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Hematological changes and antidiabetic activities of *Colocasia esculenta* (L.schatt) stem tuber aqueous extract in alloxan induced diabetic rats

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Abstract

Management of blood glucose level is the hallmark in the treatment of diabetes. Much work has not been done on the management of diabetes using the stem tuber extract of *Colocasia esculenta*. The objective of this study was to evaluate the antihyperglycemic and hematological parameter on *Colocasia esculenta* aqueous stem extract in alloxan induced diabetic rats. Sixty (60) male rats were used in the study. Seven days of acclimatization, the rats were divided randomly into six groups of five in each group. Group 1: Served as normal control, Group 2: Diabetic control group (negative control), Group 3: Diabetic group and "Glucinorm-M80" (positive control), Group 4: Diabetic group and extract at 200 mg/kg bodyweight, Group 5: Diabetic group and extract at 400 mg/kg, Group 6: Diabetic group and extract at 600 mg/kg. Diabetes was induced in albino rats by intraperitoneal injection of alloxan at a single dose of 120 mg/kg body weight in groups 2 to 6 after starving them for 24 hrs. The animals were given feed and water ad libitum. The albino rats were administered for twenty eight days with the aqueous *Colocasia esculenta* stem tuber, after which they were fasted overnight, anaesthetized with chloroform and sacrificed. The result showed that there was a significant increase ($p < 0.05$) in mean body weight of the positive control and the treatment groups (200 mg/kg to 600 mg/kg) when compared with the negative control which has a significant decrease ($p < 0.05$) in mean body weight. The result showed that there was a substantial decrease in blood glucose of animals administered with different doses of aqueous extract of *Colocasia esculenta* stem tuber and the positive control group. The result showed that a significant difference ($P < 0.05$) in the concentrations of RBC, PCV, HB, PLT and MCH of the treatment groups and negative group when compared with the normal control group. Also there was a significant difference in glycosylate hemoglobin of the treatment groups when compared with the control group and negative group. This study has demonstrated that aqueous stem tuber extract of *Colocasia esculenta* has a significant increase on body weight which may have a role of improving the states of possible weight loss following complicated diabetes. Also, aqueous stem tuber extract of *Colocasia esculenta* has an ameliorative effect on sugar level and some hematological parameters of alloxan induced diabetic rats showing effective diabetic control and management of diabetes.

Key words: *Colocasia esculenta*, Hematological, Antihyperglycemic, Diabetes, Alloxan

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Introduction

Without a symbiotic relationship existing between the use of plant and its product, the survival of man would be tampered with. The uses of plants are for present day adaptation and for future survival. World Health Organization (WHO) defines medicinal plants as any plant which in one or more way of its organs contains substances that can be used for the therapeutic purposes or which are precursors for the synthesis of useful drugs.

Medicinal plants are of great demand and there is rapid progress in acceptance. Plants play important functions in the services of the ecosystem. In absence of plants, human and other living organism cannot live and survive in same planet. However, medicinal herbs have acted as overall indicator of ecosystem health herbal products contain compounds which are synthesized through the pentose phosphate pathways, shikimic acid pathway and phenylpropanoid pathway. These metabolites play significant role in defense against pathogenic bacteria and oxidative stress in plant [1]. The different parts of the plants can be used such as seeds, root, leaf, fruit, skin, flowers or even the whole plant. These plants have active compounds which have physiological effects on living organisms. The use of the whole plant or raw materials for treatment or experiments has, many setbacks, such as changes in the plants compound in different climates, simultaneous development of synergistic compound that lead to adverse effects of antagonist or other unexpected changes in bioactivity of the plant; and changes or loss of bioactivity due to the accumulation and variability, storage and preparation of raw materials. *Colocasia esculenta* is a tropical plant grown primarily for its edible corms, roots and vegetables. It is commonly known as cocoyam and taro. The south eastern part of Nigeria precisely the Igbo's call it "ede". Cocoyam is an erect herbaceous perennial root crop widely cultivated in tropical and subtropical world, which belongs to the genus *Colocasia* in plant family called Araceae [2,3]. Cocoyam tubers are important sources of carbohydrate which is an energy source to the body. It is a good source of starch (70 to 80g/100g dry cocoyam), fibre (0.8%) and ash (1.2%). The corm of cocoyam has a relative low protein of (1.5%) and fat (0.2%). It is a good source of vitamin B6, B3,C, potassium, copper and manganese [4]. In ethnomedicine, cocoyam is used in the management of diabetes mellitus, treatment of ringworms [5,6], cough, sore throat and wounds [7]. Research has shown the hypoglycemic activity of the roots and leaves in diabetic induced rats. However, cocoyam has scientifically been documented as an antidiabetic plant, its biochemical basis and antidiabetic action has not been fully understudied [1].

Materials and Methods

Collection and identification of sample

The stem tubers of *Colocasia esculenta* locally known in the eastern parts of Nigeria as Edeofe were uprooted and collected from Uturu in Isiukwuato Local government area, Imo state Nigeria and authenticated by a plant taxonomist in Abia State University, Uturu. The Voucher specimen ABSU/FB/309 was deposited at the institution Herbarium.

Preparation of Aqueous extract of *Colocasia esculenta* stem tubers

The stem tubers of *Colocasia esculenta* were peeled with a clean knife and washed before sun drying for seven days. The dried tubers were ground using an electric blender into a powdered form. One hundred grams (100 g) of the sample was weighed out in a beaker and dissolved with 1000ml of distilled water and allowed to stand for 3 hours before heating at 100°C. Three empty beakers of 250 ml each were weighed, labeled and recorded. The sample was filtered using a clean Whatman filter paper and a funnel into the measured beakers. The beakers with the filtrates were evaporated to dryness using the water bath at 88°C to 92°C.

Animal modelling experiment

The albino rats used for this practical work were purchased at the Abia State University Uturu. A total of sixty (60) male rats weighed 133-180 g were purchased. The rats were housed using a metallic cage and placed on commercial growers feed purchased from Eke Okigwe market, Imo State as produced by Nigeria Flour Mills. The rats were allowed to acclimatize for 7 days before the commencement of the experiment. Ethical principles were strictly adhered to while handling the animals and at the end of acclimatization, the animals were grouped into six groups each containing ten (10) rats and were administered orally with different doses of the extracts according to their body weight.

Experimental Design

The animals were grouped into six groups as follows: Group 1: Served as normal control. no diabetes was induced, Group 2: Diabetic control group (negative control), Group 3: Diabetic group given oral hypoglycemic drug "Glucinorm-M80" (positive control), Group 4: Diabetic group administered orally with 200 mg/kg of aqueous extract of *Colocasia esculenta* stem tuber, Group 5: Diabetic group administered orally with 400 mg/kg aqueous extract of *Colocasia esculenta* stem tuber, Group 6: Diabetic group administered orally with 600 mg/kg of aqueous extract of *Colocasia esculenta* stem tuber.

Induction of diabetes

After 7 days of acclimatization, animals were allowed to fast for 24 hours prior to experimentation and rendered diabetic by a single dose of intraperitoneal injection of alloxan 120 mg/kg body weight. After 18 hours of injection of alloxan, diabetes was confirmed by testing blood sugar level more than 200 mg/dl were selected for the further study. Animals were maintained for four days in diabetic condition for well establishment of diabetes. The animals were divided into six groups. The animals were grouped into six groups as follows: Group 1: Served as normal control (no diabetes was induced), Group 2: Diabetic control group (negative control), Group 3: Diabetic group given oral hypoglycemic drug "Glucinorm-M80" (positive control), Group 4: Diabetic group administered orally with 200 mg/kg of aqueous extract of *Colocasia esculenta* stem tuber, Group 5: Diabetic group administered orally with 400 mg/kg aqueous extract of *Colocasia esculenta* stem tuber, Group 6: Diabetic group administered orally with 600 mg/kg of aqueous extract of *Colocasia esculenta* stem tuber. The extract (aqueous) was given orally.

Blood samples from the experimental rats were collected by the tail using pricking lancet. The collected blood samples were analyzed for blood glucose levels by the glucometer using strip technique and blood glucose levels were expressed in mg/dl. The data was represented as mean blood glucose level and standard error of mean (SEM). During the study period of 28 days the rats were weighed daily and their body weights were recorded. From this data, mean change in body weight and SEM were calculated and tabulated.

Blood collection

Twenty eight (28th) days after administering the albino rats with the aqueous *Colocasia esculenta* stem tuber, they were fasted overnight, anaesthetized with chloroform and sacrificed. Blood was collected by cardiac puncture using syringe and needle and blood samples from each animal collected into dry sample bottles for Clinical chemistry analysis. The sample bottle with the whole blood was allowed to stand for 15 minutes to clot and further spun at 12,000 rpm for 5 minutes using the centrifuge. The serum was separated from the clotted blood with Pasteur pipette into sterile sample test tubes for the measurement of biochemical parameters.

Blood glucose determination

Blood glucose was determined on day 0, 7 and 14, 21 and 28 blood samples from the experimental animal were collected by the tail using pricking lancet. The collected blood samples were analyzed for blood glucose levels by the glucometer using strip technique and blood glucose levels were expressed in mg/dl. The data was represented as mean blood glucose level and standard error of mean + SD (standard deviation).

Statistical Analysis

Results were expressed as mean + SD (standard deviation). Statistical analysis was performed by one –way analysis of variance (ANOVA) with the RTM statistic software package, version 3.03. The normal distribution of the data and the homogeneity

of variance were tested by Barlett homogeneity test. One way ANOVA with a Turkey test post-hoc was used to identify statistical differences among groups. A p-value of < 0.05 was considered statistically significant.

Results

Figure 1 result shows that there was a significant increase in mean body weight of the positive control and the treatment groups (200 ml/kg to 600 ml/kg) when compared with the negative control which has a significant decrease in mean body weight. Also, 600 ml/kg of stem tuber extract of *Colocasia esculenta* has the highest weight gain of 27.09 %. However, the negative control has a %WL of 12.04 % when compared with the normal control, positive control and aqueous stem tuber extract of *Colocasia esculenta* 200 ml/kg, 400 ml/kg and 600 ml/kg concentrations.

Figure 2 result shows that there was a substantial decrease in blood glucose of animals fed with different concentration of aqueous extract of *Colocasia esculenta* stem tuber and the positive control group. However there was a significant increase ($P < 0.05$) in blood glucose of diabetic animals in negative group when compared with the control group.

Figure 3 results of Glycosylated Haemoglobin shows elevated values in group 2 (diabetic control) in day 7 to 28. The levels of HbA1c in day 7 show significant increase ($p < 0.05$) also day 14 shows significant increase ($p < 0.05$) while Day 21 and 28 show significant increase ($p < 0.05$) in negative control and no significant increase into the treatment groups.

Figure 4 result shows that there was a significant difference ($P < 0.05$) in the concentration of RBC, PCV, HB, PLT and MCH of the treatment groups and negative group when compared with the normal control group. Also there was a significant difference in MCHC of the treatment groups when compared with the control group and negative group. However there is no significant difference ($P < 0.05$) in the WBC when compared with the control group.

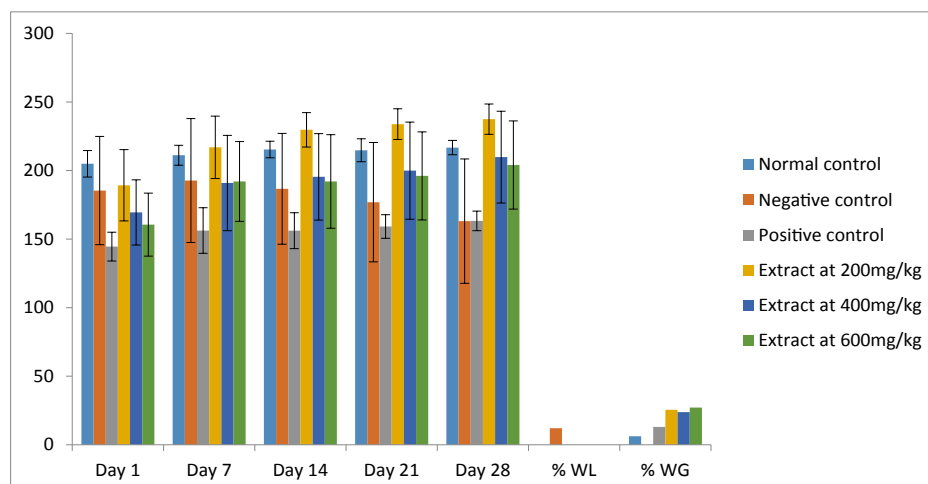


Figure 1 Effects of *Colocasia esculenta* aqueous stem tuber extract on mean body weight (g) days. (values are mean ± standard deviation (sd) n=10, %wl= percentage weight loss; %wg= percentage weight gain).

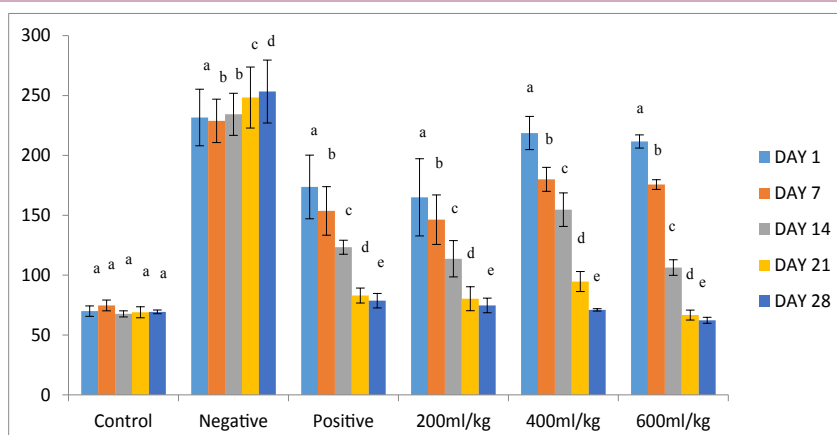


Figure 2 Effects of colocasiasculenta aqueous stem tuber extract on blood sugar level (mg/dl) days. values represent the mean \pm sd for n=10. values marked with the same alphabet (a) are not significantly different from normal control ($p < 0.05$).

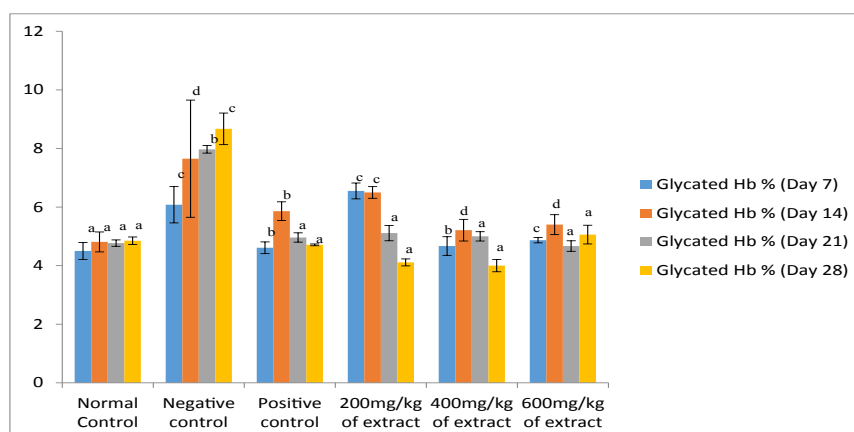


Figure 3 Effects of colocasiasculenta stem tuber aqueous extracts on glycosylated haemoglobin (hba1c) (%) day 7,14, 21 and 28. (normal 4% -5.6%) Values are mean \pm SD, (n=10). Values in the same column bearing the same letter of the alphabets are not significantly different ($P > 0.05$) from each other.

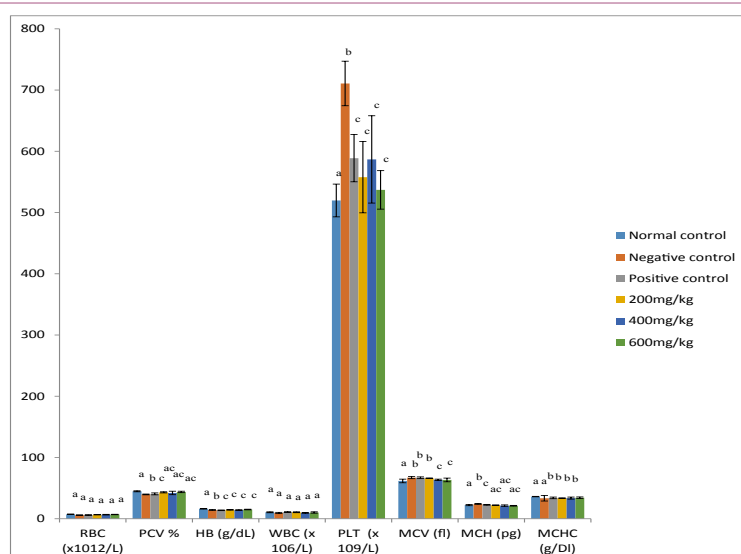


Figure 4 Effects of colocasiasculenta aqueous stem tuber extract on haematological parameters. Values represent the mean \pm SD for N=10. Values in the chart bearing the same letter of the alphabet are not significantly different from each other ($P < 0.05$).
MCV = Mean corpuscular volume; MCH = Mean hemoglobin concentration; MCHC = Mean corpuscular hemoglobin concentration; WBC = White blood cell; RBC = Red blood cell; HB = Hemoglobin, PCV= packed cell volume, PLT = platelet count test.

Discussion

Although a large number of synthetic hypoglycemic agents are available with several side effects associated with them, they have limited their clinical utilization and however the search for novel pharmacotherapy from medicinal plant to manage diabetes has gained considerable importance. The present study was evaluated to explain the effects of aqueous stem tuber extract of *Colocasia esculenta* on blood glucose level and on some hematological parameters.

From the study there was a percentage body weight gain in the control group, positive group, 200 ml/kg, 400ml/kg and 600ml/kg group. The negative diabetic group has a percentage weight loss due to its diabetic condition. The percentage weight loss by the diabetic untreated group can be explained on the basis of increased muscle wasting and loss of structural proteins that contribute to body weight while the percentage weight gain in the diabetic treated group with aqueous stem tuber extract of *Colocasia esculenta* suggests the protective action of cocoyam tuber against muscle wasting [8].

There was a substantial decrease in blood glucose level in the treatment group with aqueous stem tuber extract of *Colocasia esculenta* when compared with the synthetic drug glycinorm. Glycinorm has been used in the treatment of diabetes which carries out its activity by stimulating insulin secretion from the pancreatic beta cells. Therefore results from aqueous stem tuber extract of *Colocasia esculenta* decreased blood sugar level probably by increasing the pancreatic secretion of insulin from beta cells of islets of Langerhans. The treatment of diabetic rats with aqueous stem tuber extract of *Colocasia esculenta* led to decrease in blood glucose level indicating ameliorative effects of aqueous stem tuber extract of *Colocasia esculenta* on hyperglycemia.

Hematological Parameters are extensively used tools that help in monitoring animal health, reproductive status, and disease status and in differentiation of physiological processes [9]. From the results obtained after completion of the experiment, some parameters were observed to significantly increase or decrease and this has a relationship with the health of the animal. The red blood cells (erythrocytes) serve as a carrier of hemoglobin. Hemoglobin reacts with oxygen transported in the blood to form oxyhemoglobin during respiration [10].

From the results, it was observed that the alloxan induced diabetic untreated group (negative group) has a decrease in values of RBC, PCV and HB but as they were treated with aqueous stem tuber extract of *Colocasia esculenta* at different concentration, the values increased significantly as the dose increased. This increase suggests that some phytoconstituent present in the stem tuber extract of *Colocasia esculenta* can stimulate the formation or secretion of erythropoietin which stimulates the stem cells in the bone marrow to produce RBC [11] which is supported by the increase in MCH and MCHC [12]. However treatment of diabetic rats with aqueous stem tuber extract of *Colocasia esculenta* led to increase in RBC indicating ameliorative effects of aqueous stem tuber extract of *Colocasia esculenta* on anemia. The Packed Cell Volume (PCV) also known as hematocrit (Ht or Hct) or

erythrocyte volume fraction (EVF), is the percentage (%) of red blood cells in blood [13,14] the aqueous stem tuber extract of *Colocasia esculenta* showed an effect in the blood as there was a dose dependent increase in RBC, PCV and HB as opposed to the effect on alloxan induced diabetic rats.

Previous reports have shown that Packed Cell Volume, haemoglobin and mean corpuscular haemoglobin are vital indices for evaluating circulatory erythrocytes, and are significant in the diagnosis of anemia and also serve as useful indices of the bone marrow ability to produce red blood cells same way in mammals [15]. Also, [9] assumed that high Packed Cell Volume (PCV) reading meant either an increase in number of Red Blood Cells (RBCs) or reduction in circulating plasma volume. There was a slight decrease in the values of WBC in the negative group when compared with the treatment and control group.

The mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and mean corpuscular volume are all parts of red blood cell indices (parameters which reflect size and hemoglobin content of red blood cells) that have traditionally been used to aid in the differential diagnosis of anemia. MCH and MCHC indicate blood level conditions. A low level of MCV, MCH and MCHC is an indication of anemia however results didn't show any significant decrease in MCV, MCH and MCHC. There was an increase in MCH in the negative group when compared with the treatment groups; also there was a slight decrease in the MCHC of the negative group when compared with the treatment groups and control.

The result in this study showed significantly increased in MCH & MCHC in the negative control group, some research have been suggested that anemia occurrence in diabetic mellitus is due to increase in enzymatic glycosylation of RBC membrane proteins, which correlated with hyperglycemic. Oxidation of these glycosylated membrane proteins and hyperglycemic in diabetes mellitus cause an increase in the production of lipid peroxides causing a hemolysis of RBCs or diabetes mellitus may cause anemia as secondary disorder

However, there was a significant increase in MCV and PLT values of the negative group when compared with the control and treatment groups of synthetic drug and aqueous stem tuber extract of *Colocasia esculenta*.

Our study showed that in diabetes mellitus, platelets become more reactive and aggregable and their mean volume is increased. The increased platelet size may be one factor in the increased risk of atherosclerosis associated with diabetes mellitus and associated vascular complications in a poor managed patients.

Glycosylated hemoglobin is a form of hemoglobin that is chemically linked to a sugar. The process by which sugars attach to Hb is called glycation. HbA1c is a measure of the beta-N-1-deoxy fructosyl component of hemoglobin [16]. Glycated hemoglobin causes an increase of highly reactive free radicals inside blood cells. Radicals alter blood cell membrane properties. This leads to blood cell aggregation and increased blood viscosity, which results in impaired blood flow.

Another way glycosylated Hb causes damage is via inflammation, which results in atherosclerotic plaque (atheroma) formation.

Free-radical build-up promotes the excitation of Fe²⁺-Hb through Fe³⁺-Hb into abnormal ferryl Hb (Fe⁴⁺-Hb). Fe⁴⁺ is unstable and reacts with specific amino acids in Hb to regain its Fe³⁺ oxidation state.

Glycosylated hemoglobin is tested to monitor the long-term control of diabetes mellitus. The level of glycosylated hemoglobin is increased in the red blood cells of persons with poorly controlled diabetes mellitus.

From the result glycosylated Haemoglobin shows elevated values in group 2 (diabetic control) in day 7 to 28. This is simply because the group were not treated with extract or standard drug showing no management. The significant increase, recorded in day 14 show poor management because the level of glycosylated hemoglobin is increased in the red blood cells of persons with poorly controlled diabetes mellitus. However in day 21 and 28 the standard drug and the plant extract was able to reverse the levels of HbA_{1c} to normal showing good management of diabetes. It is therefore possible that the aqueous extract of *Colocasia esculenta* stem tuber may possess active substances which scavenges the free radicals of glucose oxidation, protein glycation and oxidative degeneration or probably an up regulation in insulin secretion.

Conclusion

This study has demonstrated that aqueous stem tuber extract of *Colocasia esculenta* has a significant increase on body weight which may have a role of improving the states of possible weight loss following complications associated with diabetes. Also, aqueous stem tuber extract of *Colocasia esculenta* has an ameliorative effect on sugar level and some hematological parameters of alloxan induced diabetic rats showing effective diabetic control and management of diabetes. Also the extract was able to reduce the glycosylated hemoglobin levels which shows good management of diabetes.

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