabetes in both developed and developing countries have challenged scientists to the discovery of various therapeutic agents that can be used to ensure efficient treatment and management of diabetes (Gupta, Mathur, Bajaj, Katariya, Yadav & Kamal, 2012). Also, with the increasing incidence of diabetes mellitus (DM) in rural population of Africa, many drugs have been formulated for the management of this chronic hyperglycemic disorder. However, there are limitations in the use of anti-hyperglycemic medications resulting from the side effects, high cost, limited action and secondary failure rates. There is a clear need for the development of indigenous, inexpensive herbal sources for diabetic treatment (Atmakuri & Dathi, 2010). There is presently no cure for diabetes and the drugs available for the treatment and management of this disease are still unable to impair insulin deficiency. Less privileged diabetic patients are unable to purchase expensive drugs to manage this conditions or maintain their life style Amod, Ascott-Evans, Berg, Blom, Brown, Carrhill, Dave, Distiller & Ganie, 2012).

Diabetes mellitus is a chronic metabolic disorder that has now become an epidemic, with a worldwide incidence of 5% in the general population. The fundamental defect in diabetes mellitus is an absolute or relative lack of biologically active insulin, which results in the impairment of uptake and storage of glucose, reduced glucose utilization for energy purpose (Sangeeta, Rahul Jain, Saxena, Chaurasia, & Rajeev Shrivastava, 2010). According to World Health Organisation (WHO)
projection, the prevalence of diabetes is likely to increase by 35%. Currently there are over 150 million diabetics worldwide and this is likely to increase to 300 million or more by the year 2025 (World Health Organisation, 2013).

Diabetes Malitus (DM) remains a major global public health problem. It is a metabolic syndrome of multiple aetiologies characterized by chronic hyperglycemia resulting from defects in insulin, insulin actions or both. Hyperglycemia in Diabetes has been associated with increased formation of Reactive Oxygen Species (ROS) and inflammatory mediators. Consequently, if this metabolic syndrome is left untreated, it can lead to severe complications. Globally, increase in weight, obesity and sedentary lifestyle is gradually becoming prevalent resulting to diabetes. This may be due to a change of diet. Diabetes is projected to become the seventh leading cause of death in experimental animals. Diabetic in animals is expected to rise by more than 50% in the next 10 years. (Ansari & Dash, 2013). Many drugs have been designed for the treatment and management of this disease. However, there are limitations in the use of anti hyperglycemic medications due to the side effects, high cost, limited actions and secondary failure rates (Baggio & Drucker, 2007).

*Moringa oleifera* well known for its pharmacological actions and is used for the traditional treatment of diabetes mellitus (Babu & Chaudhuri, 2005). *Moringa oleifera* known in English by the names miracle tree, horseradish, drumstick, benzoilive tree and named in respective native languages in other regions where it is grown. In Hindu, it is called Sajjan, in Yoruba Ewe ile, in Filipino Mulanggay, in Hausa Zagole and in Igbo Oduduoyingbo (Muhammad & Soriani, 2014). MO belongs to the family of *Moringaceae*, which is widely distributed in the tropics and subtropics of Asia and Africa (Sreelatha, Jeyachitra & Padma, 2011). The *oleifera* species has been in existence as far back as early 2000 BC, and is one of the world’s most useful plants because of its medicinal properties (Kumar, Mishra, Ghosh& Panda, 2010). MO has its origin in Agra and Oudh, in the northwest region of India, south of the Himalayan Mountains (Mishra, Singh, Verma, Kumar, Srivastav, 2011). There are twelve (12) other known species: *stenopetala* which is a staplefood of the indigenes of Ethiopia, *pygmaea, peregrine* (Forsk.), *rivae, arborea, borziana, ruspiliana, longituba, concanensis, droshardii, hilde- brandii, and ovalifolia.*

MO is called miracle tree because every part of this plant is useful, has high nutritive value, and possesses numerous medicinal properties that can be used in treating or managing various diseases. MO is a plant which can be eaten as a vegetable and used as beverages. A wide variety of nutritional and medicinal potentials have been attributed to its roots, bark, leaves, flowers, fruits, and seeds (Kumar, Mishra, Ghosh & Panda, 2010). Various parts of MO such as the leaves roots, seeds bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess antitumor, antiucler, antipyretic, antiepileptic activities (Farooq, Rai, Tiwari, Khan, Farooq, 2012). In addition, MO has been used for the treatment and management of different ailments in traditional medicine because of the antihypertensive, antioxidant, antimicrobial, antibacterial, antispasmodic, antifungal, anti inflammatory, anti-tuberculosis, analgesic, anti diabetic, diuretic, cholesterol lowering, and hepatoprotective properties (Kumar,etal, 2010). MO shows hypolipidaemic, antiatherosclerotic and immune boosting effects (Chumark, Kunawat, Sanvarinda, Phornchiraslip, Morales &Phivthong-ngam, 2006). Moringa Oleifera (MO) has been used for centuries as a folk remedy for stomach complaints, catarrh, cancer, gastric ulcers, skin diseases, lowering blood sugar, increasing bone density, nervous conditions, diabetes, fatigue, increase lactation, hay fever, impotence, oedema, cramps, hemorrhoids, headaches, sore gums; liver, gall, digestive, respiratory and immune system, as a blood cleanser, blood builder and wound healing (Rathi, Bodhankar & Baheti, 2011). The leaves of MO are anthelmintic aphrodisiac (increases sexual desire), cures hallucinations, dry tumors, hiccouc and asthma (Goyal, Agrawal, Goyal & Mehta, 2007). MO is also used as stimulants, expectorant, and antilithic. Furthermore, it also provides a local solution to malnutrition (Mishra, Singh, Verma, Kumar & Srivastav, 2011). MO is well known traditionally for the treatment of diabetes mellitus, hepatotoxicity, rheumatism, venomous bites and also for cardiac stimulation (Soliman, 2013).

People in many developing countries, especially in Africa have been using *Moringa oleifera* to treat and manage the symptoms of diabetes for years. The International Diabetic Federation (IDF), in a report stated that over 246 million people worldwide were suffering from the disease and the prevalence is expected to rise to380 million by the year 2026 (Malviya & Jain, 2010). It is interesting to note that this plant can also grow in any type of soil and can be grown in the garden space at home. MO grows best in sandy or loamy soil with a slightly acidic pH and has a height ranging from 5 to 12 m with a straight trunk 10 - 30 cm thick (Hussain, Malik & Mahmood, 2014).

The leaves of MO contain phytochemicals such as niazirin and niazirin(Goyal, Agrawal, Goyal & Mehta, 2007). In addition, MO contains high level of vitamins A, B and C minerals (especially iron) amino acids (leucine, glutamic, valine, asparitic, alanine and so on), fatty acid, carotenoids (carotene, lutein xanthin, flavonoids, polyphenols (tannins), high in protein and antioxidants (Kumar, Mishra, Ghosh, Panda, 2010). MO contains glucosinolates and flavonoids, anthocyanins, pranoanthocyanidin and cinnamates (Bennett, Mellon, Foidl, Pratt, Dupont& Perkins, 2013). The leaves contain 4-(α-Lhamnopyranosilyloxy) – benzy glucosinolate and three monoacetyl isomers of glucosinolate. Quantitative analysis carried out on the leaves revealed the presence of...
The biological actions have been studied in rabbits were randomly allocated. (2005) identified that the growth range from 70% to 90% and 4.8 to 6.5 respectively. Also, ethanolic and freeze dried leaves extract of MO has been reported to contain quercetin and kaempferol (Siddhuraju & Becker, 2013).

Gongronema latifolia Benth called amaranth in English while called ‘utazi’ and ‘arokeke’ in South Eastern and Western Nigeria, is of West African origin (Nielsen, 1965 as cited in Daniel, 2012). It is abundantly available in virgin forests in many parts of sub Saharan Africa and some parts of China (Nielsen, 1965; Ying and Ping-tao, 1997 as cited in Daniel, 2012). It is used as a leafy vegetable and spice in South Eastern Nigeria (Agbo, Baiyeri & Obi, 2005). It is a herbaceous shrub of the tropical rain forest of the family Asclepiadaceae. It is a climbing shrub up to 5 m long. The leaves are commonly used either as vegetable or as a spice. Various pharmacological actions have been reported on this plant and include antioxidant potential (Atawodi, 2005, as cited in Eke, Omoja & Echema, 2018), anti-asthmatic (Sonibare & Gbile, 2008), antimalarial, anti-inflammatory and anti-sickling activities (Eguyoni, Moody & Eletu, 2009; Etetim, Useh & Okokon, 2008). The plant has also been reported to cure cough, loss of appetite, and stomach disorders.

Agboet al. (2005) identified the crop Gongronema latifolium to be nutritionally high in iron, zinc, vitamins, protein and amino acids and thus could complement the inadequacies of these substances in feed. Unfortunately, its beneficial inclusion as a source of vitamins and minerals in rabbits production is still at a very low ebb. The levels of vitamins A, C, E and β-carotene in the crop species are relatively high, measuring 40.82 mg/100 g, 15 mg/100 g, 3.71 mg/100 g and 6.80 mg/100 g, respectively. Results show that the lipid extract, ash, crude fibre and nitrogen free extractives, oxalate, phytate and tannin of the plant are within expected ranges. They however had unexpectedly high crude protein content: 27.20 per cent. Potassium, phosphorus, calcium and cobalt were the most abundant mineral elements. G. latifoliunare 50.22 per cent saturated; 39.38 per cent polysaturated, degrees of unsaturation are 0.46. Palmitic and oleic acids were the major monounsaturated fatty acids. Major essential amino acids are leucine, valine and phenylalanine. Proportions of essential to non-essential amino acid are 43.37 and 49.84 per cent, respectively.

Therefore, this study is bent on evaluating the effect of Moringa oleifera and Gongronema latifolia (Utazi) on the defense system of diabetic rabbits. Hence, the following questions were formed:

1. Will the intake of Moringa oleifera by diabetic rabbit have positive effects on the defense system of rabbits?
2. Will the intake of Gongronema latifolium by diabetic rabbit have positive effects on the defense system of rabbits?
3. Will the combination of Moringa oleifera and Gongronema latifolia have higher effects on the defense system of rabbits?

II. MATERIALS AND METHODS

2.1 Location of the Study

This study was carried out in the Teaching and Research Farm, University of Uyo, Uyo in Akwaibom State (16.14°N and 7.45°E). The area lies in South –South zone of Nigeria with a prevailing high average rainfall ranging from 2400 to 3600mm. The average temperature of the area range from 26 – 280°C during the rainy season. The relative humidity range from 70 – 90% and 4.8 to 6.5 respectively. The state is bounded on the east with Cross River state, west with Rivers state and Abia state and on the South with Atlantic Ocean. The state has 31 local government areas and a population of 5 million people leaving in the state.

2.2 Experimental Animals

The Sire and the Does between the ages of 10-12 weeks and weighing 120-140 g were blocked by weight and randomly allotted to five treatment in a completely randomized design. The animals were confined to individual hutches made up of concrete floors and a roof, fed and watered ad libitum. They were acclimatized and dewormed by administering Thiabendazole 7 days before the commencement of the experiments and dipped a weekly in ascaricde (Rhoicidoxides) solution to control ectoparasites throughout the period of the experiment. All animal experiments were in accordance with the guideline stipulated by the National Institute of Health for Care and use of laboratory animals (Pub. No. 85: 23 revised 1985).

2.3 Experimental Procedures

At first the moringa leaves and Gongronema latifolia leaves were obtained from the farm around Uyo and was authenticated by a plant taxonomist. The leaves were washed differently, air dried and blended to powdery form to have Moringa leaf meal and Gongronema latifolia leaf meal. Secondly, the 30 rabbits were divided into five groups, four experimental groups and one controlled group and housed in a well-ventilated animal facility in stainless steel cages (beddings composed of ground sterilized maize cobs) with 6 rats per cage to allow free mobility. The rabbits were fed with standard rabbit chow and water ad libitum. Conducive temperature of 22°C, humidity 55% and a normal period (12 h light/12 h dark) was maintained.

Lastly, the diabetic rabbits were randomly assigned into five groups six in each group making a total of fifteen rabbits (n = 30). The experiment went as follows:
2.5 Statistical Analysis

Blood glucose levels were expressed in mg/dl as mean±SEM. The statistical analysis of data was done using one way analysis of variance (ANOVA), followed by Dunnett’s test using the software “PRIMER OF BIOSTATISTICS”. P value less than 0.05 was considered to be significant.

2.4 Growth Performance Indices

The rabbits were weighed before and after each daily feeding. Feed intake was determined daily as the difference between the quantity given and the left over the following morning. At the end of the experiments, the difference between the final live weight and initial live weight were determined and recorded as weight gain. The mean weight gained per replicate was further divided by the period of experiment to determine the average daily gain. Feed conversion ratio was calculated by dividing average daily feed intake of each replicate by the average daily weight gain. Mortalities if any was recorded on daily basis and summed up at the end of the experiment. Cost of feed and other inputs were estimated using the prevailing market prices while the experiment lasted.

The cost per kilogram of each diet was estimated by using glucometer and results were expressed as mg/dl.

2.5 Haematology and Biochemical Indices

As each rabbit was bleeding, 2 ml of blood were collected into sample bottles treated with ethylenediaminetetraacetic acid (EDTA). Another 2 ml was collected into plain bottles for serum indices assessment.

2.5.1 Haematological Assay

Red Blood Cell Count: The red blood cell count was determined via the improved Neubauer ruled chamber after diluting with 0.02 ml of blood mixed with EDTA. 4 ml of formaldehyde citrate solution was used as the diluting liquid at 1: 200 using a Pasteur pipette. The two ruled areas of the improved Neubauer chamber were filled with diluted blood and allowed to stand for 3 minutes for the cells to settle. The cells were counted in accordance with the procedures of WHO (1980).

\[ \text{Cell count (1012/L)} = \frac{N \times (D/A) \times 10^9}{10} \]

Where
- \( N \) = Total number of cells counted
- \( D \) = Dilution factor of blood
- \( A \) = Total counted area (mm\(^2\))
- \( 10 = \text{Factor to convert area to volume (iμ) \text{ area to volume (iμ) \text{ area to volume (iμ)}} \]
- \( 10^9 = \text{Factor to convert count per iμ to count per litre} \]

Packed Cell Volume (PCV): Blood was filled into microcrt tube 3/4th and sealed. The blood sample was centrifuged in a hematocrit centrifuge at 200 rpm for 4-5 minutes. The values were read using a hematocrit reader and recorded.

\[ \text{PCV} = \frac{\text{Height of red cell column}}{\text{Height of total blood column}} \]

Haemoglobin Concentration: 2ml of haemoglobin standard tube was labelled. 5ml of Drabkin reagent were dispensed into tubes labelled blank, control and sample. Deionized water was added and thoroughly mixed and allowed to stand at room temperature for 3 minutes. Adjustment was made at zero absorbance at 540nm using reagent black. The values were read.

Red Cell Indices: The Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) were expressed in picogram (pg), femtolitre (fl) and grams / 100 ml respectively. The MCH, MCV and MCHC were determined from RBC, PCV and haemoglobin (Hb). These hemotological constant were calculated using the appropriate formulae as described by Jain (1986).

\[ \text{MCV (fl)} = \frac{\text{PCV}}{\text{RBC}} \]
\[ \text{MCH (pg)} = \frac{\text{Hb}}{10} \times \text{RBC} \]
\[ \text{MCHC (g/l)} = \frac{\text{Hb}}{\text{PCV}} \]

White Blood Cell Count: The white blood cell count was obtained using a haemocytometer with Natt and Hendricks diluent to obtain a 1: 200 blood dilutions. The diluents and samples were mixed and carefully loaded into a counting chamber. This was left for 2 – 3 minutes for the cells to settle before they were counted using improved Neubauer haemocytometer at magnification of × 40. All the cells in the entire central square (1 mm\(^2\))
were thereafter estimated in accordance with method of Schalm et al. (1975).

\[
\text{Cell count (109 / L)} = \frac{N \times (D/A) \times 10 	imes 10^9}{109} 
\]

Where
\( N \) = Total number of cells counted
\( D \) = Dilution factor of blood
\( A \) = Total counted area (mm\(^3\))
10 = Factor to convert area to volume (iμ)
109 = Factor to convert count per iμ to count per liter

3.5.2 Serum Biochemical Assay Serum Urea: 4 ml of freshly prepared urea colour reagent was pipette into each tube and mixed vigorously. The content of each tube was incubated at 100\(^\circ\)C for 15 minutes. The absorbance was read using green filter 520nm colorimeter.

\[
\text{Urea mmol/l} = \frac{\text{Absorbance of test}}{\text{Absorbance of } 10\text{mmol/l}} \times 10
\]

Serum Glucose: Four tubes were labelled blank, standard and treatment tube. 1.5 ml of protein precipitant was pipette into all the tube samples. 1.5ml of colour reagent was added and thoroughly mixed and incubated at 37\(^\circ\)C for 10 minutes. Spectrophotometer was zeroed with blank sample and absorbance of all tubes read at 520 nm. The glucose level was thereafter calculated as;

\[
\text{Concentration of glucose (mg / dl)} = \frac{\text{Abs.of treatment}}{\text{Abs.of std}} \times \frac{\text{concentration of std.}}{100}
\]

2.6 Carcass Weights
At the end of the experiment, two rabbits whose live weights were closest to the mean live weight of each replicate were randomly selected from each replicate. The pigs were starved for 24 hours but water was provided ad libitum. The birds were individually weighed, then slaughtered by severing the jugular vein and were allowed to bleed to death in a vertical position (head down). After this, they were scalded in hot water, dressed and eviscerated. The carcass was cut into the constituent parts following the methods described by Okeudo et al., (2005). Each carcass part was weighed and the dressing percentage calculated as dressed weight divided by the live weight multiply by 100. The internal organs were carefully separated and weighed. The carcass cuts and internal organs were expressed as percentage of the live weight.

III. RESULT

Table 1: Percentage Composition of Experimental diets

| Ingredients          | T1     | T2     | T3     | T4     | T5     |
|----------------------|--------|--------|--------|--------|--------|
| Maize ofal           | 39.40  | 60.00  | 60.00  | 60.00  | 60.00  |
| Wheat ofal           | 6.68   | 6.18   | 6.18   | 6.18   | 6.18   |
| PKC                  | 24.43  | 21.46  | 21.46  | 21.46  | 21.46  |
| Soysabeans           | 23.19  | 12.18  | 12.18  | 12.18  | 12.18  |
| Eleuciala Indica     | 0.00   | 40.00  | 0.00   | 0.00   | 0.00   |
| Tropical Kudzu       | 0.00   | 0.00   | 40.00  | 0.00   | 0.00   |
| Moriga Oleifera      | 0.00   | 0.00   | 0.00   | 40.00  | 0.00   |
| Gongronema Latifolium| 0.00   | 0.00   | 0.00   | 0.00   | 40.00  |
| Bone Meal            | 3.00   | 3.00   | 3.00   | 3.00   | 3.00   |
| Limestone            | 2.00   | 2.00   | 2.00   | 2.00   | 2.00   |
| Methnine             | 0.25   | 0.25   | 0.25   | 0.25   | 0.25   |
| Lysine               | 0.25   | 0.25   | 0.25   | 0.25   | 0.25   |
| Salt                 | 0.25   | 0.25   | 0.25   | 0.25   | 0.25   |
| VMPa                 | 0.25   | 0.25   | 0.25   | 0.25   | 0.25   |
| Total                | 1.00   | 1.00   | 1.00   | 1.00   | 1.00   |

Calculated Analysis

| Component       | T1    | T2    | T3    | T4    | T5    |
|-----------------|-------|-------|-------|-------|-------|
| Protein         | 14.00 | 13.95 | 13.95 | 13.95 | 13.95 |
| Energy (kcal of ME/Kg) | 20.35 | 20.45 | 20.45 | 20.45 | 20.45 |
| Crude fibre (%) | 4.95  | 4.69  | 4.69  | 4.69  | 4.69  |

T1= Control group, T2= 40% of Eleuciala Indica, T3= 40% of tropical Kudzu meal, T4=40% of Moringa oleifera Leaf Meal, T5=Gongronema latifolia Leaf Meal, VMP= Vitamin Mineral Premix.
Table 2: Composite Pronximate of Experimental Diets

| Descrip. | M (%) | CP (%) | CF (%) | Ash (%) | EE (%) | ME Kg |
|----------|-------|--------|--------|---------|--------|-------|
| T1       | 9.70  | 19.00  | 10.00  | 11.83   | 4.93   | 2910  |
| T2       | 11.01 | 15.00  | 10.21  | 9.56    | 6.25   | 2902  |
| T3       | 9.00  | 16.20  | 12.01  | 11/01   | 5.60   | 2826  |
| T4       | 10.00 | 18.36  | 11.10  | 10.50   | 5.60   | 2756  |
| T5       | 12.70 | 17.73  | 13.10  | 11.12   | 4.60   | 2415  |

CP=Crude protein CF=Crude fibre Ash EE= Ether extract ME=Metabolizable energy, T1= Control group, T2= 40% of Elueciala Indica, T3= 40% of tropical Kudzu meal, T4=40% of Moringa oleifera Leaf Meal, T5= Gongronema latifolia Leaf Meal.

Table 2 above summarizes the proximate composition of the experimental diets. The experimental diets consisting of 40% inclusion of air dried of Elueciala Indica, tropical Kudzu, M. oleifera leaves, G. latifolia leaves, and the proximate composition, nutritional factors and vitamin content of air-dried Elueciala Indica, tropical Kudzu, M. oleifera leaves, G. latifolia leaves were separately analysed. The proximate analysis was carried out in the Animal Science Biochemistry laboratory. The proximate analysis was conducted based on the methods of the Association of Official Analytical Chemists (AOAC, 1990) for determination of moisture, crude fibre, protein, ash and vitamin content of the samples. The proximate values were reported in percentage. The chemical analysis was carried out according to the AOAC (1990) procedure. Energy values of feeds were calculated using the prediction equation cited in NRC (1994).

Table 3: Growth performance of rabbits fed on dried leaves of tropical herbs

| Parameters          | T1  | T2  | T3  | T4  | T5  | SEM (±) |
|---------------------|-----|-----|-----|-----|-----|---------|
| Ave. initial weight(kg) | 9.32| 8.88| 9.00| 9.00| 9.00|         |
| Ave. final weight(kg)  | 23.84 b | 24.67 a | 22.00 c | 26.67 d | 25.78 d | 0.46    |
| Ave. total weight gain(kg) | 14.52 b | 16.79 a | 13.00 d | 12.50 cd | 11.84 d | 0.44    |
| Ave. weekly weight gain(kg) | 2.07 b  | 2.40 a | 1.86 c | 1.78 cd | 1.69 d | 0.64    |
| Feed intake(kg)       | 35.00| 35.00| 35.00| 35.00| 35.00| 0.01    |
| Feed conversion ratio | 2.41 c | 2.06 d | 2.69 b | 2.80 b | 2.96 a | 0.68    |
| Protein efficiency ratio | 2.18 b | 2.52 a | 1.95 c | 1.88 ed | 1.78 d | 0.08    |
| Mortality (%)         | -   | -   | -   | -   | -   |         |

a, b, c, d, e means along the same row with different superscripts are significantly (p< 0.05) different from each other, Ave: Average, SEM: Standard error of mean.

Initial live weight of weaner rabbits ranged from 8.88kg to 9.32kg, average final weight gain of the weaner rabbits ranged from 22.00 to 26.67 as shown in table 2. The result revealed that, experimental animals were significantly (P<0.05) affected by the experimental diets. Animal fed 40% inclusion of Moringa oleifera leaf meal (T4) and Gongronema latifolium leaf (T5) gave the highest final weight (26.67kg and 25.78kg) respectively, followed by control group (T10 (23.84kg), and while values obtained from T2= 40% of Elueciala Indica, T3= 40% of tropical Kudzu meal had the weight gain of 24.67kg and 22.00kg respectively. However, similar significant (P<0.05) differences were recorded in average total weight gain and weekly weight gain of rabbits fed with the various experimental diets that ranged from T1 (2.40) to T5 (1.69kg) respectively. Feed intake values were not significantly affected at the different phase; results were the same across the groups.

Feed conversion ratio differed significantly (P<0.05) in the experimental animals, while animals on T4 (2.06) diet gave the best compared to other diets with corresponding values of T1 (2.41), T5 (2.69), T2 (2.80) and T3 (2.96) respectively. Protein efficiency ratio of weaner rabbits was significantly (P<0.05) influenced by the experimental diets, highest value was recorded in T4 (2.52) diet, followed by control (2.18), T5 (1.95), T2 (1.88) and T3 (1.78) in that order. There was no mortality throughout this phase of feeding trial.
The results for haematology and serum analysis are presented in tables 3. No significant differences (P>0.05) were found for haemoglobin content, packed cell volume and red blood counts while there were significant differences in (P<0.05) among treatments in mean corpuscular volume (MCV), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), platelet count, total white blood counts (WBC) and differential white cell counts.

Table 5 above describes the hypoglycemic effect of supplemented doses of 40% of Elueciala Indica, 40% of tropical Kudzu meal, 40% of Moringa oleifera Leaf Meal and Gongronema latifolia Leaf Meal on fasting blood glucose level of diabetic rabbits, 40% of Elueciala Indica and 40% of tropical Kudzu meal showed significant increase in blood glucose only at 1h, 2h and 4h respectively (P>0.05). However, treatment groups with 40% of Moringa oleifera Leaf Meal and Gongronema latifolia Leaf Meal showed significant decrease in blood glucose only at 1h, 2h (P<0.001) and 4h (P<0.05). Maximum reduction in blood glucose level was 14.01 % at 2h as compared to control. This means that Moringa oleifera and Gongronema latifolium has anti-hypoglycemic effects on diabetic rabbits in that it causes glucose reduction in the blood upon administered while Elueciala Indica and tropical Kudzu meal are not.

Table 6: Combined effect of aqueous leaves extract of Moringa oleifera and Gongronema latifolium (Utazi) on the defense system of diabetic rabbits

| Treatment | Blood glucose level (mg/dl) |
|-----------|---------------------------|
| 0h | 1h | 2h | 4h |
| 100% of concentrates | 129.60±1.02 | 128.40±1.28 | 128.60±1.03 | 128.0±0.89 |
| 40% of Elueciala Indica | 126.40±2.13 | 128.80±2.17 | 130.0±3.13 | 121.20±2.87 |
| 40% of tropical Kudzu | 128.40±5.08 | 138.0±5.17 | 138.0±3.17 | 135.60±2.92 |
| 40% of Moringa oleifera Leaf Meal | 129.60±1.02 | 127.40±1.28** | 112.40±0.87** | 110.0±1.58* |
| 40% of Gongronema latifolia Leaf Meal. | 128.40±5.08 | 125.0±5.17** | 108.0±1.37** | 115.60±2.92* |

Values are mean±SEM, n=5, * P<0.05, ** P<0.001.
Table 6 above describes the hypoglycemic effect of supplemented doses of 40% of *Moringa oleifera* Leaf Meal and *Gongronema latifolium* Leaf Meal on fasting blood glucose level of diabetic rabbits. The combined meal showed significant decrease in blood glucose only at 1h, 2h (P<0.001) and 4h (P<0.05). The reduction in was very clear between the combined leaf meal and the *Moringa oleifera* as well as *Gongronema latifolium* administered differently to the diabetic rabbits. In 1hour the reduction of glucose caused by the combined effect of the leaf meal was 108.40 as compared to 127.40 in *Moringa oleifera* alone and 125.00 in *Gongronema latifolium* alone. This trend continue till the fourth hours. This means that when *Moringa oleifera* and *Gongronema latifolium* are combined together it produces more anti-hypoglycemic effects on diabetic rabbits than when both leaf meal are administered differently.

IV. DISCUSSION

The finding of the study reveals that treatment of diabetic rabbits with MO showed a significant decreased glucose level when compared to diabetic control and other leaf meals. This implies that *Moringa oleifera* is able to increase the ability of insulin to lower plasma glucose, suggesting its anti-diabetic activity. These results are consistent with other studies Navarro-González and Mora-Fernández (2008). Increased kidney size is a sign of acute inflammation and was observed in diabetic rabbits when compared to normal controls (Table 1). This study agrees with the findings of previous authors who reported that kidney enlargement may be due to hyperplasia (rapid production of the cell leading to enlarged tissues) and hypertrophy (enlargement of cell components) of the kidney Rodríguez-Pérez, Quirantes-Piné, Fernández-Gutiérrez and Segura-Carretero (2015). Treatment with MO reduced kidney size gained, showing a hypolipidemic effect of MO in the kidneys of diabetic rats.

Also, the findings of the study revealed that treatment of rats with GL showed a significant decreased glucose level when compared to diabetic control. This implies that *Gongronema latifolium* is able to increase the ability of insulin to lower plasma glucose, suggesting its anti-diabetic activity. These results are consistent with other studies Mora-Fernández (2014). Increased kidney size is a sign of acute inflammation and was observed in diabetic rats when compared to normal controls.

The findings of the study was consistent with the study Rai and Watal (2007), Singh, Prakash, Dhakarey, Upadhyay and Singh, (2009), Gupta, Dubey, Kannan & Flora, (2007), Hussain, Malik & Mahmood, 2014; Stohs & Hartman, (2015), Soliman, (2013) etc, who also found that aqueous extract of moringa has reducing effects in the amount of glucose present in the diabetic rabbits. Hypoglycemic and anti-hyperglycemic activity of the leaves of *Moringa oleifera* may be probably due to the presence of terpenoids, which appears to be involved in the stimulation of the β-cells and the subsequent secretion of preformed insulin. One or more of the other chemical constituents of the plant especially flavonoid is also likely to have played a crucial role in the hypoglycemic action of the plant extract.

Furthermore, the result of the study showed that aqueous extract of *Gongronema latifolium* (Utazi) leaves have glucose lowering effect in studies on normoglycemic and hyperglycemic rats. Doses of 100, 200 and 300 mg/kg of the extract showed significant decrease in the blood glucose levels at 2 h both in normal (p<0.001) and diabetic rats (p<0.001), but maximum reduction was seen with 200mg/kg. At 1h, 2h and 4h, 300 mg/kg of the extract showed significant (P<0.05) decrease in the blood glucose levels. Maximum reduction in blood glucose level was 14.01 % at 2h with 200mg/kg, whereas it was 10.03 and 10.50% with 100 and 300mg/kg respectively as compared to control. Such a phenomenon of less hypoglycemic response at higher doses is common with indigenous plants thereby confirming the first hypothesis which stated that *Gongronema latifolium* (Utazi) will have a positive effects on the defense system of diabetic rabbits.

The findings of the study was consistent with the study Ogunidpe, Moody, Akinyemi and Rama (2003), Nwanjo et al, (2006), Nwinyi, Chinedu and Ajani (2008) etc, who reported that the leaves of *Gongronema latifolium* has a hypoglycemic effect by decreasing activity of glucok kinase enzyme and levels of hepatic glycogen.

Finding of the study showed significant differences in the mean final body weights (P<0.05) of the rabbits among treatments with rabbits on 40% of *Moringa oleifera* Leaf Meal and 40% of *Gongronema latifolium* Leaf Meal diets having higher values than others. Feed consumption is always affected by level of crude fibre of the diet. The higher the crude fibre of the feed, the lower the feed consumed (Arthur, 1975). However, studies on the influence of fibre in the diets, on the growth rate and digestibility of nutrients in rabbits have indicated that animals fed on high fibre diet had reduced digestibility of dry matter, protein and fat, and exhibited significantly lower growth rates than animals fed on low fibre diet (Zyl Van et al., 1999). An increase in fibre levels in the diets of rabbits has been found to be associated with a decrease in the digestibility of DM, protein and fat in rabbits leading to reduction in growth rate (Zyl Van et al., 1999). This could be the reason for the lowest feed intake on T2 GLLM that has the highest crude fibre level in this study. The unpalatability of GLLM may be due to the presence of bitter substances in the leaves of *G. latifolium*, this must have caused the reduction on the quantity of feed consumed by the rabbits and hence their weight. Tannin extracts from the leaves of *G. latifolium* have also been shown to be strong inhibitors of oxidative enzymes present in feedstuffs (Kubicka and Troszunski, 2003). These could also be the cause of the lowest growth and feed intake in T2 (GLLM). In a similar
study GLSD (*G. latifolium* supplemented diets) decreased the weight of rats that consumed it due to its unpalatability (Iweala and Obidioa, 2009). It was observed that the lowest feed intake was recorded in rabbits fed on GLLM and the highest feed intake was recorded in rabbits fed on MOLM, followed by the rabbits fed on OGLM diets (P<0.05). Feeds that have been pelleted also increase feed intake by most farm animals and the palatability, taste, the amount of vitamins and phytochemicals in the leaf may also influence performance. The highest weights were recorded for rabbits fed on MOLM (1.52±0.81kg) followed by that of CONTROL (1.35±0.32kg) and finally GLLM (1.21±0.42kg). This work agrees with the works of Annor *et al.*, (2008), and slightly differs from the works of Karikari *et al.*, (2009), carried out in Nigeria and Ghana, respectively. Animals on treatment T1 had a higher average total feed intake and had a high average total weight gain similar to the animals on treatments 3 as opposed to treatments 2 and 4. This finding on average daily weight gain can compare favourably with the findings of (Oppong *et al.*, 2008). The reason for the higher weight gain of growing rabbits on the MOLM diet could be because of the higher feed intake response on that diet. Thus, animals on treatment 2 that exhibited a lower average total feed intake had a lower average total weight gain and it could be justified that performance of animals depends mostly on the nutritional value of the feed, palatability, taste and not on the quantity of the feed consumed. The mean final weights of animals fed on T1 were higher than those on T2 and T3. However, there was significant difference in the total weight gain (P<0.05). Similarly, the mean daily live weight gain of animals fed on MOLM was higher than those fed on GLLM (P<0.05). The average daily weight gain of growing rabbits 7.20 to 10.23g obtained in this experiment was comparable to 8 to 13g (Jori *et al.*, 2001) and 7 to 12g (Mensah, 1995) reported in other studies. The differences in growth response of rabbits among treatments suggest that growing g rabbits may be relatively sensitive to different types of dietary protein supplements. What may be observed as the sensitivity of the growing rabbits to dietary concentration in this experiment may probably be explained by the ability of rabbits to adjust their feed intake to meet their nutrient requirement. Further explanation may be based on the role of microbes of the caecum and large intestine in fermentation, which convert fiber and simple nitrogen compounds to volatile fatty acids and microbial cells, which can be utilized by the rabbits as substrates for protein synthesis (Danfaer *et al.*, 1995), which is necessary for growth. The practice of coprophagy established in rabbits, which may be another source of protein, may help adjust the protein requirement of rabbits. Feed conversion ratio was higher on control diet followed by treatment 2 (GLLM) and lowest on treatment 3 closely followed by treatment 1 (MOLM). The values obtained in this study for feed conversion ratio were comparable with the findings of Oppong *et al.*, (2008). Results obtained in this study in feed and dry matter intake demonstrate that MOLM is palatable and highly preferred by rabbits. These findings were inconsistent with those reported in other leaf meals by (Vohra 1972; Ravindran *et al.*, 1986; Osieet *et al.*, 1990 and Bhatnagar *et al.* 1996) who observed a depression in intake when laying chickens were fed diets containing various levels of *Leucaena leucocephala* (LLM.). These variations probably suggest lower anti-nutritional factors and toxic materials in MOLM (Makker and Backer 1997) than in other leaf meals. In areas where MOLM can be obtained for free and quality of animals fetch higher premium complete substitution (20%) with MOLM is highly recommended (Kakengiet *et al.*, 2007). Feed cost/kg weight gain were significantly (P<0.05) different among treatments with treatment 1 which were fed on MOLM having the lowest cost of feed per kg weight gain of 40.34 compared to other groups fed on (GLLM) 46.89, (OGLM) 43.75 and the (control) 51.17 respectively. These results fall within the range of results reported by Wogaret *et al.*, (2007). These authors obtained feed cost/kg gain ranging from 48.49 to 75.64 in grass cutters fed on various combinations of elephant grass and concentrate diets. There were no mortalities recorded during the application of the experimental diets.

The packed cell volume in the blood of rabbits at the end of the experiment for the control group was 55.5% on the average and 53.33% (OGLM), 54.00% MOLM on the average for the experimental groups. This shows that the packed cell volume (PCV) was not significantly (P<0.05) affected by treatment. These packed cell volume values were significantly higher than those values (20.3% to 30.4%) reported by Ogunsami *et al.* (2002) in captive rabbits. Probably the rabbits were able to maintain constancy in the amount of PCV in the blood despite the presence of antinutritional and other chemicals in the experimental diets containing the leaf meals. The red blood cell counts in this study were 9.6ul for the control group and mean values of 7.70ul, 11.06ul, 9.45ul for the experimental groups. The values indicate an increase RBC counts on treatment 2 (GLLM) compared to the control group. Treatment 1 (MOLM) had similar values with the control group. The increased RBC values obtained in treatment 2 were comparable with the findings of Ogunsami *et al.* (2002), these authors reported RBC values of (12.36ul ± 0.52) in captive rabbits. The increased condition is known as polycythemia or erythrocytosis. High red cells can increase the delivery of oxygen to the tissues, and the animal experience full calories of energy (Clarke and Myra, 1975). However, the increased number of cells in blood causes the blood to become thickened and sticky. This may put a huge strain on the heart, and can cause heart attacks and heart failure. Clarke and Myra (1975) reported that red blood cell count is an indication of feed quality. The hemoglobin values in the blood of the rabbits s at the end of the experiment were (18.09 g/dl) on the average for the control group and (16.22, and 17.65 g/dl)
on the average for the experimental groups. This shows a reduction in the hemoglobin level, and this is in consonance with the finding of (Ephraim et al., 2000) in which the hemoglobin value decreases significantly after administration of aqueous extract of *moringa* to rabbits. The hemoglobin values were higher than the reports Opera et al., (2006) in rabbits (14.17 ± 0.52g/dl) and the reports of Ogunsami et al., (2002) in captive rabbits (12.36 ± 1.65g/dl). Thus haemoglobin (Hb) concentration in this study fell within the normal range of (12 – 18g/dl) normally contained in the blood of rabbits. Haemoglobin has the unique property of combining reversibly with oxygen and is the medium by which oxygen is transported within the body. It takes up oxygen as blood passes through the lungs and releases it as blood passes through the tissues. The observed difference in control group and T2 (GLLM) group suggests that the oxygen carrying capacity of the blood was high in rabbits on control diet, suggesting that GLLM leaf meal caused a decline in the oxygen carrying capacity.

The white blood cell count gave a mean value of 6.75uL for rabbits in the control group at the end of the experiment and mean values of 8.53uL and 11.77uL respectively for rabbits on MOLM and GLLM diets. A significant increase in the WBC count was observed in the experiment group as compared to the control. Ephraim et al. (2008) reported a decrease in WBC count by their work on captive grass cutters. The presence of high levels of vitamin A, vitamin C, vitamin E and phytates that are known to have antioxidant properties and useful in maintaining good health, may have been responsible for the increase in WBC values reported in the experimental groups. According to Iweala and Obidoa (2009), phytochemicals and flavonoids in *G. latifolia* leaves possibly interferes with the process of WBC synthesis resulting to increased presence of WBC in the blood. In addition, Duthie et al. (1996) reported that antioxidant phytochemicals play a protective role on the lymphocytes and also decrease their destruction in the blood. The presence of high levels of vitamin A (40.82 mg/100 g), vitamin C (15 mg/100 g), vitamin E (tocopherol) (3.71 mg/100 g), ß-carotene (6.80 mg/100 g) and phytate (6.5 mg/100 g) that have been indicated to have antioxidative properties (Traber and Atkinson, 2007) and useful in maintaining good health, may have been suggested as possible causes of increased WBC in the blood. Leukocyte counts have been reported to increase due to any form of stress, exercise, feeding, age, breed and wide variety of other conditions (Dellmann and Brown, 1987). The mean corpuscular haemoglobin concentration MCHC, MCH, MCV had no significant differences between the control group and the experimental groups suggesting that the amount and quantity of leaf meals (20%) added to the diets did not have any detrimental effect on the internal physiological milieu of the rabbits. Thus the rabbits on some of the experimental diets were able to perform better in some parameters compared with the control. In the present study, the lymphocyte counts of the reared young rabbits were higher than neutrophil counts. The mean values of neutrophil (NEU), monocyte (MON), Eosinophil (EOS) and Basophils (BAS) in the blood of the rabbits were not significantly different (P>0.05) between the control group and the experimental groups suggesting that the amount and quantity of the leaf meal (20%) added to the diets did not have any effect on the physiology of the rabbits. The mean value of urea was 5.24mg/dl for the control group and mean value of (11.77, 12.87, 11.95mg/dl) in experimental groups 1, 2 and 3 respectively. These values are lower than the findings of Ogunsanmi et al., (2002), and Opara et al. (2006) for wild rabbits. Opara reported mean urea values of 21.87mg/dl on wild rabbits and Ogunsanmi had mean urea values of 27.00mg/dl in their work on captive g rabbits. The cause of the higher blood urea in the captive rabbits fed on the experimental diet may be due to increased production of urea in the liver, or to increased protein breakdown or decreased blood flow through the kidney. Urea is a function of protein quality and high urea level depicts low protein quality fed. In the present study, lower concentration of urea in the rabbits served the experimental diets indicated that the quantity of the leaf meals given to the rabbits were not harmful to the rabbits, concentrations of tannin, phytate and hydrocyanic acid were appreciable in the experimental diets fed to the rabbits. Liver is the predominant source of urea production in the body. Urea is a waste product produced during protein synthesis in the liver; the kidney plays a major role in the filtrating process to help remove the waste found in the body, which primarily leaves through the urine. Therefore too much protein can put extra strain on these organs (Rao et al., 2007). If blood urea nitrogen is high, it often means eating too much protein. Factors that could be responsible for BUN increment are, increased catabolism, increased production of urea in the liver due to high protein diet, increased protein breakdown, decreased elimination of urea due to decreased blood flow through the kidney (Atkinson and Bourke, 1987). These authors also suggested that hepatic urea synthesis, which consumes HCO3, plays an important role in acid-base homeostasis. The results obtained in this work for creatinine, albumin, serum globulin were not significantly different among treatments. The values for these parameters were similar to the findings of Oyewale, (1997) for reared rabbits and Opara et al. (2006) for rabbits. Variations in the haematological indices of animals may occur due to genotype differences, age, physiological condition and nutrition (Machebe et al., 2009).

Finally, the findings of the study also revealed that when *Moringa oleifera* and *Gongronema latifolium* are combined together and fed to the diabetic rabbits, the anti-hyperglycemic effect was higher than that of the extracts of *Moringa oleifera* and that of *Gongronema latifolium*. This means that diabetic patients taking both *M. oleifera* leaves and *G. latifolium* leaves simultaneously are likely going to have better glycemic
control than those taking either extracts of *M. oleifera* leaves or *G. latifolium* leaves alone. The combination protocol did not produce overt hypoglycemia showing that there is less risk of dangerous herbs-interaction. The reason for the outcome of the result is that the phytochemicals of both herbs and the biochemical effects are more higher when taken simultaneously than when taken differently and such explains the higher anti-glycemic effects on diabetic rabbits.

V. CONCLUSION

Diabetes mellitus is becoming a leading cause of death globally in both rural and urban areas. MO and *Gongronema latifolium* has been used in traditional medicine to treat diabetes and various diseases. Its pharmacological properties have drawn the interest of researchers to this plant. It can be used as an anti-diabetic, cholesterol lowering, anti-inflammatory, analgesic, hepatoprotective, anti-oxidant, anticancer, antiviral and wound healing agent. MO and *Gongronema latifolium* has shown beneficial effects in various pathological conditions in experimental animal models by acting as an anti-oxidative and anti-inflammatory agent through different mechanisms. The flowers, leaves, bark, and seeds of this plant are shown to possess active compounds that can help combat the issue of malnutrition, and to prevent and treat many disease conditions and promote good health. In view of the evidences of the potential effects of MO and *Gongronema latifolium* as revealed in previous studies, there is still the need for further studies to be done on the standardization of the extracts, and isolation of various active compounds present in the plant and their possible mode of action. Therefore, the study recommends the following:

1. Pharmacists in general should make a vaccine containing high doses of *Moringa oleifera* and *Gongronema latifolium* which will be used mainly for the treatment of diabetics mellitus in humans.

2. The general public should inculcates the habits if ingesting *Moringa oleifera* and *Gongronema latifolium* either as food supplements or as extract to reduce their and check their sugar levels.

Agriculturists should make supplements from *Moringa oleifera* and *Gongronema latifolium* and give to their animals as it will aids in weight gain and performance growth as revealed by the study.

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