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Effect of Ethanolic Leaf Extract of Sida Corymbosa (Wire Weed) on Alloxan Induced Pancreatic Damage in Adult Male Wistar Rats

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Abstract
Sida corymbosa is a semi shrubby perennial plant used in herbal concoctions. This study evaluated the effects of ethanolic leaf extract of *Sida corymbosa* (ELESC) on alloxan induced pancreatic damage in adult male wistar rats. LD_{50} test showed that ELESC was orally non-lethal at 5000mg/kg. 25 adult male albino wistar rats weighing between 200-300g were used for this study, and were divided into 5 groups (1-5) of 5 rats each. Group 1 served as control and received feed and distilled water *ad-libitum* throughout the experiment. Group 2 served as diabetic group, and received a single intraperitoneal dose of 150mg/kg of alloxan. Pancreatic damage in groups 3 and 4 were induced with a single intraperitoneal injection of 150mg/kg of alloxan and subsequently treated daily with 500mg/kg and 250mg/kg of ELESC respectively. Group 5 received 500mg/kg of ELESC only. Administration of ELESC was oral and for 28 days. Blood samples were collected weekly and glucose level assessed. At the end of the experiment, serum samples were assayed for insulin level, while pancreases were harvested for histological observations. Alloxan elicited characteristics similar to that of type 1 diabetes, with histological alterations in the pancreas. However, administration of ELESC caused a dose dependent decrease in blood glucose level, with increases in insulin levels in all ELESC treated diabetic rats, when compared to control. Histological findings revealed regenerative changes in pancreatic tissues of ELESC treated diabetic treated rats. This study showed that ELESC has hypoglycemic properties as well as soothing effects on alloxan induced pancreatic damage.
Keywords: Pancreas, Insulin, Diabetes, Hypoglycemia.

1.0 Introduction

The pancreas is a mixed glandular organ located behind the stomach. Light microscopy reveals two different types of parenchyma tissues, the acinar cells, that are dark stained in H&E located mainly at the intercalated duct of the pancreas which function as the exocrine pancreas secreting digestive enzymes that aid in carbohydrate, protein, fats and starch metabolism; and the pancreatic cell (islet of langerhans), that are numerous at the tail of the pancreas and comprises of three main types of hormone producing endocrine cells (Young, 2006). These cells include the alpha cells that produce glucagon, beta cells that produce insulin and delta cells that produce somatostatin and gastrin (Singh, 2011). Insulin and glucagon control blood sugar levels, while somatostatin and pancreatic polypeptide have diverse functions that are vital for the body's survival (Constanzo, 2006; Young, 2006).

Some factors have been known to harm the pancreas upon exposure, thereby leading to its damage. These factors that damage the pancreas include expose to toxins, in the form of chemicals, medicines, and herbal concoctions, as well as disease conditions that alter the functionality of the pancreas, resulting in clinical conditions such as abdominal pain, nausea, cystic fibrosis, pancreatic cancer, pancreatitis which may be acute or chronic, exocrine pancreatic insufficiency and diabetes mellitus (Laffan *et al*., 2008).

Diabetes mellitus is a clinical condition, which could be due to either the pancreas not producing enough insulin, a hormone that normalizes the glucose level by promoting glucose uptake into body cells (Diabetes type 1) or due to the cells of the body not responding properly to the insulin stimulation (Diabetes type 2) (Shoback, 2011). This condition causes hyperglycemia (Kitabchi *et al.,* 2009), and produces classical symptoms of such as polyuria, polydipsia, and polyphagia, weakness, and coma, amongst others (WHO, 2014).

Chemicals such as alloxan are used widely in the laboratories to induce diabetes mellitus in experimental animals (Szkudielsi, 2001). Alloxan (2, 4, 5, 6-tetraoxypyrimidine; 2, 4, 5, 6- pyrimidinetetrone) is an oxygenated pyrimidine derivative that selectively destroys the insulin-producing beta cells of the pancreas, causing an insulin-dependent diabetes mellitus (Danilora *et al.,* 2015). Alloxan also causes pancreatic beta cell toxicity and diabetogenicity that may be attributed to alloxan-induced redox cycling and reduced oxygen specie (ROS) generation. The mechanism underlying this cytotoxic action of alloxan to insulin-producing cells is due to

the reduction by interaction with intracellular thiols such as glutathione (Elsner *et al*., 2006). The resultant formation of cytotoxic ROS is the result of a cyclic reaction between alloxan and its reduction product, dialuric acid, which by autoxidation generates superoxide radicals, hydroxyl radicals and H_2O_2 (Das *et al*., 2012). Continuous generation of these ROSs causes an increase in the level of the ROSs which results in significant damage to cell structures such as the DNA of the pancreatic islet thereby leading to the damage of the pancreas' islet of langerhans (Elsner *et al*., 2006).

Alloxan also elevates cytosolic free Ca2+ concentration in the beta cells of pancreatic islets (Elbert *et al*., 2000). The calcium influx is resulted from the ability of alloxan to depolarize pancreatic beta cells that further opens voltage dependent calcium channels and enhances calcium entry into pancreatic cells. This increased concentration of Ca2+ ion further contributes to supraphysiological insulin release that along with ROS has been noted to ultimately cause damage of beta cells of pancreatic islets (Etuk, 2010).

Management of damage to the islet cells and its resultant characteristics similar to that of type 1diabetes mellitus has been carried out with drugs such as metiformin and canagliflozin (Olokoba *et al*., 2012). However, these applications have often led to complication such as nausea, diarrhea, yeast infection, urinary tract infection, hypotension and other infections (Irons and Minze, 2014). As a result, herbal remedies have been employed successfully in management of pancreatic damage as well as diabetes mellitus (Shan and Khan, 2014; Sedigheh, *et al*., 2011).

Sida corymbosa commonly known as "wire weed" is a semi shrubby woody perennial weed, found on the roadsides, tracks, waste place and overgrazed pasture, with great medicinal value (John-Africa *et al*., 2014). It is one of the species of *sida* plants, which belongs to the family *malvaeceas* (Kumar *et al.,* 2013). Its leaves contain calcium, copper, iron, magnesium, manganese, phosphorus, sodium, and zinc (Aworinde *et al*., 2012); as well as phytochemicals such as tannins, saponins, alkaloids, flavonoids, terpens and sterols (John-Africa and Aboh, 2015). Available literatures show that *Sida corymbosa* extracts posses uterotonic property (Attah *et al.,* 2012), anti-ulcer, wound healing and anti-hemorrhagic activities **(**John-Africa *et al.,* 2014, John-Africa and Aboh, 2015). Since other species of *Sida* weed have been found to possess anti-hyperglycemic activity (anti-diabetic) and pancreatic repair in established diabetic conditions, there may be a possibility that *Sida corymbosa*, which is the most assessable specie of *Sida* in the southeastern part of Nigeria, could possess similar properties.

2.0 MATERIALS AND METHODS

2.1 Collection of *Sida Corymbosa* Leaves and Preparation of Ethanolic Extract

Fresh leaves of *Sida Corymbosa* were obtained from farms at Ndimgbu-Otolo, Nnewi, in Nnewi-North Local Government Area of Anambra State of Nigeria. These leaves were identified and authenticated at the department of Botany, Nnamdi Azikiwe University, Akwa. Ethanolic leaf extract was prepared according to the method described by Sundaraganapathy *et al.,* (2013), with slight modifications. The leaves were washed with distilled water, air-dried and pulverized using laboratory grinder. The ethanol extract was obtained by soaking 25g of each of leaves in round bottom flasks containing 200ml of absolute ethanol for forty-eight hours with shaking. The ethanolic extracts were filtered using 40mm Whatman filter paper and evaporated using rotary evaporator (model:TT22, USA) at 65°C. A portion of this freshly prepared extract was used for phytochemical analysis using standard protocols.

2.2 Procurement and Reconstitution of Alloxan Solution

Alloxan powder in the form of Alloxan monohydrate was obtained from the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Agulu, Anambra State, Nigeria. 0.5grams of this salt was dissolved in 5ml of normal saline to form a stock solution, and 150mg/kg was withdrawn and injected intraperitoneally to the experimental animals were applicable.

2.3 Procurement and Housing of Experimental Animals

A total of thirty (30) apparently healthy male Albino Wistar rats weighing between 200-300g were used. They were obtained from a private farm in Nnewi, Anambra state, Nigeria and were transported to the Animal House of the College of Health Sciences, Nnamdi Azikiwe University, Nnewi campus where they were acclimatized for two (2) weeks before commencement of substrate administration. During acclimatization, the rats were fed rat feeds (growers mash) and distilled water *ad-libitum.* All procedures were carried out in strict accordance with the institutional guidelines on the care and use of experimental animals.

2.4 Acute Toxicity Test (LD_{50}) For *Sida corymbosa*

The LD_{50} for ethanolic leaf extract of *Sida corymbosa* was determined by employing the method of Lorke (1983) as modified by Doera *et al*., (2010). Findings from this test showed that oral administration of ethanolic leaf extract of *Sida corymbosa* to male adult Wistar rats showed no death at a dose level of 5,000mg/kg.

2.5 Experimental Design and Protocols

2.5.1 Housing and Grouping of Experimental Animals

After acclimatisation and toxicity test, 25 experimental rats weighing between 200-300g were divided into five (1-5) groups of five (5) rats each and were housed in plastic rat cages with regularly change sawdust that served as bedding. Group 1 served as normal control group, group 2 served as the diabetic control group, groups 3-5 were used as test groups. The entire groups were fed with rat feed (growers feed, manufactured by Guinea Feeds PLC) and water *ad-libitum* on daily basis and weighed on weekly basis throughout the duration of the experiment.

2.5.2 Induction of Pancreatic Damage and Subsequent Treatment

Alloxan was used to induce pancreatic damage in groups 2-4, with consequent development of characteristics similar to that of type 1diabetes in humans. This was achieved by a single intraperitoneal injection of freshly prepared 150mg/kg of alloxan. Diabetes mellitus was confirmed by the presence of high fasting plasma glucose level (above 180mg/dl) 48 hours after alloxan injection.

After the induction of pancreatic damage with alloxan, group 2 were left untreated and were diabetic throughout the duration of the experiment, group 3 and 4 animals were treated with 500mg/kg and 250mg/kg of the ethanolic leaf extract of *Sida corymbosa* respectively*,* while group 5 were given only 500mg/kg of the ethanolic leaf extract of *Sida corymbosa*. All extract administration was via the oral route and lasted for 28 days. Blood samples were collected from the tail artery of the rats on weekly basis, and fasting blood glucose determined after a 12 hour fast by using ACCU-CHEK Blood Glucose Monitoring system, recorded, and expressed in mg/dl.

2.6 Termination of Experiment, Serum Blood collection, and Organ collection

The experimental animals were injected with ketamine hydrochloride 24hours after the last administration of extract so as to anaesthetize the animals. Blood samples were collected from the animals immediately before sacrificing by ocular puncture for insulin hormone assay. These blood samples were put in plain sterilized glass tubes with no anticoagulant. The samples were centrifuged with a laboratory ultracentrifuge machine (New life model) and serum collected and stored in the refrigerator.

After the collection of blood samples, the animals still under the influence of anaesthesia were dissected through the anterior abdominal region and their pancreases harvested and put into beaker containing freshly prepared normal saline to wash off blood before weighing and transferring them into universal bottles containing 10% formal saline for preservation prior to histological processing. The remains of the experimental animals were properly disposed.

2.7 Procedure for insulin Hormone Test

An Accu-Bind ELISA Microwells was used to test for insulin. The microplates wells for calibrator, control and specimen to be assayed were formatted. 0.05mls of the fluids that served as appropriate calibrators, controls and samples were poured into the assigned wells. 0.1 ml of the Insulin Enzyme Reagent was added to each well containing the calibrators, controls and samples. The microplates was then swirled gently for 20-30 seconds to mix and then covered with a plastic wrap. The microplates containing the calibrators, controls and samples were incubated for 120 minutes at room temperature (20-27°C). The contents of the microplates were discarded by decantation and the plates blotted dry using absorbent paper. After blotting the plate dry, 350μl of wash buffer was used to decant the plate twice making it a total of three (3) washes. 0.1 ml of working substrate solution was thereafter added to all wells. The microplates were incubated at room temperature for fifteen (15) minutes after adding the working substrate. After incubating, 0.05ml of stop solution was added to each well and mixed gently for 15-20 seconds. The absorbance in each well was then read at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in the microplates reader.

2.8 Processing the Pancreas for Microscopic Viewing

The harvested pancreases were fixed in 10% formal saline in a container with light fitting lids for three days to prevent autolysis; improve staining quality and to aid optical differentiation of cell. The fixed pancreatic tissues were then dehydrated to remove water using different ascending grades of alcohol ranging from 50% to absolute Alcohol for 30 minutes each. Afterwards, the dehydrated tissues were cleared by removing the alcohol from the tissue by immersing it through three changes of xylene for 30 minutes each. The cleared tissues were then impregnated and infiltrated to remove the clearing agent (xylene) by passing it through three changes of molten paraffin in a hot oven temperature of 60°C for 30 minutes.

The infiltrated tissues were embedded with molten paraffin wax in an embedding mould and allowed to solidify. The paraffin blocks of tissue obtained after embedding prior to sectioning, were first trimmed so as to expose the surface of the tissue by adjusting the microtome knife to 15µm. These blocks were afterwards attached to a wooden block and sections were cut with rotary microtome and the ribbons placed on 20% alcohol

on a large slide (5cm X 7.5cm slide) to remove minor folds and creases from sections. The ribbons were gently placed on a water bath preheated to about 45°C so as to float out the tissues, after which the tissues were collected with clean slides, allowed to dry, and labelled using diamond pencil. The slides were then dried on a hot plate at 5°C for the tissue to adhere to the slide.

The tissues were dewaxed in xylene for 10 minutes and then rinsed in descending order of alcohol ranging from absolute 95%, 90%, 70% and 50% for two seconds each. The tissue was then washed in two changes of water and differentiated in 1% acid alcohol. Afterwards, the tissues were stained in Hematoxylin and washed in tap water until the tissues changed to blue colour. The tissue were then counterstained in Eosin for 5 minutes and cleared in xylene before mounting in DPX and dried for micrograph and interpretation.

2.9 Statistical Analysis

The mean and standard deviation for the body weight during administration was generated using the IBM SPSS Statistics 20, and data were analyzed statistically. Comparison was done using one- way analysis of variance (ANOVA), to compare means, Values were considered significant at $P < 0.05$. Post Hoc multiple comparisons for differences between groups within groups were established using least significant difference (LSD). With results presented as Mean ± S.E.M.

3.0 RESULTS

3.1 Phytochemical Screening

Phytochemical screening of ethanolic leaf extract of *Sida corymbosa* revealed the presence of alkaloids, carbohydrates, tannins, flavonoids, glycosides and saponins. Test were performed on freshly prepared extract and recorded in Table 3.1

Table 3.1 Phytochemical Screening of Ethanolic Leaf Extracts of *Sida corymbosa*

Phytoconstituents	**Presence**
Carbohydrates	+
Alkaloids	+
Saponins	+
Tannins	-
Fixed oils	+
Phytosterols	+
Flavonoids	+

+ = present, - = Absent

3.2 Physical and Behavioural Observations

At the beginning of the experiment, all animals were apparently healthy and agile. During the two weeks of acclimatization, their stool was dark brown and they adapted well to their environment. During the first week following induction of pancreatic damage using alloxan, and subsequent onset of diabetes, the rats showed signs of weakness, reduction in food and water intake and polyuria. In the group that was left diabetic (group 2) without treatment, feeding habits improved moderately, although they became weaker as the experiment progressed. However, in diabetic treated groups, feeding habits and urine outputs returned to normal as the experiment progressed. In group 5 (that received only 500mg/kg of the ethanolic leaf extract of *Sida corymbosa*), they became weaker towards the end of the experiment.

3.3 Effect of Alloxan and Ethanolic leaf extract of *Sida Corymbosa* on Body Weights of the Experimental Rats

Table 3.2: Effect of Alloxan and Ethanolic leaf extract of *Sida Corymbosa* on Body Weights of Various Experimental Groups

Group	Initial Body Weight (g)	Final Body Weight (g)	Weight Difference (g)	Mean Weight Difference ± SEM	t-value	p-value
1	208	246	38	35.25 ± 2.46	14.316	<0.001
	210	240	30			
	205	248	43			
	209	239	30			
2	220	170	-50	- 46.25 ± 2.14	21.653	<0.001
	215	175	-40			
	218	168	-50			
	220	175	-45			
3	280	270	-10	- 8.00 ± 7.03	1.138	0.299
	300	295	-5			
	285	274	-11			
	290	284	-6			
4	270	263	-7	- 12.25 ± 1.49	8.205	<0.001
	275	260	-15			
	272	261	-11			
	275	259	-16			
5	200	263	63	58.5 ± 2.51	23.322	<0.001
	205	263	58			
	208	268	60			
	210	263	53			

Table 3.2, shows that while comparing weight differences within groups, there were significant weight gains in groups 1 (control) and 5. There was however significant decreases (p>0.05) in weights of Groups 2 (diabetic) and 4 (diabetic treated with 250mg/kg of ELESC) during the course of the experiment, while in group 3 (diabetic treated with 500mg/kg of ELESC), the weight loss was not statistically significant when initial weights were compared to that of final weights of the experimental animals.

Table 3.3: Effects of Alloxan and Ethanolic Leaf Extract of *Sida Corymbosa* on Mean Body Weights of Various Experimental Groups When Compared With Control

GROUP	MEAN OF WEIGHT DIFFERENCE ±SEM	F-value	Sig
1	35.25 ± 3.19	320.751	
2	- 46.25 ± 2.39		.000
3	- 12.25 ± 2.05		.000
4	- 8.00 ± 1.47		.000
5	58.5 ± 2.10		.000

Table 3.3 shows that there were significant weight gains observed in groups 1 (control) and 5 (treated with 500mg/kg of ELESC only). Weight gain observed in group 5 was statistically significant when compared to that of the control (group 1). There was however significant decreases (p>0.05) in weights of Groups 2 (diabetic control), 3, and 4, when compared to that of control.

3.4 Effect of Alloxan and Ethanolic Leaf Extract of *Sida Corymbosa* on blood glucose Level

Table 3.4.1 Comparison of Blood Glucose levels (mg/dL) in each of the Experimental Groups studied at the end of the experiment.

Group	Week	Mean	Std. Error	F-value	Sig.
Group 1	Week 1	76.20	0.10	843.785	-
	Week 2	81.80	0.09		<0.001
	Week 3	87.60	0.30		<0.001
	Week 4	78.71	0.08		<0.001
Group 2	Week 1	289.20	0.10	46830	-
	Week 2	180.00	0.30		<0.001
	Week 3	132.00	0.50		<0.001
	Week 4	202.14	0.14		<0.001
Group 3	Week 1	289.20	0.00	3453000	-
	Week 2	180.00	0.07		<0.001
	Week 3	132.00	0.00		<0.001
	Week 4	84.53	0.11		<0.001
Group 4	Week 1	293.20	0.07	1147000	-
	Week 2	200.00	0.00		<0.001
	Week 3	136.00	0.10		<0.001
	Week 4	90.14	0.11		<0.001
Group 5	Week 1	74.00	0.80	1394	-
	Week 2	69.00	0.30		<0.001
	Week 3	55.00	0.00		<0.001
	Week 4	38.36	0.06		<0.001

Table 3.4.2 Comparative Analysis of Changes in Blood Glucose Level (mg/dL) Studied in the Various Groups of Experimental Animals.

Groups	Mean Glucose Level ±SEM	F value	Sig.
1	78.71 ±0.08	1876.550	
2	202.14 ±0.14		.000
3	84.53 ±0.11		.011
4	90.14 ±0.11		.000
5	38.36 ±0.06		.000

Results obtained as shown in tables 3.4.1, and 3.4.2 showed that there was a statistically significant increase ($p>0.05$) in blood glucose levels of Group 2 (diabetic) when compared to control. Results on final mean blood glucose level also showed that although there was statistically significant reduction in blood glucose level when initial levels (week 1) were compared to final levels (week 4) in Groups 3 (diabetic treated with 500mg/kg of ELESC), and 4 (diabetic treated with 250mg/kg of ELESC) when compared to control (group 1), as animals in these groups had their glucose level returned to near normal values, although their final blood glucose levels were still higher in groups 3, and 4, with the increase in glucose level in group 4 being statistically significant when compared to control. However, very low glucose levels were observed in group 5 rats (that received 500mg/kg of ELESC) when initial levels were compared to final levels. This reduced glucose level observed in group 5 when compared to control was statistically significant.

3.5 Effect of Alloxan and Ethanolic Leaf Extract of *Sida Corymbosa* on Serum Insulin Level

Table 3.6 Comparative Analysis of Serum Insulin Levels (µU/ml) between the Various Experimental Groups Studied.

Group	Mean(µU/ml) ±SEM	F-value	Sig.
1	16.21 ± 0.78	85.498	-
2	5.59 ± 0.39		0.000
3	12.50 ± 0.76		0.000
4	10.08 ± 0.35		0.000
5	18.75 ± 0.29		0.006

Table 3.6 shows the result of comparative analysis of insulin levels. There was statistically significant difference in mean serum insulin levels between the various experimental groups ($F = 85.498$, $p < 0.05$). Mean serum insulin levels were observed to be highest in Group 5 (that received only 500mg/kg of ethanolic leaf extract of *Sida Corymbosa*), followed by control (group 1). Diabetic treated rats had reduced serum insulin levels when compared to control, while group 2 (diabetic group) had the least mean serum insulin levels.

3.6 HISTOLOGICAL FINDINGS

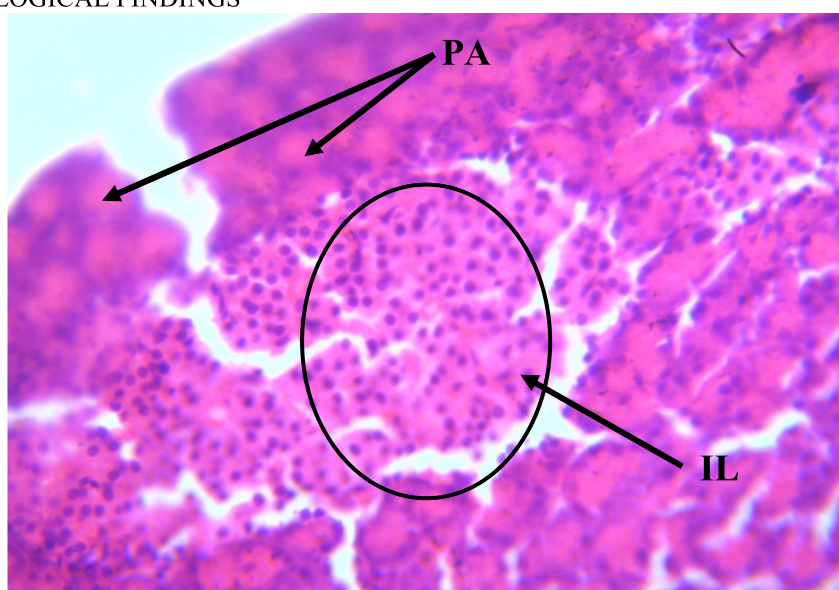

PLATE 1

PLATE 1 shows photomicrograph of the pancreas of group 1(normal control) section of pancreas (x150)(H/E) that were fed with normal rat feed and water *ad-libitum* showing normal pancreatic parenchyma with islets of langerhans (IL), and pancreatic acini (PA).

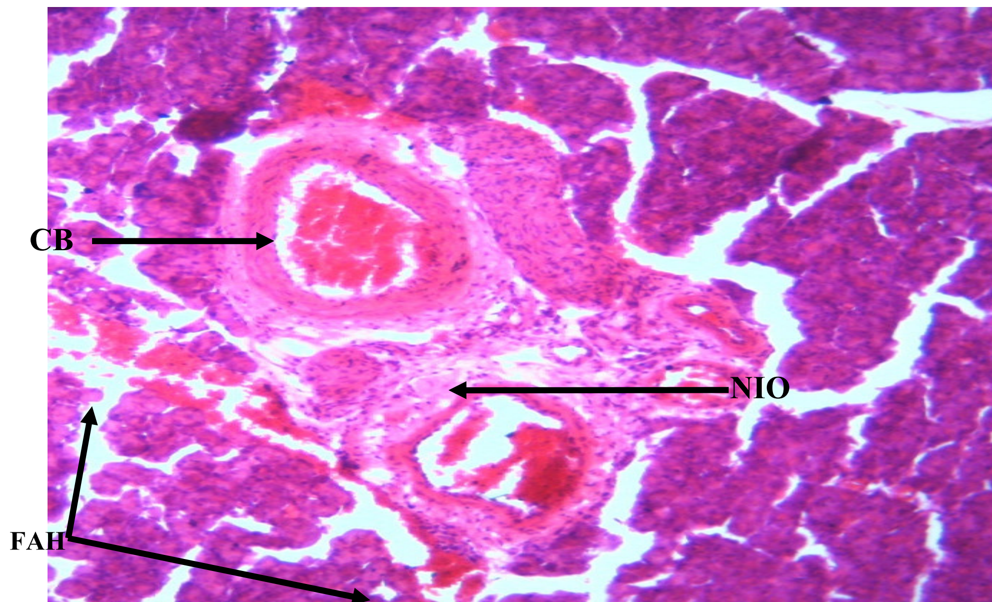

PLATE 2: Photomicrograph of the pancreas of group 2

Plate 2 shows Group 2 section of the pancreas (X150)(H/E) that were administered alloxan only without treatment, showing pancreatic tissue with congestion of the blood vessel (CBV), necrosis of islet of langerhans (NIOL), and pancreatic tissue with focal area of hemorrhage (FAH) indicating pancreatic damage.

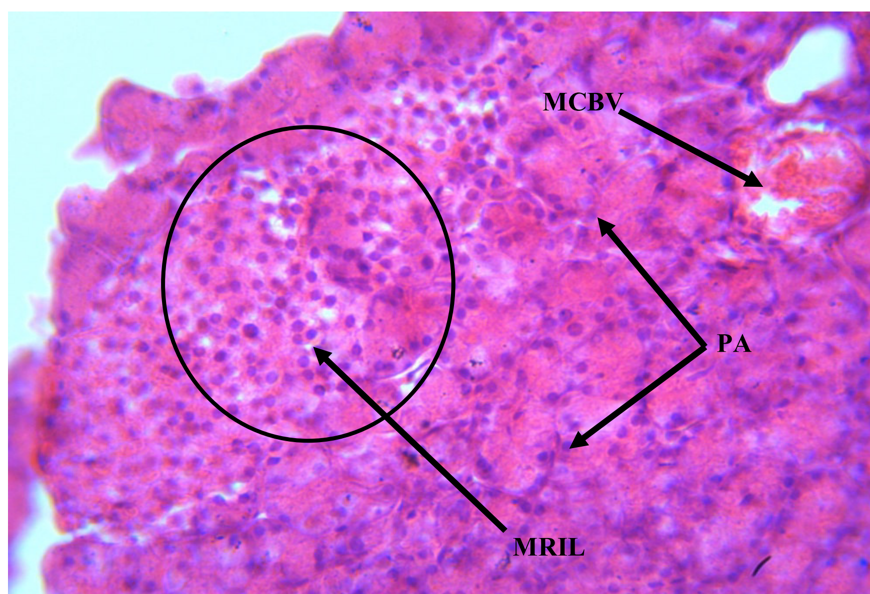

PLATE 3: Photomicrograph of the pancreas of group 3

Plate 3 shows Photomicrograph of Group 3 section of pancreas (X150)(H/E) that were administered 500mg/kg ethanolic leaf extract of *Sida corymbosa* after alloxan induction (150mg/kg) showing regeneration of damaged pancreatic tissue with moderate regenerated Islet of Langerhans (MRIL), and presence of pancreatic acinar cell (PA). However, there are mild congestions of blood vessels (MCBV).

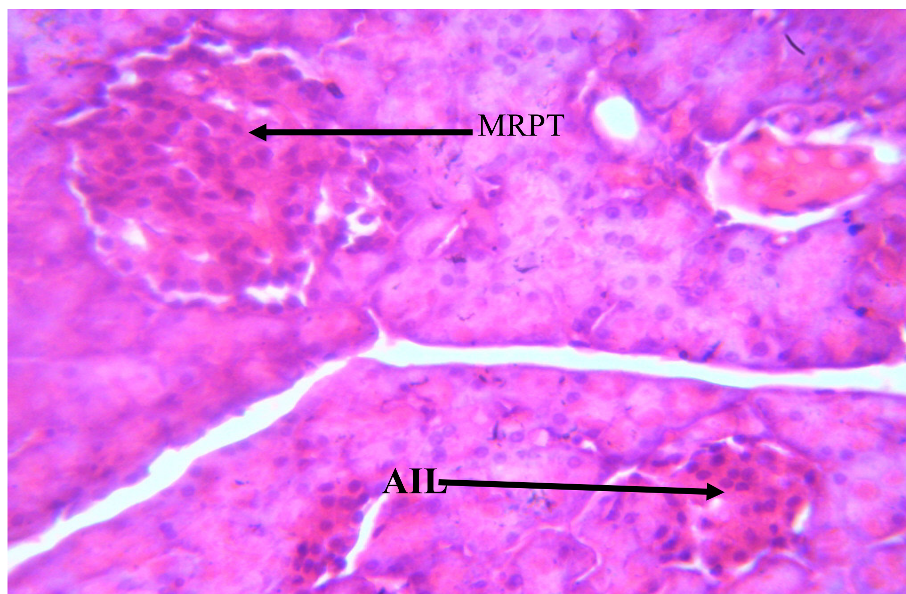

PLATE 4: Photomicrograph of the pancreas of group 4

Plate 4 shows photomicrograph of Group 4 section of pancreas (×150)(H/E) that were administered 250mg/kg of ethanolic leaf extract of *Sida corymbosa* after induction with alloxan (150mg/kg) showing moderate regeneration of damaged pancreatic tissue (MRPT) although there are still areas showing atrophy of islets of langerhans (AIL).

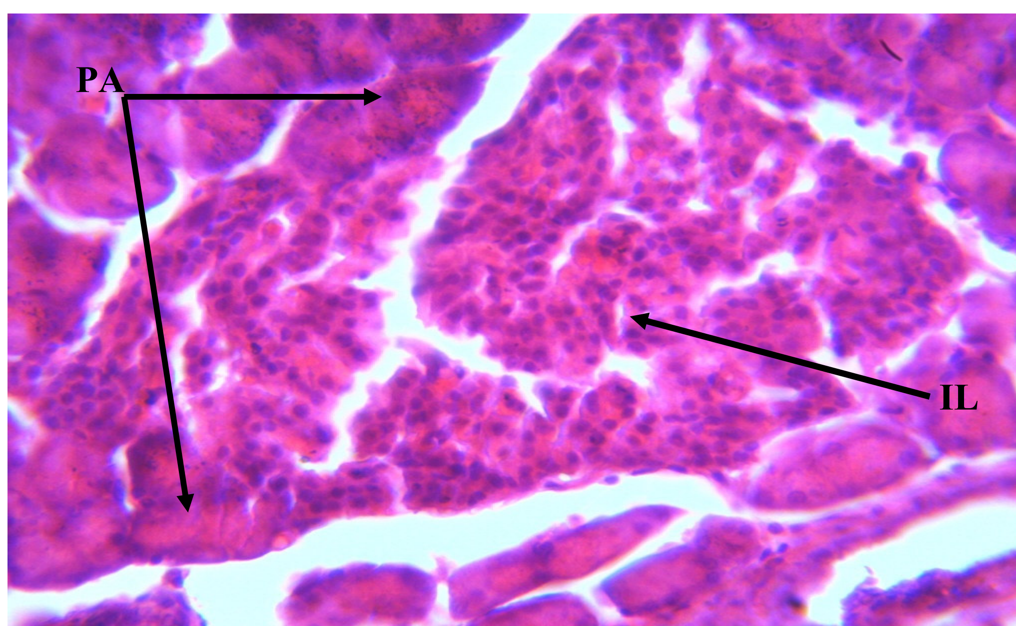

Plate 5: Photomicrograph of the pancreas of group 5

Plate 5 photomicrograph shows Group 5 section of Pancreas (×150)(H/E) that were administered 500mg/kg of ethanolic leaf extract of *Sida corymbosa* only, showing normal pancreatic architecture, with the presence of islet cells (IL) and pancreatic acinar cells (PA).

4.0 DISCUSSION

While exposure to some chemicals have the potentials to elicit diabetes mellitus, some herbal compounds have been used to provide soothing effects and in the process aiding quick recovery following development of diabetic symptoms. Reductions in body weight observed in the alloxan alone treated rats (group 2) could be as a result of impairment in insulin's action in the conversion of glucose into glycogen, which causes catabolism of fats and inhibition of lipolysis due to unavailability of insulin, resulting from destruction of beta cells (Gillesprie, 2006), as shown in Plate 2. This observation is in agreement with work done by Balamurugan *et al.,* (2014), whose report showed that there was decrease in body weight of alloxan induced diabetic rats when compared to

positive control. Insulin deficiency has been shown to accelerate muscle proteolysis and consequent muscle mass reduction and weight loss. This is caused by a decrease in the activity of phosphatidylinositol 3-kinase (PI3K) (enzymes involved in cellular functions such as cell growth, proliferation, differentiation, motility, survival and intracellular trafficking) (Xiaonan *et al*., 2006; Lee *et al*., 2004; Du *et al*., 2004). A decrease in PI3K activity reduces the level of phosphorylated Akt (pAkt). A low pAkt has been shown to relieve the inhibition of the expression of specific E3 ubiquitin-conjugating enzymes atrogin-1/MAFbx and MuRF1 in muscle (Xiaonan *et al*., 2006; Lee *et al*., 2004, Sandri *et al*., 2004, Stitt *et al*., 2004). This causes expression of E3 enzymes which have been implicated in several conditions that cause loss of lean body mass (Xiaonan *et al*., 2006; Lecker *et al*., 2004). Decrease in muscle PI3K activity has also been documented to activate Bax (a protein that is encoded by the *BAX* gene and functions as an apoptotic activator), which stimulates the activity of caspase-3 leading to muscle protein loss by providing substrates for the ubiquitin-proteasome proteolytic pathway (UPP) (Xiaonan et al., 2006; Lee *et al*., 2004; Du *et al*., 2004). The increase in weight observed in the groups that received graded doses of *Sida corymbosa* alone, or after treatment with alloxan when compared to the alloxan alone treated group, could have been made possible by modulatory effects of active ingredients domiciled in the leaves of *Sida corymbosa* such as saponins, carbohydrates, Saponins, and flavonoids. These active ingredients have been documented to increase appetite and body weight (Gillesprie, 2006). These phytochemicals have also been found to suppress and cancel out effects of reactive oxygen species (ROS) produced by toxic chemicals (Elsner *et al*., 2006).

The highest blood glucose level recorded in group 2 (diabetic group) when compared to control and other groups, may be as a result of specific cytotoxic impact of alloxan on pancreatic beta cells, producing free radicals. Continuous generation of these reactive oxygen species (ROS) such as superoxide's, hydroxyls and hydrogen peroxide, results in significant damage to cell structures such as the DNA of the pancreatic islet, leading to significant rise in blood glucose level (Elsner *et al*., 2006; Carvalho *et al*., 2003). It has been noted also, that alloxan elevates cytosolic free Ca2+ concentration in the beta cells of pancreatic islets (Elbert *et al*., 2000). This increased calcium influx is as a result of the ability of alloxan to depolarize pancreatic beta cells that further opens voltage dependent calcium channels and enhances calcium entry into pancreatic cells. This elevated concentration of Ca2+ ion further contributes to supraphysiological insulin release that along with ROS has been noted to ultimately cause damage of beta cells of pancreatic islets (Etuk, 2010). However, the significant decrease in blood glucose levels in ELESC treated diabetic rats when compared to the diabetic group may well be due to the presence of hypoglycemic bioactive phytochemicals contained in ELESC. These phytochemicals such as saponin, sterol and alkaloids have been reported to possess hypoglycemic properties (Switi *et al*., 2014; Onyeka *et al*., 2013). A member of the same genus *Prema Corymbosa* was found to be effective in reducing glucose levels of diabetic treated rats (Radhika *et al*., 2013). Other species of *Sida* have also been documented to posses similar hypoglycemic properties (Padmaja and Tebogo, 2015; Naseer and Muhammad, 2014). This hypoglycemic effect of *Sida corymbosa* observed in this study, is evidenced in the statistically significant reduction in glucose level in group 5 (treated with 500 mg/kg of ELESC) when compared to control. This could be a result of ELESC, being able to improve the structural architecture of islet cells, increase insulin production, as well as possibly enhance glucose uptake by tissues. Padmaja and Tebogo (2015), while working on another member of *Sida* specie noted that methanol extract *of Sida rhombifolia* reduced the glucose levels at 2 hours after its administration in moderately diabetic rats at a dose of 200 mg/kg and in severely diabetic rats at a dose of 300 mg/kg. The effect was more pronounced in the severely diabetic rats. Long-term administration of methanol extract *of Sida rhombifolia* to diabetic rats resulted in the normalization of glucose levels especially in moderately diabetic rats. In the present study, effects of ELESC was found to be dose dependent, with doses of 500 mg/kg showing more pronounced soothing effects when compared to doses of 250 mg/kg. Similar dose dependent activities of other species of *Sida* have been documented (Ahmad *et al*., 2014; and Naseer and Muhammad, 2014).

The decrease in insulin level in group 2 (diabetic group), could be directly linked to the obliteration of islet cells population that produce insulin by alloxan. Alloxan causes pancreatic beta cell toxicity and diabetogenicity, by selectively destroys the insulin-producing beta cells of the pancreas, causing an insulin-dependent diabetes mellitus (Danilora *et al.,* 2015). Smirnov and Snigur (2012), as well as Ikechukwu and Obri (2009) reported swollen, hyperemic and collapsed beta cells, with focal sclerotic changes in the islets cells following intraperitoneal administration of alloxan at a dose of 120mg/kg. In the current study, results of histological investigations as shown in Plate 2 showed pancreatic tissue with congestion of the blood vessel, necrosis of islet of langerhans, and pancreatic tissue with focal area of hemorrhage, indicating pancreatic damage in alloxan alone treated rats. This could be as a result of the ability of alloxan to continuously generate reactive oxygen species (ROS), leading largely to increased rates of cell apoptosis and consequent reduction in cell number. Beta cell apoptosis and replication rates, islet size, and islet neogenesis are the major determinants of pancreatic endocrine capability for insulin secretion and glucose homostasis (Montanya and Tellez, 2009). Intrinsic and extrinsic pathways are routes for the activation of apoptosis, with the former activated by stress factors such as

growth factor deprivation, cell cycle disturbance, and DNA damage, that cause the mitochondria to release cytochrome c and subsequently stimulating caspase-9; and the latter beginning with death of cell receptors and the associated activation of caspase-8 (Forouzanfar et al., 2013; Sharma et al., 2009). Significantly higher serum insulin levels observed in group 5 (that received only 500mg/kg of ethanolic leaf extract of *Sida Corymbosa*), could be attributed to *Sida Corymbosas'* arousal of beta cell, which preceded the increased insulin secretion, with resultant hypoglycemia observed in this group. This caused general weakness observed in this particular group (group 5), towards the end of the experiment. **Other species of *sida* have been found to possess protective and anti-hyperglycemic activity against ROS induced toxicities (**Padmaja and Tebogo, 2015; Naseer and Muhammad, 2014; Datusalia *et al*., 2012)**.** In addition, this stimulating and soothing effect of *Sida Corymbosa* may well be attributed to the higher serum levels of insulin triggered by improved pancreatic histology, observed in diabetic groups treated with 500mg/kg and 250mg/kg of the ethanolic leaf extract of *Sida corymbosa* (groups 3, and 4 respectively) as shown in Plates 3 and 4, when compared with observations made in the diabetic group (Plate 2). Administration of extracts of other species of *Sida* have been documented to improve tissue microarchitecture following alloxan induced damage **(**Padmaja and Tebogo, 2015; Naseer and Muhammad, 2014). Ethanolic leaf extract of *Sida corymbosa* contains phytochemicals such as tannins, saponins, alkaloids, flavonoids, terpens and sterols. These substances are important and are undeniably behind the dose dependent antioxidant properties as well as soothing effects observed in the diabetic treated groups (groups 3 and 4). It is believed that beta cell regenerate via the replication of already existing beta cells and neogenesis from stem cells and progenitor cells, a process that depends on extra-pancreatic activators including phytochemicals (Bouwens and Rooman, 2005). Exposure of cells to low doses of phytochemicals has been reported to increase the resistance of the cells to a range of stressors by activating adaptive cellular stress pathways. These antioxidant effects also mediate anti-apoptotic action against beta cell (Gao *et al*., 2011; Wu *et al*., 2011).

5.0 Conclusion

Result from this study reveals that alloxan induced deleterious histopathological changes in the pancreas of adult male albino wistar rats, with resultant alterations in body glucose and insulin levels, as well as pancreatic architecture. These effects were however ameliorated by oral administration of graded doses of ethanolic leaf extract of *Sida corymbosa*. This ameliorative effect of was dose dependent, as its therapeutic effect was more pronounced at a dose of 500mg/kg body weight.

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