



ALTERATIONS IN HISTO-ARCHITECTURE OF SELECTED TISSUES IN CO-ADMINISTRATION OF *IPOMOEA BATATA* EXTRACT AND ANTI-OXIDANT VITAMIN TO DIABETIC WISTAR RATS

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ABSTRACT

Reports on the importance of potato leaf (*Ipomoea Batata*) extract on various physiological systems of the human body are rife; with most showing it to be medicinal, pharmacological, and efficacious in the management of disease conditions, including blood related ailments. However, little or no record(s) have detailed its hypoglycaemic effect on body weights in relation to changes in the histo-architecture of selected tissues. Current study examined the effect of *Ipomoea Batata* leaf aqueous extract on blood sugar levels, body weights and histology of selected visceral [pancreas, liver and kidney]; in alloxan induced diabetic wistar rats. Twenty five (25) healthy Wistar rats of an average weight of between 140 – 200g were procured from the animal unit of the Ambrose Alli University, Ekpoma, Edo State. They were then acclimatized for two (2) weeks and randomly grouped into five (5) [G1, G2, G3, G4 and G5]; G1 (Control) received standard rat diets *ad libitum*, While G2 was fed daily with calculated doses of aqueous extract of sweet potato leaf per kg body weight after inducing diabetes mellitus (DM) with alloxan monohydrate. G3 and G4 (DM induced) were orally given (by mixing with diets, daily for two weeks) vitamin C and extract + vitamin C (Co-administration) respectively, group 5 (G5) received calculated doses of extract + vitamin E per kg body weight for 2weeks. For each group, Body weights were checked weekly, at the end of which animals were sacrificed with their liver, pancreas and kidneys harvested for histo-architectural changes. Blood samples were also obtained (using cardiac puncture) for analysis of changes in glucose levels. Following comparison (using the student t-test), study found a statistically significant increase ($p < 0.05$) in body weights and blood sugar levels of experimental groups [G2 – G5] compared with control [G1]; study also observed ameliorative changes in pancreatic, liver and renal histo-architectures (using H and E x 40) of DM induced rats as against non-diabetic (control) rats.

KEYWORDS: *Ipomoea Batata*, haematological indices, Wistar rats.

INTRODUCTION

In recent times, research findings have shown that over 1.70 million of such global cases are seen in Nigerians above 15 years old; with about 70,000 of them occurring as type I in children under the ages of 15 years.^[1,2] Diabetes is prevalently rising daily as a result of obesity, population growth, and sedentary lifestyles; projecting it to be over 360 million cases by 2030.^[3] Though efforts have been made to prevent its life threatening complications through the use of oral hypoglycemic (blood sugar reducing) agents like sulphonylureas, Diabetes remains an incurable endocrine disorder. Cost, undesirable and adverse effects associated with these drugs promote the use of suitable herbs with minimal effect hypoglycemic activities.^[4] Over 50% of such herbs

now serve traditional medics in fighting and ameliorating ailments like dysentery, diarrhea, toothache, skin infections, and diabetes; which greatly affects humans. One of such plants often alleged to be of great importance is *Ipomoea Batata* (Sweet potato).

Sweet potato (*Ipomoea batatas*), taxonomically belongs to the morning glory family, *Convolvulaceae*, and the only member of the genus *Ipomoea* whose roots are edible. It is speculated to be a native of South America but presently grown throughout the tropical and subtropical regions of the world.^[5] Sweet potatoes form a large part of the food of the people in many countries and are the sixth most important food crop of the world with an annual production of about 126.19 million tonnes

from 9.26 million hectares. Several reports have shown its phytochemicals to possess multifaceted actions, including anti-oxidant, anti-mutagenic, anti-inflammatory, antimicrobial and anti-carcinogenesis and thus are important for several health-promoting functions in humans.^[6] It is reported to be rich in carbohydrates, cellulose and beta carotene (an active ingredient of vitamin A). *Ipomoea batatas* is also a great source of vitamins B6 and C with numerous mineral nutrients like Zn^{2+} , K^+ , Na^+ , Mg^{2+} , Ca^{2+} and Fe^{2+} .

Available reports suggest *I. batata* to contain major phytochemicals like flavonoids, terpenoids, tannins, saponins, glycosides, alkaloids, steroids and phenolic acids. These constituents may vary with varieties, depending on flesh and skin color.^[7] Orange varieties are particularly rich in beta-carotene, while purple sweet potato contains higher anthocyanin than other varieties of sweet potato. Beta carotene is a terpenoid with a strongly colored red-orange pigment abundant in plants and fruits. Anthocyanins are members of the flavonoid group of phytochemicals responsible for the red, purple and blue pigments in many fruit and vegetables. Today, the antioxidant activities of sweet potato have mostly been attributed to their anthocyanin and beta-carotene contents.

Numerous studies have also reported the different medicinal potentials of sweet potato. These properties have been attributed to either a single or combined effect of the phytochemicals present in the plant.^[8,9] In traditional medicine, sweet potato has been used to treat many diseases such as oral infections, inflammatory diseases and also in the management of diabetic conditions. In recent times, pharmacological potentials of sweet potato has been investigated and demonstrated by different *in vitro*, animal models and a few human studies. However, little or no record(s) are available on the hypoglycaemic effect of *I. batata* on body weights in relation to changes in the histo-architecture of selected tissues. Current study therefore aimed at examining the effect of aqueous extract of *Ipomoea Batata* leaf on blood sugar levels, body weights and histology of selected visceral [pancreas, liver and kidney], using wistar rats as experimental model.

MATERIALS AND METHOD

Study Location

The study was carried out in Pharmacology department of the faculty of clinical sciences, Ambrose Alli University, Ekpoma, Edo State.

Study Design

A total of twenty five (25) wistar rats of approximately the same age and an average body weight of between 140–250g were purchased and acclimatized for two weeks, following which they were grouped into five (5) of five rats each. Group 1 received normal rat feeds (control), While Group 2 got (for 2weeks) specific doses (based on body weight) of aqueous extract of sweet potato leaf. Rats in groups 3 and 4 were given (through

diets) vitamin C and extract + vitamin C (Co-administration) respectively, whereas, group 5 rats got calculated doses of extract + vitamin E per kg body weight for 2weeks. worth mentioning is that substrate treatment of groups 2 to 5 rats was done after inducing diabetes mellitus (DM) with alloxan monohydrate, and confirming them to be diabetic.

Procurement, Preparation and Identification of Plant

Before experiment proper, sweet Potato (*Ipomoea batatas*) leaf extract was obtained from local farms within Ekpoma. The leaf was then taken to the Department of Botany for identification by experts. Next, leaf was macerated and made into extract through sun-drying, crushing (in pestle and mortar) and dissolution in distilled water.

Ethical Clearance

Animal handling was performed with regard to CPCSEA guidelines, and the University's research ethics. Procedures were performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Ambrose Alli 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.^[13]

Inducing Diabetes Mellitus

After the two (2) weeks of acclimatization, Alloxan monohydrate was used to induce type I diabetes mellitus in experimental animals. Intraperitoneal administration of 100mg/kg body weight of Alloxan monohydrate was administered once. A mild pressure was then applied at the spot of injection to enhance absorption. After 3 days of administration animals' fasting blood glucose levels were checked, using the glucose monitoring device (Acu-check).^[10]

Preparation of Stock Solutions of *Ipomoea Batata* Extract

After weighing 2g of *Ipomoea Batata* with electronic balance, the substance was then homogenized in pestle and mortar using 10ml of distilled water, and then filtered with Wattmann filter paper. This gave a 200mg/ml stock solution.

Collection of Blood Sample

After the 14th day of administration, animals were euthanized by cervical decapitation, and blood samples obtained via cardiac puncture, a 2ml syringe.

Drug Preparation and Administration

Five hundred milligram (500mg) of Vitamin C tablets were obtained from local pharmacy stores in Ekpoma, Edo State, Nigeria. Each tablet (500mg) was dissolved in 100ml of distilled water, with mixture centrifuged to obtain clear Vitamin C solution. This was then administered orally in combination with potato extract as detailed in study design.

Vitamin E

Due to the solubility of vitamin E on lipid, rather than water, obtained vitamin E was dissolved in groundnut oil to aid its dissolution and transport across membranes. 150mg/kg of it was then administered orally in combination with potato extract as detailed in study design.

Harvesting of Organs/Tissues

Following blood collection, animals were then dissected with selected organs [Liver, Kidneys and Pancreas] were harvested and ripped off unnecessary tissues. Harvested organs were then stored in formaldehyde and transported to the laboratory for histological analysis.

Histological Analysis

In the laboratory, harvested tissues were immediately opened and fixed in Bouin's fluid, whilst soaking in formal saline so as to preserve the various constituents in their normal micro-anatomical positioning and prevent them from degeneration or analytic changes. The following processes were then carried out sequentially.

Dehydration

The tissues were allowed to fix in 10% formal saline for 48hours. They were then grossed and cut into smaller pieces of 3mm thick in pre-labelled tissue cassettes. They were processed using Automatic tissue processor (LEICA TP1020) where they passed through various reagents including alcohol (of various concentrations starting from 70%, 80%, 90%, 95%, and two 100% or absolute alcohol) for dehydration, two changes of Xylene and three changes of molten Paraffin wax set at 65 degree centigrade. The processing time was 12 hours.

Embedding

The tissues were embedded in Paraffin wax by burying the tissues in a metal mold containing molten paraffin

wax and allowed to cool and form tissue paraffin blocks, ready for microtomy.

Microtome (Sectioning)

The tissues were sectioned at 4 microns using Rotary microtome (LEICA RT2115) and the sections were floated on hot water bath to attach the sections to pre-labelled slides. The sections were dried on hot plate and ready for staining using Haematoxylin and Eosin, and Giesma staining technique.

Procedure for Haematoxylin and Eosin (H&E) Technique

Dewax was soaked in Xylene for 15mins.

Mixture was then placed in Absolute Alcohol, 95% and 70% Alcohol, Next, section of test tissue was rinsed in distilled water and stain in Harris haematoxylin for 5mins.

Combination was then differentiated in 1% acid alcohol briefly and rinsed in distilled water again (for 10mins).

Tissue was then counterstain with 1% aqueous Eosin for 2min and rinsed in distilled water for the third time.

Product was then dehydrated in ascending grades of alcohol, next, cleared in xylene and Mount in Microscope for viewing.

Statistical Analyses of Data

Results from study were presented as mean \pm Standard Deviation (SD). Using the one way analysis of variance (ANOVA), Average values of obtained data were statistically compared. Here, p-values < 0.05 was considered to be statistically significant.

RESULTS

Table I: Comparative Effect(s) of *Ipomoea Batata* on Blood Glucose levels between and

Group	Blood Glucose (mg/dl)				Remark
	IGL	7 th Day GL	14 th Day GL	ANOVA (p-value)	
I	74.20 \pm 5.98	86.60 \pm 9.07	85.20 \pm 7.05	80.20 \pm 7.50	Insignificant
II	80.60 \pm 9.74	185.40 \pm 40.87	179.20 \pm 40.87	150.40 \pm 49.58	
III	76.80 \pm 9.76	174.0 \pm 39.29	150.80 \pm 41.17	115.00 \pm 19.82	Significant
IV	71.60 \pm 7.57	169.80 \pm 36.06	146.60 \pm 21.42	111.2 \pm 16.17	Significant
V	71.80 \pm 7.56	356.60 \pm 49.30	397.80 \pm 87.79	278.60 \pm 71.51	Insignificant
ANOVA	0.46333 [#]	2.65614 [#]	0.00265 [*]		

* = statistically significant, # = statistically insignificant at p-value $< .05$ =. GL = Glucose Level, IGL = Initial Glucose Level.

Table II: Comparative Effect of Extract on average Body Weights of Wistar Rats.

Group	Body Weights (g)				Remark
	IBW (g)	Week 1 (g)	Week 2 (g)	ANOVA (p-value)	
I	160 \pm 8.09	130 \pm 4.37	120 \pm 2.83	0.01363	Significant
II	160 \pm 8.09	120 \pm 2.83	130 \pm 4.37	0.01363	Significant
III	130 \pm 4.37	125 \pm 3.96	126 \pm 2.36	0.34384	Insignificant
IV	110 \pm 2.29	125 \pm 3.96	130 \pm 4.37	0.04942	Significant
V	120 \pm 2.83	140 \pm 7.39	135 \pm 5.56	0.03842	Significant
ANOVA	0.00013 [*]	0.00293 [*]	0.00316 [*]		

* = statistically significant at p-value $< .05$, IBW = Initial Body Weight. Result is presented as Mean \pm Standard Deviation.

Table III: Comparative Effect of Extract on Body Weights Changes of Male and Female Rats.

Group	Body Weights (g)				Remark
	Male	Female	t-Cal	t-test (p-value)	
I	136	92	-1.383	0.00234	Significant
II	136	123	-2.463	0.00373	Significant
III	127	128	2.233	0.10336	Insignificant
IV	122	133	1.393	0.00332	Significant
V	132	125	1.452	0.06273	Insignificant
ANOVA	0.30393 [#]	0.00284 [*]			

* = statistically significant at p-value < .05, [#] = statistically insignificant at p-value > .05.

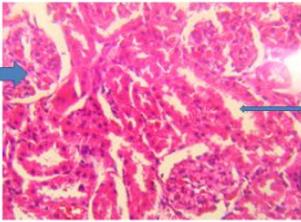
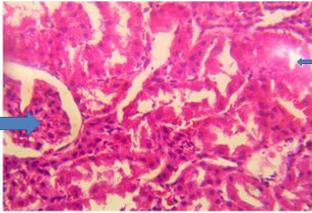
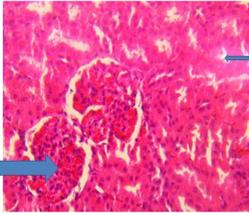
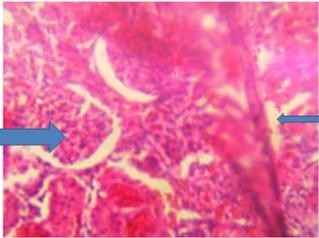
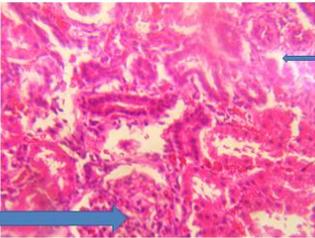
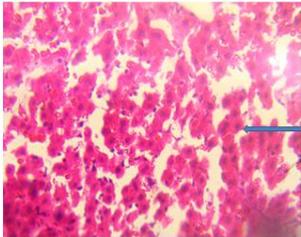
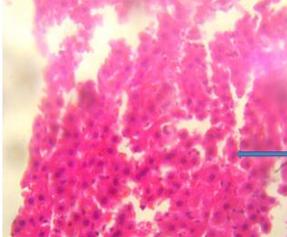
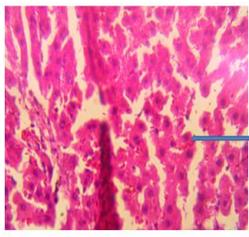
Group I	Group II	Group III
KID 1B X40 	KID 2B X40 	KID 3B X40 
<i>thick arrow shows normal glomerulus and thin arrow shows normal tubules</i>	<i>thick arrow shows normal glomerulus and thin arrow shows mild acute tubular necrosis</i>	<i>thick arrow shows normal glomerulus and thin arrow shows mild acute tubular necrosis</i>
Group IV	Group V	
KID 4B X40 	KID 5B X40 	
<i>thick arrow shows normal glomerulus and thin arrow shows mild acute tubular necrosis</i>	<i>thick arrow shows normal glomerulus and thin arrow shows normal tubules</i>	

Figure I: Photomicrograph of Kidney after Two weeks of Extract Administration.

Group I	Group II	Group III
LIV 1B X40 	LIV 2B X40 	LIV 3B X40 
<i>Arrow shows normal hepatocytes</i>	<i>Shows degeneration of hepatocytes</i>	<i>Arrow shows normal hepatocytes</i>
Group IV	Group V	

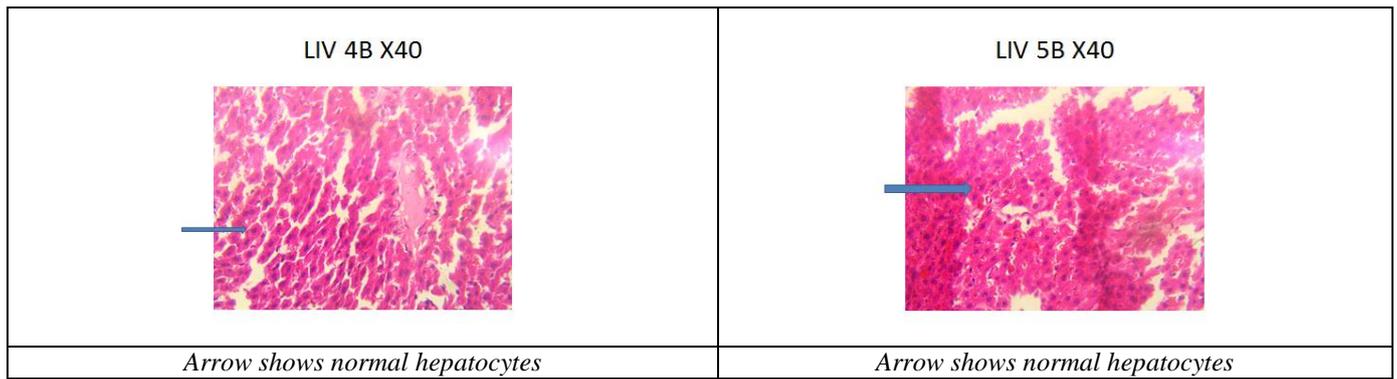


Figure II: Photomicrograph of Liver after Two weeks of Extract Administration.

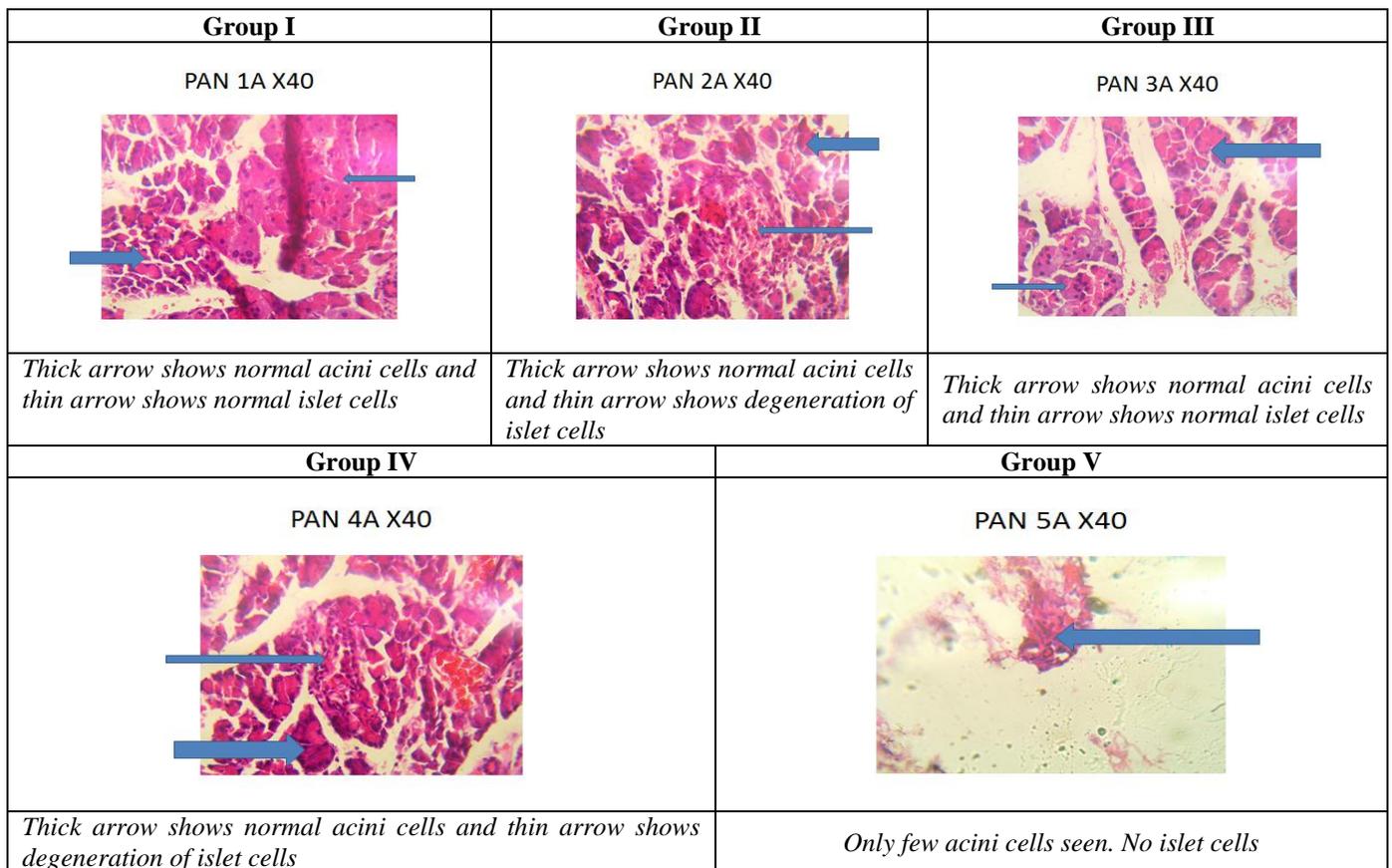


Figure III: Photomicrograph of Pancreas after Two weeks of Extract Administration.

DISCUSSION

Overtime, sweet potato herbal extracts have been suggested to be potent in blood glucose lowering.^[11] In some animal and human studies, different forms of sweet potato have been reportedly helps to maintain blood sugar levels and lowering insulin resistance. For instance, 'Caiapo', a dietary supplement and a crude extract of white skinned sweet potato has been sold and consumed for a long time in Japan as a remedy for diabetes. 'White star', a sweet potato cultivar; indigenous to Pakistan and 'Beauregard' is also known to lower glucose blood level in diabetic humans.^[12] From this study, the aqueous leaf extract of sweet potato was observed to significantly reduce blood glucose levels in Alloxan-induced diabetic wistar rats. This agrees with the result of a recent study by Pal *et al* who reported that

the aqueous extract of the leaves of sweet potato shows significant improvement in the blood glucose profile of diabetic rats.

Again from this study, a blood glucose lowering effect of sweet potato is seen to significantly cause an increase in body weights of wistar rats with duration across groups (with the exception of group III) when compared with control (table II). In humans, available reports have shown that subjects with poorly-regulated insulin metabolism and insulin insensitivity resulting to DM have lower body weights, and individuals with healthier insulin metabolism tend to have higher weights on the average. Generally, results from current study re-enforces available literatures on the anti-diabetic property of sweet potato as seen in the result of table II.

Contrary to result of this study however, Zhao *et al* had isolated flavone from the leaves of sweet potato and evaluated its effects on different markers of diabetes; there, they reported a statistically significant decrease in the fasting plasma insulin and blood glucose level and significant increase in the insulin sensitive index in non-insulin dependent diabetic rats.^[13,14]

Moreover, when extract administered rats were compared with diabetes untreated group, there was significantly decreased blood glucose level in all experimental groups. This implies that treatment with *Ipomoea Batata* at all doses and Vitamin E separately and combined significantly improves blood glucose level in animals. Results from our study is partially supported by Preetha *et al.*, (2012), who though worked on ethanolic extract, showed that the mature liquid of *Ipomoea Batata* has hypoglycemic activities in alloxan-induced diabetes.^[15,16] *Ipomoea Batata* leaf extract alleviates hyperglycemia in diabetes and improves glucose tolerance probably by its antioxidant effect which consequently leads to improvement of insulin secretion as seen in this study. Other possible mechanisms include: improvement of thyroid function and improvement of lipid metabolism.

Also, the extraction of *Ipomoea Batata* leaf is thought to be more beneficial than usually prepared sweet potato because its mode of extraction retains more biologically active components such as alpha tocopherol (vitamin E) and polyphenols (Nevin & Rajamohan, 2004). For the effect of *Ipomoea Batata* extract treatment on body weight (g) of Wister rats, Result shows a significant loss in body weight (g) for all experimental groups when compared with control. This implies that treatment with *Ipomoea Batata* at all doses with Vit. E and separately does not improve body weight (g) in alloxan induced diabetes. These findings corroborate the conclusions made by Anosike and Obidoa (2010).^[17,18] They revealed that *Ipomoea Batata* promotes weight loss.

The Photomicrograph of figure III for normal pancreas reveals presence of islet cells, without any significant reduction of islet cells. However photomicrograph of untreated rats pancreas reveals absence of islet cells, marked perivascular fibrosis, inflammation and congested blood vessels. Shrunken islets cells and depleted with a great reduction of islet cells. However, treatment for 2 Weeks on the pancreas reveals very few islet cells with peripheral infiltration by mild to moderate population of lymphocytes. Also there is perivascular lymphocytic infiltration and fibrosis across tissues. Again, the Photomicrograph of rats treated with *Ipomea batata* + Vitamin E for 2 Weeks reveals perivascular inflammation with stromal fibrosis and few depleted islet cells with more islet cells and very minimal absence of inflammation.

CONCLUSION

From this study, treatment of diabetic rats with *Ipomea batata* extract significantly improved metabolic outcomes in diabetic rats. *Ipomea batata* treatment was seen to rival the beneficial effects of vitamin E in almost all parameters measured, suggesting that *Ipomea batata* treatment and Vitamin E has possible similar anti-oxidant activities. More so, *Ipomea batata* treatments showed a dose- dependent effect on most parameters measured, with more significant outcomes in higher dose. These discoveries were orchestrated by a cascade of events within various mechanisms germane to physiological outcome. The beneficial effects on metabolism are possibly achieved via multiple metabolic reversal effects sufficient enough to counter balance complications due to diabetes.

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