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Evaluation of the hematological effect of alloxan induced diabetic albino rat treated with ethanol extract of sweet potato (*Ipomoea batatas*)

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ABSTRACT

In most part of the world sweet potato (*Ipomoea batatas*) is being heavily cultivated in some of these areas, local population is putting parts of the plant to a good medical. Therefore this study investigated the hematological effect of alloxan induced diabetic albino rat treated with ethanol extract of sweet potato (*Ipomoea batatas*). Twelve albino rats were used for acute toxicity test while forty eight were divided randomly into six groups of eight in each group. The animals were starved for 48hrs before the commencement of treatment. Group A: served as normal control, Group B: served as diabetic control, Group C:was treated with a standard drug (Glucophage) at 100mg/kg body weight, Group D:was treated extract 200mg/kg body weight, Group E was diabetic group treated with extract at 400mg/kg body weight and Group F 600mg/kg body weight by oral administration respectively and water *adlibitum*. Diabetes was induced in albino rats by intraperitoneal injection of alloxan at a single dose of 120mg/kg body weight in group B, C, D,E and F. Group D, E,F were fed with ethanol extract of ipomoea batatas (except groupA, B,&C) for a period of 28days serum biochemical parameters were analyzed. The result revealed that alloxan-induced diabetic untreated (group B) rats showed some abnormalities in the hematological parameters (packed cell volume, haemoglobin and red blood cells) when compared to normal control and treated (group C–F) rats. In conclusion, the heamatological parameters were improved in diabetics' rats group, especially group E which were treated with 400 mg/kg body weight of the extract.

Keywords: Hematology, alloxan, diabetic rats, sweet potato and *Ipomoea batatas.*

INTRODUCTION

Diabetes mellitus (DM) is a critical general medical condition, considered one of the greatest difficulties in our century owing to the quantity of individuals suffering from DM has enormously increased over the most recent 20 years [1]. DM is a metabolic problem that is described by chronic high blood glucose level that prompts complications in the eyes, kidneys, heart, vessels and nerves [2]. Elevated diabetes level is a consequence of uncontrolled glucose and prompts hazardous injury to many of the body's systems(WHO, 2018). Glucose happens either when the pancreas doesn't deliver adequate insulin (type I diabetes) or when the body can't utilize the insulin it produces (type II diabetes).Patients with glucose type II constitute about 90%-95% in around the world [3].This chronic complex illness requiring permanent clinical consideration involving hazard reduction techniques is beyond glucose control [4]. More treatment drugs are financially accessible in the administration of glucose however they have results and extravagant, subsequently the requirement for natural items as a substitution treatment [5]. Sweet potato is being intensely developed in many pieces of the world and in a portion of these spaces, local people use portions of the plants for medicinal purposes. In the Philippines for instance, the plant is professed to be valuable in the management of diabetes without logical proof [6]. The plant is plentiful in dietary fiber, minerals, vitamins and compound of substance with organic impact, for example, β-carotene, phenolic

corrosive and anthocyanin which gives it the one of a kind tissue tones (cream, yellow,orange and purple) [7]. The anthocyanin found in sweet potato could control the blood glucose level by inhibiting the alpha-glucosidase [8] and could likewise increase the phosphorylation of insulin receptor [9]. Blood which is an imperative extraordinary circulatory tissue is made out of cells suspended in a liquid intercellular substance (plasma) with the significant capacity of looking after homeostasis. Hematological boundaries, which comprise of red platelets or erythrocytes, white platelets orleucocytes, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscularhaemoglobin concentration are important in checking the blood just as the wellbeing status of creatures [6].

Aim and objective of the Study

The aim of this study is to evaluate the hematological effect of alloxan induced diabetic albino rat treated with ethanol extract of sweet potato (*Ipomoea batatas*).

MATERIALS AND METHODS

MATERIALS

STUDY SUBJECTS

The study subjects were make wiser rats with varying size or weight ranged between 100g to 200g.

Plant Materials

Fresh tubes of *Colocasia esulanta* was punrchased from nkwo market in mgbakwu town, Awka North Anambra State. It was sent to the department of Zoology. Nnamdi Azikiwe University, Awka for proper identification by Dr. Bibian Aziagba, a taxonomist Voucher specimens were number: NAUH: 147[A] and was deposited at the departmental *Herbarium*.

Animals

Sixty male wister albino rats weighing between 100 and 120 was purchased from Chris Research farms, Awka and used for the LD_{50} while Male rats weighing between 150g and 200g was used for the antidiabetic studies

Procurement, identification and preparation of plant

Fresh tubers of *I.batatas*were purchased from major market in Enugu south Local Government Area, Enugu State. The tubers were then taken to the Department of Botany, Nnamdi Azikwe University, Awka for identification by an expert. Dry cooked tubers of *I. batatas* were macerated and made into extract through sun-drying, crushing (in pestle and mortar) and dissolution in 4L of70% ethanol for 2 hours. The filtrate was concentrated using water bath (k420) at 50°C before use.

METHODS

Study design

A total of forty-eight male Wister albino rats of approximately the same age and an average body weight of between 150 and 200g were purchased from Chris Research farm, Ngbakwu Awka. They were housed in standard aluminum cages (4 per cage, such that the number per cage will not interfere with clear observation of eachrats), in a 12-hour light and dark cycle with temperature of 22±2˚C. The rats were allowed two weeks' period of dietary accommodation to acclimatize before they were randomly grouped into six (group A, B, C, D, E, and F) as shown in Table 1 below:

Table 1: Protocol for treatment

Group	Treatment
A	Normal control (fed with rat growers feed+ H_20 adlibitum)
B	Diabetic untreated (negative control fed with rat growers feed + H_20 adlibitum)
C	Diabetic (positive control) – treated with standard drug e.g Glucophage + fed with rat growers feed+ H_20 adlibitum)
D	Diabetic treated with 200mg/kg of *ipomoea batatas* + growers feed
E	Diabetic treated with 400mg/kg of *ipomoea batatas* + growers feed
F	Diabetic treated with 600mg/kg of *ipomoea batatas* + growers feed

All the rats were given standard feed and water *ad libitum*. The rats in group A were not induced with diabetes; group B were induced but not treated; group C were induced but treated with standard drug (Glucophage); group D to F were induced but treated with extracts at 200, 400 and 600mg/kg body weight. The extracts were prepared with distilled water and given daily by oral route (cannula feeding).The standard drug dose were equivalents of human therapeutic dose of the drug and were prepared using standard methods.

Induction of Diabetes Mellitus

Following fourteen days of acclimatization, alloxan monohydrate was utilized to initiate type II diabetes in exploratory creatures. Intraperitoneal administration of 100mg/kg body weight of alloxan monohydrate was managed once. A gentle pressing factor was then applied at the spot of infusion to improve ingestion. Following three days of administration, creatures' fasting blood glucose levels were checked utilizing the glucose observing gadget (Acu Check).

Sample collection

At the end of experimental administrations, the wistar rats were anesthetized in a desiccator containing cotton wool soaked with chloroform. After they had attained deep anesthesia, they were brought out of the desiccator and a laparotomy was carried out (by making a V-shape incision in the abdominal region with the aid of a surgical scissors) and the visceral organ (liver) were then exposed and harvested for analysis.

Ethical clearance

Animal handling was performed with regard to Guide for the Care and Use of Laboratory Animals, and the University's research ethics. Procedures were performed in strict accordance with the recommendations in the Guide of the Chukwuemeka Odumegwu Ojukwu University Animal Ethical Committee and the protocols were appropriately approved. Study was also conducted in accordance with the Current Animal Care Regulations and Standards approved by the institute for Laboratory Animal Research.

Haematological parameters

Determination of total RBC using haemocytometer

A 1:200 dilutions were made by diluting 20µl of EDTA anticoagulated blood in 3.98ml of Gower's solution and mixed for 3minutes. The counting chamber and cover glass was cleaned appropriately. 10µl of the diluted fluid was used to both chambers of the haemocytometer avoiding air bubble, it was allowed to stand for 3minutes prior to counting. The haemocytometer was carefully placed on the microscope stage, the condenser on the microscope was lowered and the chamber was scanned using 10X objective lens. The cells were counted using the 40X objective lens.

Determination of packed cell volume

Using micro haematocrit method, a well-mixed anticoagulated whole blood was allowed to enter capillary haematocrit tubes until appropriately 2/3 filled with blood. Blood filling was done for each tube. One end of each tube was sealed with Bunsen flame and placed in the medial grooves of the haematocrit centrifuge head exactly opposite each other, with the open end towards the center. The lid was replaced and centrifuged for five minutes at 11,000rpm. The tubes were removed as soon as the centrifuge had stopped spinning. And the value of the packed cells was read off using the micro-haematocrit reader.

Determination of haemoglobin concentration

Using Cyanmethaemoglobin method, exactly 5.0ml of Drabkin's reagent was pipetted into two test tubes 1 and 2. A well-mixed sample of EDTA blood (0.02ml) was pipetted into the tubes, rinsing the pipette five times with the reagent, until all the blood was removed from the pipette. The solutions were well mixed and allowed to stand at 25 ℃ for 10 minutes in order to allow the formation of cyanomethaemoglobin. The mixtures were transferred into cuvettes and read in a spectrophotometer at a wavelength of 540nm. The Drabkin 's reagent in tube 1 was used as the blank (setting the percentage transmittance at 100%). The readings from each tube were recorded and the actual Hb values in g/dl were determined from a pre- calibrated chart.

Determination of white blood cells

The blood specimen was mixed approximately for one minute, using the white blood cell pipette, blood will be drawn to the 0.5mark in the pipette. Blood was removed from the outside of the pipette with clean gauze. The tip of the pipette was placed into the counting diluting fluid to draw it slowly until it reached the 11 mark. The counting chamber and the cover glass were cleaned with a cloth. The counting chamber was filled with diluted blood. The four corners of the chamber was visualized under a low power (10X) objective and the cells were counted in all the four marked corner squares.

Determination of differential cells

A drop of well mixed anticoagulated blood was placed on a clean, grease free slide, using a spreader the blood was smeared on the slide and allowed to air dry. The slide was flooded with Leishman stain and allowed to stand for 2minutes. The stain was diluted with twice its volume of buffered distilled water. It was mixed by blowing air gently on the stain to ensure uniform mixing. The stain was allowed to stand for 8minutes. Excess stain were rinsed off with buffered distilled water, the back of the slide was wiped to remove all traces of the stain. The slide was drained and stood upright in a draining rack to dry. The slide was examined microscopically with 100X oil immersion objective lens.

Statistical analysis

Data were presented as mean ± standard deviation (SD) following one-way analysis of variance (ANOVA) and Tukey-HSD test using Microsoft Excel 2016. Differences between $p < 0.05$ were considered significant.

RESULTS

HAEMATOLOGICAL PARAMETERS

Table 2: Effects of *Ipomoea batatas* ethanolic extract on PCV andhemoglobin

Group	Treatment	PCV (%)	Haemoglobin (g/dL)
A	Normal control	37.67±1.37	12.53±0.45
B	Diabetic (negative control)	33.80±0.98[a]	11.24±0.29[a]
C	Diabetic (positive control)	36.60±0.80[b]	12.18±0.24[b]
D	Diabetic + 2000mg	38.0±1.41[b]	12.63±0.47[b]
E	Diabetic + 4000mg	36.60±0.80[b]	12.18±0.24[b]
F	Diabetic + 6000mg	36.67±1.03[b]	12.20±0.31[b]

Hints: values are Means±SD, n = 6.The superscript letters indicate a significant difference ($p<0.05$). Group D has the highest PVC and haemoglobin concentration while group B has the lowest PVC and haemoglobin concentration.

Table 3: Effects of *Ipomoea batatas* ethanolic extract on RBC, and WBC

Group	Treatment	RBC (mm^3)	WBC (mm^3)
A	Normal control	$4.53x10^{12}$±$4.50x10^{11}$	5216.67±75.28
B	Diabetic (negative control)	$3.24x10^{12}$±$2.94x10^{11}$[a]	6440±120[a]
C	Diabetic (positive control)	4.18 $x10^{12}$±2.4 $x10^{11}$[b]	6200±63.25[ab]
D	Diabetic + 2000mg	4.63 $x10^{12}$±4.68 $x10^{11}$[b]	6050±83.67[ab]
E	Diabetic + 4000mg	4.18 $x10^{12}$±2.4 $x10^{11}$[b]	6360±80[acd]
F	Diabetic + 6000mg	4.20 $x10^{12}$±3.1 $x10^{11}$[b]	6416.67±98.32[acd]

Hints: values are Means±SD, n = 6. The superscript letters indicate a significant difference ($p<0.05$).

In Table 3, group D has the highest volume of red blood cells while group B has the lowest volume. In the same way, group B has the highest white blood cells while group A has the lowest.

Table 4: Effects of *Ipomoea batatas* ethanolic extract on neutrophils, eosinophils and basophils

Group	Treatment	Neutrophils (%)	Eosinophils (%)	Basophils (%)
A	Normal control	52.83±2.32	3.67±1.75	0.92±0.80
B	Diabetic (negative control)	52.60±1.85	3.60±1.50	0.40±0.49
C	Diabetic (positive control)	51.60±1.96	4.00±0.63	1.10±0.66
D	Diabetic + 2000mg	52.17±2.48	3.83±1.94	1.03±0.59
E	Diabetic + 4000mg	52.60±2.24	3.40±1.62	1.10±0.66
F	Diabetic + 6000mg	52.50±2.07	3.17±1.33	1.17±0.82

Hints: values are Means±SD, n = 6.

The percentage of neutrophils, eosinophils and basophils were highest with group A, C and F respectively, and lowest with group D, F and B respectively.

Table 5: Effects of *Ipomoea batatas* ethanolic extract on monocytes and lymphocytes

Group	Treatment	Monocytes (%)	Lymphocytes (%)
A	Normal control	4.17±2.48	39.00±2.96
B	Diabetic (negative control)	3.80±1.47	39.60±2.24
C	Diabetic (positive control)	5.00±0.00	38.80±2.40
D	Diabetic + 2000mg	4.67±2.94	38.83±3.19
E	Diabetic + 4000mg	4.00±2.45	39.6±2.58
F	Diabetic + 6000mg	4.00±1.55	40.17±2.40

Hints: values are Means±SD, n = 6.

The percentage of monocytes and lymphocytes were highest with group C and F respectively, and lowest with group B and C respectively.

DISCUSSION, CONCLUSION AND RECOMMENDATION

Discussion

The *Convulvulaceae* is an important family in traditional medicine for the treatment of many ailments *I.batata s*tubers which is a member of the family as a vegetable has great economic importance. In the present study, ethanol extract of *I. batatas* tuber was found to have significant effect on serum hematological parameters and biochemical parameters. This present study revealed that alloxan-induced diabetic untreated (group B) rats showed some abnormalities in the hematological parameters (packed cell volume, haemoglobin and red blood cells) when compared to normal control and treated (group C–F) rats. This is in line with the report of [7]. Some of these abnormalities might be due to the destruction of mature red blood cells, leading to the low haemoglobin counts accompanied by the fall in the red blood cells and packed cell volume. Administration of the extracts elicits a positive change in the hematological parameters suggesting that it may not contribute further diabetic complications to hematological parameters [4]. Blood examination is a good way of assessing the health status of animals as it plays a vital role in physiological, nutritional and pathological status of organisms submitted that assessment of hematological parameters can be used to determine the extent of deleterious effect on blood constituents of an animal [4,6]. Also, hematological parameters were used to explain blood relating functions of chemical compounds plant extract [6]. Peripheral blood leukocytes produce polymorpho nuclear cells, including monocytes as well as lymphocytes. Some previous studies [4,7] showed that peripheral white blood cell count might be associated with type II diabetes, coronary artery disease, stroke, micro and macro vascular complications. Increased differential cell counts, including counts of eosinophils, neutrophils, and monocytes, also indicate the future incidence of coronary artery disease. [2,4,6] showed that the white blood cells might play a role in the development and progression of diabetic complications. However, there is no investigation into the differential leukocyte count in relation to diabetic nephropathy.

CONCLUSION

From the obvious results, it could be concluded that the heamatological parameters were improved in diabetics' rats' group, especially group E which were treated with 400 mg/kg body weight of the extract.

REFERENCES

1. Crossby, D.G. and L.J. Anderson (1984): The organic constituents of food II J. Food . Science 29, 287-293.
2. Rohilla, A. and S. Ali, 2012. Alloxan induced diabetes: Mechanisms and effects. Int. J. Res. Pharmaceut. Biomed. Sci., 3: 819- 823.
3. Niwa, A., T. Tajiri and H. Higashino, 2011. Ipomoea batatas and Agarics blazei ameliorate diabetic disorders with therapeutic antioxidant potential in streptozotocin-induced diabetic rats. J. Clin. Biochem. Nutr., 48: 194-202.
4. Ivorra, M.D., M. Paya and A. Villar, 1989. A review of natural products and plants as potential antidiabetic drugs. J. Ethnopharmacol., 27: 243-275.
5. Ijaola, T.O., A.A. Osunkiyesi, A.A. Taiwo, O.A. Oseni, Y.A. LanreIyanda, J.O. Ajayi and R.T. Oyede, 2014. Antidiabetic effect of Ipomoea batatas in normal and alloxaninduced diabetic rats. IOSR J. Applied Chem., 7: 16-25.

6. Islam, S., 2006. Sweetpotato (Ipomoea batatas L.) Leaf: Its potential effect on human health and nutrition. J. Food Sci., 71: R13-R21
7. Nishikant, A.R., R.K. Alkesh and J.G. Naresh, 2014. Evaluation of antidiabetic potential of Ipomoea turpethum R.Br. and Ipomoea batata L. (Convolvulaceae) in alloxan induced diabetes in rats: A comparative study. Res. J. Pharma. Biol. Chem. Sci., 5: 137-141.
8. Oliveira, H.C., M.P. dos Santos, R. Grigulo, L.L. Lima and D.T.O. Martins et al., 2008. Antidiabetic activity of Vatairea macrocarpa extract in rats. J. Ethnopharmacol., 115: 515-519.
9. Wild, S., G. Roglic, A. Green, R. Sicree and H. King, 2004. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care, 27: 1047-1053.