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Histological and Biochemical Alterations in the Pancreas of Diabetic Wistar Rats Treated with Garcinia Kola and Tetracarpidium Conophorum Extracts

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Abstract
To ascertain the stated effects, phytochemical and LD_{50} studies of the extracts were determined. Thirty-six adult male Albino Wistar rats weighing 180 – 200 g were divided into 6 groups (n =6). Diabetes mellitus was induced by single intraperitoneal injection of streptozotocin (45 mg/kg bw) reconstituted in citrate buffer pH 4.5, and rats with fasting blood glucose (FBG) ≥ 11.3 mmol/L after three days of induction were used for the study. Glibenclamide significantly ($p < 0.05$) reduced FBG from 22.10 ± 1.65 mmol/L to 4.48 ± 0.29 mmol/L, *G. kola* from 22.04 ± 4.06 mmol/L to 7.40 ± 2.41 mmol/L, *T. conophorum* from 14.26 ± 2.38 mmol/L to 5.98 ± 0.57 mmol/L, and combined from 17.54 ± 1.72 mmol/L to 11.58 ± 2.11 mmol/L. Glibenclamide and the extracts significantly increase the insulin levels. *G. kola* significantly ($p < 0.05$) lowered the urea and raised the chloride and creatinine levels. *T. conophorum* significantly ($p < 0.05$) reduced the urea and increased the sodium and chloride levels, while the combined treatment significantly lowered the urea and raised the sodium levels. Glibenclamide and the extracts significantly ameliorated the histological alterations in the pancreas but only the plant extracts significantly attenuated the alterations in the kidneys. It may be concluded that the combined administration of *G. kola* and *T. conophorum* extracts and their single treatments showed hypoglycaemic and nephroprotective effects.
Keywords: Albino Wistar rats, Streptozotocin, Glibenclamide, *Garcinia Kola, Tetracarpidium conophorum,* Pancreas, Kidney
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1. Introduction

Diabetes mellitus (DM) commonly known as diabetes, is a group of metabolic diseases characterised by elevated blood sugar level over a prolonged period (American Diabetes Association (ADA), 2004;WHO, 2016). The disease is due to insufficient production of insulin or loss of cellular response to insulin or both (Shoback, 2011). Diabetes was first mentioned by the Egyptian physician Hesy-Ra of the 3rd Dynasty in 1552 BCE.

Before insulin was discovered by Banting and Best in 1922, diabetes was initially treated by low carbohydrate diet and by feeding fresh veal pancreas by mouth (Menon *et al*., 2015). Symptoms of diabetes mellitus include frequent urination, glycosuria, increased thirst and hunger (Kitabchi *et al*., 2009; International Diabetes Federation, 2017). Diabetes is not withoutcomplications. The acute complications include diabetic ketoacidosis, non-ketotic hyperosmolar coma, seizures or loss of consciousness and infections (Kitabchi *et al*., 2009; IDF, 2017).

The pancreas is a vital organ with both exocrine and endocrine functions. Its exocrine secretion contains enzymes necessary for digestion while the endocrine secretions – insulin, glucagon, and somatostatin are important in the regulation of glycaemia (Saladin, 2012). Insulin is an important hormone which helps to regulate the body glucose levels. It lowers blood glucose levels by acting on insulin receptors throughout the body, mainly skeletal muscles, liver and adipose tissues to stimulate glucose uptake and inhibit hepatic glucose production (Klien-Schwartz *et al*., 2015). This hormone is produced by the beta cells in the pancreas, an organ found behind the stomach in the abdomen (Khan, 2014). In diabetic condition, the ability of the pancreas to produce insulin is hampered mainly by autoimmune destruction of the beta cells (Matthew, 2014).

The treatment of diabetes involves dietary, exercise, insulin therapy and drugs (Prabhakar, 2015; IDF, 2017). The synthetic drugs used in the management of diabetes cannot be said to be ideal, due to their toxic side effects and sometimes diminution in response after prolonged use (Lepzem and Tongun, 2017). The limitations and side effects associated with the existing synthetic oral hypoglycaemic agents have necessitated the search for newer drugs. Thus, natural agents from plants and plant products have been the alternative target to source for new antidiabetics (Lepzem and Tongun, 2017).

Walnut is very important because of the nuts and timber most of their representative genus produces. Walnut comprises such families as *Juglandaceae* (English walnut), *Euphorbiaceae* and *Olacaceae* (African

walnut). All the families produce nuts in common (Innocent *et al*., 2013). *Tetracarpidium conophorum* commonly called African walnut is a woody perennial climber found in large tree forest in southern Nigeria, Africa in general (Amaeze *et al*., 2011) and India (Edem *et al*., 2009). In Nigeria, it is found in Abak, Uyo, Etinan, Akpabuyo, Akamkpa, Lagos and Ibadan (Edem *et al*., 2009).

Garcinia kola (*G. kola*) commonly known as bitter kola is an evergreen tree belonging to the Gluttiferae family. It grows in the rain forest of West African countries such as Nigeria. *G. kola* is nutritious containing carbohydrate, ash, protein, vitamins and minerals.

Animal models have for long played a pivotal role in the exploration and characterisation of diseases pathophysiology, target identification and in the evaluation of new therapeutic agents for treatments *in vivo* (Amin *et al*., 2016). The early experimental models of diabetes was based on partial or total pancreatectomised animals. Selection of species was more or less spontaneous but small animals (mostly rats and mice) were used because of handling or space, size, short generation interval, easy availability, and affordability (Srinivasan and Ramarao, 2007; Lukacinova *et al*., 2008; Lukacinova *et al*., 2011). There are several animal models available for the study of diabetes. These are the spontaneous autoimmune induced, surgical removal of the pancreas, genetically induced, virally induced and chemically induced.

Though several studies exist about the hypoglycaemic effects of *G. kola* and *T. conophorum* extracts, none has compared the effects of these extracts or considered the effects of co-administration of the two extracts.

This study was set out to investigate if the combination of these extracts *G. kola* and *T. conophorum* would have a better hypoglycaemic effects or better ameliorative effect on diabetes induced pathological alterations in the pancreas and kidney than the individual extracts or glibenclamide, for the management of diabetes mellitus. This is a worthwhile effort considering the fact that the number of people with diabetes mellitus kept increasing with its attendant noxious complications and high cost of management worsen by the poor socioeconomic background of the people which have the highest prevalence.

The pancreas are affected by diabetes mellitus (Dahiru *et al*., 2016; IDF, 2017). The pancreas is at the centre in the development of diabetes mellitus (Menon *et al*., 2015). Destruction of β cells in the pancreas may result in low or complete lack of insulin secretion. This leads to type 1 diabetes mellitus. A situation whereby the body cells do not respond to insulin results in type 11 diabetes mellitus (Shoback, 2011; Saladin, 2012). In Nigeria, diabetes mellitus is one of the major predisposing factors to premature birth, obstetric complications, neonatal mortality and lower extremity amputation (Odatuwa-Omagbemi and Adiki, 2012; Olufemi and Samuel, 2015). It is one of the costliest diseases to manage largely because of the associated complications (Adisa *et al*., 2009).

Reports show that *G. kola* seed and *T. conophorum* leaf are useful in the management of blood sugar (Adaramoye, 2012; Donatus *et al*., 2014; Adedara *et al*., 2015; Aneke *et al*., 2016; Lepzem and Tongun, 2017) which might have impact on the kidney and pancreas health in diabetes. Most people commonly eat *G. kola* and *T. conophorum* either singly or together but there is dearth of information about the histological and biochemical effects of these plants in the pancreas and kidney. Therefore, this study investigates the histological and biochemical alterations in the pancreas and kidney of diabetic Wistar rats treated with *G. kola* seed and *T. conophorum* leaf extracts.

The aim of this study was to assess and compare the effects of the co-administration of *G. kola* seed and *T. conophorum* leafextracts on the pancreas of diabetic Wistar rats.

The objectives were to assess and compare:

i. The effects of combined extracts of *G. kola* seed and *T. conophorum* leaf, their single administration, and glibenclamide on blood glucose level of STZ – induced diabetic rat.

ii. Morphological changes in the pancreas and kidneys of diabetic rats treated with glibenclamide, combination of *G. kola* seed and *T. conophorum* leaf extracts and their single treatments, using H&E staining method.

iii. Changes in the pancreatic insulin expression using insulin antibody immunohistochemical method; and glycogen accummulation in the kidneys using PAS, of diabetic rats treated with glibenclamide, combination of *G. kola* seed and *T. conophorum* leaf extracts and their single treaments.

iv. The serum insulin profile, electrolytes, urea and creatinine of diabetic rats treated with glibenclamide,

This study provides data on the histological and biochemical alterations in the pancreas and kidney of diabetic Wistar rats administered with *G. kola* seed and *T. conophorum* leaf extracts. This information may be useful to diabetic patients or health care providers.

2. Research Design

Thirty six adult male Albino Wistar rats of body weight between 180 – 200 g were used for the study. They were assigned into well ventilated wooden cages under 12 hours night/12 hours day cycles and standard temperature. The animals were allowed to acclimatise for two weeks and had free access to tap water and livestock feed (Vital grower®) *ad libitum* throughout the period of the experiment except on the days prior to the measurement of fasting blood glucose when feeds were withdrawn overnight.

2.1. Induction of Diabetes Mellitus

Thirty (30) adult male Wistar albino rats were induced with diabetes by a single dose of intraperitoneal injection (i.p.) of streptozotocin (STZ) (45mg/kg body weight) reconstituted in 0.1 M of citrate buffer at a pH of 4.5 (Arikawei *et al*., 2012; Nagayach *et al*.,2014; Lawal *et al*., 2017; Iwara *et al*., 2017). The STZwas given after the rats had fasted overnight. This was to reduce or eliminate the competitive affinity of glucose with the STZ by the beta cells of the pancreas. To avoid fatal hypoglycaemia, the rats were given 30% glucose solution after theSTZ administration (Ofor *et al.,* 2013). To confirm diabetic induction, the rats were fasted overnight and the fasting blood glucose levels of the rats were measured using a glucometer with strips after seventy-two (72) hours of STZ administration and rats with glycosuria and hyperglycaemia with glucose levelof 11.3 mmol/L and above were used for the experiment (Wang *et al*., 2010). The diabetic rats were then divided into 5 diabetic groups.

2.2. Grouping of Animals

The remaining six (6) normal adult male Wistar albino rats and the diabetic rats were divided into six (6) different groups, each comprising of six (n = 6) rats, as follows:

Table 1: The grouping and treatment of experimental animals

S/N	Group	Treatment	Dose
1	Normal control	distilled water	2 ml/kg
2	Diabetic control	distilled water	2 ml/kg
3	Diabetic treated	glibenclamide	5 mg/kg
4	Diabetic treated	*G. kola* extract	548 mg/kg
5	Diabetic treated	*T. conophorum* extract	524 mg/kg
6	Diabetic treated	*G. kola* + *T. conophorum*	548 + 524 mg/kg

Source: Field data (2019).

2.3. Determination of Serum Insulin concentration

The desired number of coated wells was secured in a holder. 50µL of standards, specimens and control were dispensed into appropriate wells. Estradiol biotin reagent (100 µL) was dispensed into each well and mixed thoroughly for 20-30 seconds then covered with a plastic wrap. The mixture was incubated at room temperature (20 – 27 °C) for 120 minutes. The content of microplate was discarded by decantation and the plates blotted dry with absorbent paper. Wash buffer (350 µL) was added to the wells then decanted by tapping and blotting. This was repeated twice for a total of 3 washes. 100 µL of working substrate solution was added to all the wells. The mixture was incubated for 15 minutes at room temperature. Stop solution (50 µL) was then added to each well and gently mixed for 15-20 seconds. Absorbance at 450 nm (using a reference wavelength of 620 – 630 nm) was read within 30minutes with a microplate reader (Eastham, 1985).

2.4. Tissue processing

The harvested tissues were processed for histological studies as follows:

The pancreas were fixed in 10 % neutral buffered formalin for one week then dehydrated using ascending grades (70 %, 95 % and 100 %) of alcoholfor 1.5 hours each in 2changes. Tissues were cleared of the alcohol using xylene for 1.5 hours in two changes each, and then infiltrated in molten paraffin wax in a hot air oven for 1.5 hours. The infiltrated tissues where placed in moulds where molten paraffin wax was poured and allowed to set.The embedded tissues were sectioned with the rotary microtome at 5 µm thick slices and floated on a water bath at 65 ºC. The tissue ribbons were then picked up on glass microscopic slides from the water bath and allowed to air dry for 15 minutes (Hani *et al*., 2016).

2.4.1. H & E Staining Procedure

The tissue sections were dewaxed in xylene, hydrated in descending grades (100 %, 95 % and 70 %) of alcohol and the nuclei were stained in haematoxylin for 15 minutes. Sections were rinse in running tap water till it blued, then differentiated in 1 % acid alcohol for 5 minutes. Sections were again rinsed in running tap water, counterstained in1 % eosin for 2 minutes and rinsed in running tap water. Sections were dehydrated in ascending grades of alcohol, cleared in xylene and mounted in dipolycysteinxylene (DPX) (Dhurba, 2015).

2.4.2. Avidin Biotin Complex (ABC) Immunoperoxidase Method

The paraffin sections were taken into water and rinse in phosphate buffered saline (PBS)-Tween 20 for 2 changes in 2 minutes. Antigen retrieval was performed using IHC-TekTm Epitope Retrieval solution (cat # 1w-1100). Sections were incubated in normal serum then in the insulin IHC antibody in IHC-TekTm antibody diluent (cat# 1W-1000) for 1 hour at room temperature. Sections were rinsed in PBS-Tween 20 for 3 changes in 2 minutes each. For peroxidase blocking, sections were incubated in peroxidase blocking solution for 10 minutes at room temperature then rinsed in PBS-Tween 20 for 3 changes in 2 minutes each. Sections were then incubated in biotinylated secondary antibody in PBS for 30 minutes at room temperature and rinsed in PBS-Tween 20 for 3 changes in 2 minutes each. Sections were incubated in ABC-peroxidase solution for 30 minutes at room

temperature for detection and then rinsed in PBS-Tween 20 for 3 changes in 2 minutes. Sections were incubated in peroxidase substrate solution, rinsed in PBS-Tween 20 for 3 changes in 2 minutes., Counterstain with Mayer's Haematoxylin in 30 seconds, rinsed in running tap water for 2-5 minutes, dehydrated through 95 % ethanol for 1 minute, 100 % ethanol for 2 changes in 3 minutes., Cleared in xylene for 2 changes in 5 minutes and Coverslip with mounting medium (IHCWorld, 2011).

2.5. Statistical Analysis

Data collected were organised in tabular form and presented as MEAN ± SEM. The data were analysed using IBM SPSS 20.0 (IBM Corp, 2012). The results were considered significant at ($p < 0.05$).

3. RESULTS

Table 2: Phytochemical screening of *G. kola* seed extract

Test	Observation	Inference
Alkaloids	Red ppt. observed	+ +
Flavonoids	Orange ppt. observed	+ +
Saponins	Persistent frothing observed	+
Tannins	Bluish-black colouration observed	+ + +
Cardiac glycosides:		
1.Lierbermann	Reddish brown ring observed	+
2.Keller-killiani	Brown ring formed	+
3.Salkowski	Reddish brown ring formed	+

Key: + Means slightly present, + + means moderately present, and + + + means highly present.
Source: Field data (2019).

Table 3: **Phytochemical screening of *T. conophorum* leaf extract**

Test	Observation	Inference
Alkaloids	Red ppt. observed	+ + +
Flavonoids	Orange ppt. observed	+ +
Saponins	Persistent frothing	+
Tannins	Bluish-black colouration observed	+ + +
Cardiac glycosides:		
1.Lierbermann	Reddish brown ring formed	+
2.Keller-killiani	Brownish ring formed	+
3.Salkowski	Reddish ring formed	+

Key: + means slightly present, + + means moderately present, + + + means highly present.
Source: Field data (2019).

3.1. Haematoxylene and Eosin (H&E) of the Pancreas

The H&E section of the pancreas in the normal control (group 1) shows normal islet nuclei in the islet of Langerhans (Figure 4.1).The section of the pancreas of the diabetic control (group 2) revealed pathologic alterations such as atrophied nuclei in the islet and vacuolisation (Figure 4.2). Sections of the diabetic pancreas treatedwith glibenclamide 5 mg/kg (group 3) and *G. kola* 548 mg/kg (group 4) shows restored normal islet nuclei (Figures 4.3 and 4.4 respectively) and sections of the diabetic pancreas treated with *T. conophorum* 524 mg/kg (group 5) and the combined (group 6) revealed restored normal islet nuclei ameliorated vacuolisation (Figure 4.5 and 4.6 respectively).

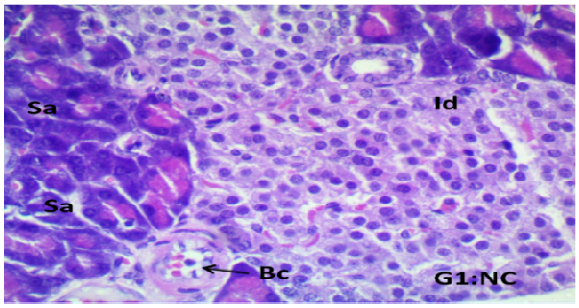


Figure 4.1

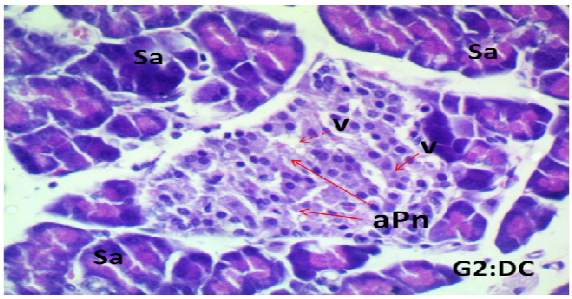
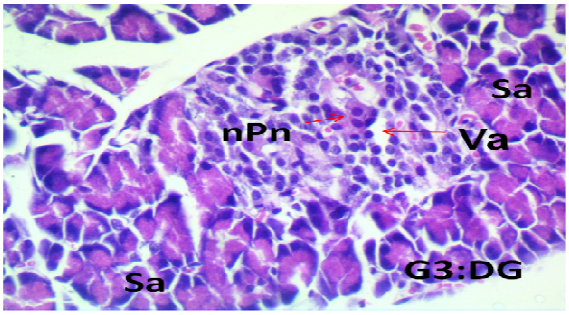


Figure 4.2



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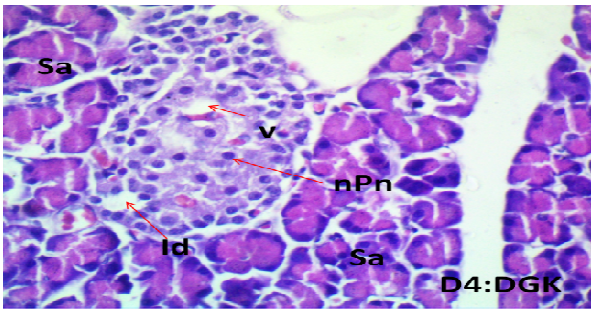

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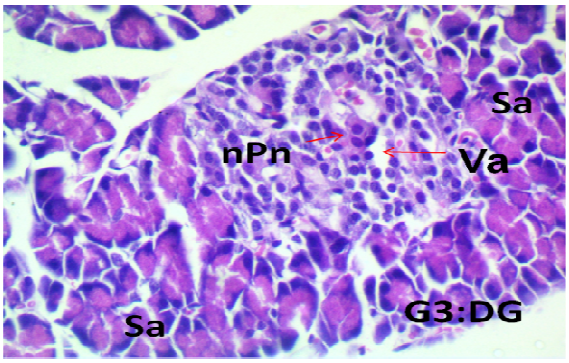

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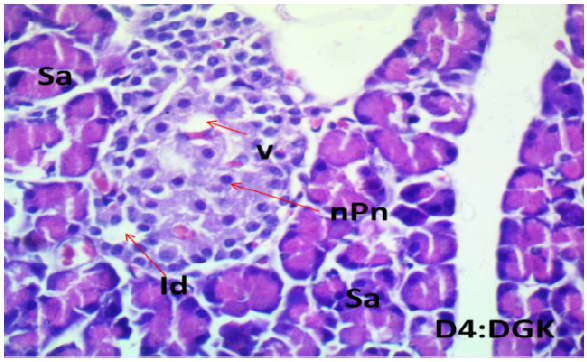

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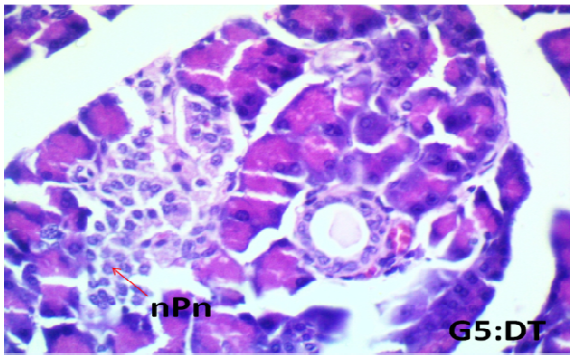

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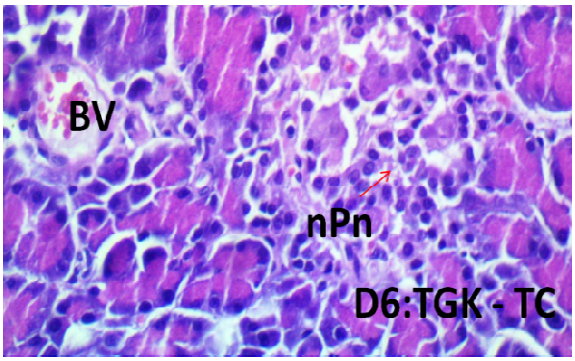


Figure 4.6

Figure 4.1: Photomicrograph of a cross-section of the pancreas of the normal control rat (group1) indicating normal islet nuclei (I), intercalated duct (Id), blood capillary (Bc) and serous acini (Sa). H&E. X 400. Source: Field data (2019).

Figure 4.2: Photomicrograph of a cross-section of the pancreas of diabetic control rat (group 2) indicating vacuolisation (v), and atrophied pancreatic nuclei (aPn).H&E. X 400. Source: Field data (2019).

Figure 4.3: Photomicrograph of a cross-section of the pancreas of diabetic rat treated with glibenclamide 5 mg/kg body weight (group 3) indicating normal pancreatic nuclei (nPn) and vacuolisation (V). H&E. X 400. Source: Field data (2019).

Figure 4.4: Photomicrograph of a cross-section of the pancreas of diabetic rat treated with *G. kola* 548 mg/kg

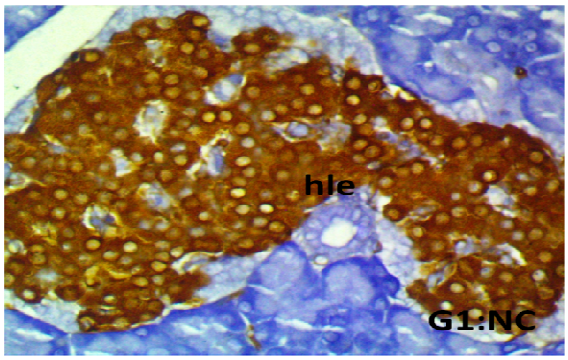
body weight (group 4) indicating normal pancreatic nuclei (nPn), vacuolisation (V) and normal intercalated duct (id). H&E. X 400. Source: Field data (2019).

Figure 4.5: Photomicrograph of a cross-section of the pancreas of diabetic rat treated with *T.conophorum* 524 mg/kg body weight (group 5) indicating normal pancreatic nuclei (nPn). H&E. X 400. Source: Field data (2019).

Figure 4.6: Photomicrograph of a cross-section of the pancreas of diabetic rat treated with *G. kola* 548 mg/kgand *T. conophorum* 524 mg/kg (group 6) indicating normal pancreatic nuclei (nPn). H&E. X 400. Source: Field data (2019).

3.2. Immunohistochemistry (Insulin Antibody) of the Pancreas

A section of the pancreas of the normal control (group 1) shows high insulin expression (Figure 4.7), while a section of the pancreas of the diabetic control (group 2) shows very weak insulin expression (Figure 4.8). A section of the pancreas of diabetic rat treated with glibenclamide (group 3) shows strong insulin expression (Figure 4.9), while sections of the pancreas of diabetic rats treated with *G. kola* seed, *T. conophorum* leaf and the combined extracts demonstrated moderate insulin expressions (Figures 4.10 to 4.12 respectively).



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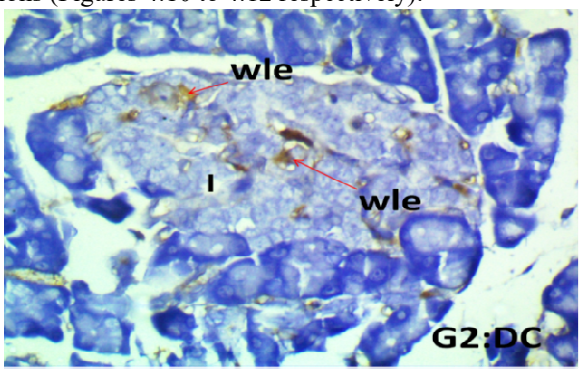

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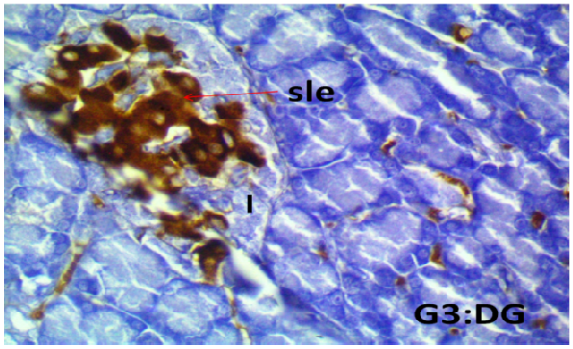

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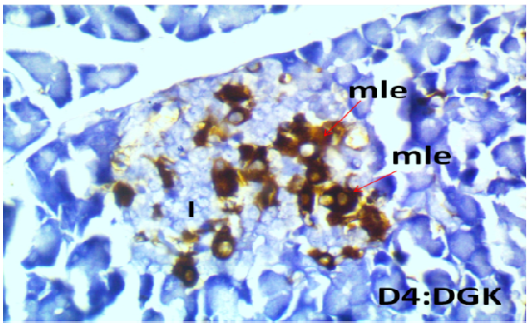
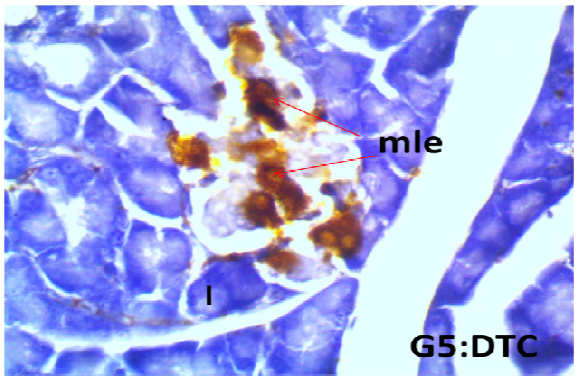


Figure 4.10



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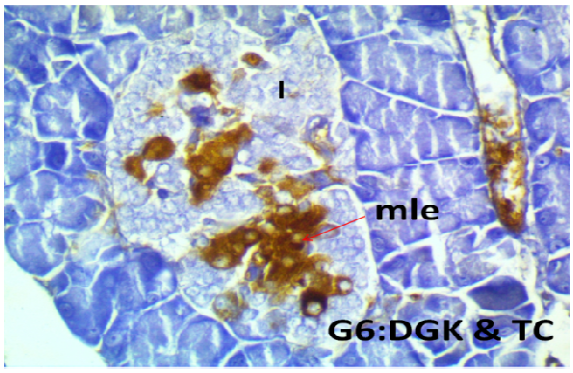

Figure 4.12

Figure 4.7: Photomicrograph of a cross-section of the pancreas of normal control (group 1) indicating high insulin expression (hIe). IHC (insulin antibody). X 400. Source: Field data (2019).

Figure 4.8: Photomicrograph of a cross-section of the pancreas of diabetic control at (group 2) indicating very weak insulin expression (wIe) in the islet of Langerhans (l). IHC (insulin antibody). X 400. Source: Field

data (2019).

Figure 4.9: Photomicrograph of a cross-section of the pancreas of diabetic rat treated with glibenclamide 5 mg/kg bodyweight (group 3) indicating strong insulin expression (sIe) in the islet of Langerhans (l). IHC (insulin antibody). X 400. Source: Field data (2019).

Figure 4.10:Photomicrograph of a cross-section of the pancreas of diabetic rat treated with G. Kola 548 mg/kg body weight (group 4) indicating moderate insulin expression (mIe) in the islet of Langerhans (l). IHC (insulin antibody). X 400. Source: Field data (2019).

Figure 4.11: Photomicrograph of a cross-section of the pancreas of diabetic rat treated with T. conophorum 524 mg/kg body weight (group 5) indicating moderate insulin expression (mIe) in the islet of Langerhans (l). IHC (insulin antibody). X400. Source: Field data (2019).

Figure 4.12: Photomicrograph of a cross-section of the pancreas of diabetic rat treated with G. kola 548 mg/kgand T. conophorum 524 mg/kg (group 6) indicating moderate insulin expression (mIe) in the islet of Langerhans (l). IHC (Insulin Antibody). X 400. Source: Field data (2019).

3.3. Biochemical Studies

3.3.1. Effect of Glibenclamide, *G. kola* Seed Extract, *T. conophorum* Leaf Extract and Combined Extracts on the Fasting Blood Glucose

Treatment groups; 3 to 6 had a significant ($p < 0.05$) reduction in the FBG levels compared with the diabetic control (group 2). All the diabetes induced groups: 2 to 6 had a significantly ($p < 0.05$) higher FBG levels compared with the normal control (group 1). Glibenclamide (5 mg/kg, group 3) reduced FBG from 22.10 ± 1.65 mmol/L to 4.48 ± 0.29 mmol/L, *G. kola* seed extract (548 mg/kg, group 4) lowered FBG from 22.04 ± 4.06 mmol/L to 7.40 ± 2.41 mmol/L, *T. conophorum* leaf extract (528 mg/kg, group 5) reduced FBG from 14.26 ± 2.38 mmol/L to 5.98 ± 0.57 and the combined *G. kola* seed and *T. conophorum* leaf extracts (group 6) reduced FBG from 17.54 ± 1.72 mmol/L to 11.58 ± 2.11mmol/L. At base line, it was found that the highest FBG level was in group 3 (22.10 ± 1.65) and the lowest in group 1 (6.08 ± 0.42) giving a range of 16.02 ±1.23 mmol/L (Table 4.3) (Appendix III)

Table 4. Effect of glibenclamide, *G. kola* Seed extract *T. conophorum* leaf Extract and Combined Extracts on Fasting Blood Glucose (mmol/L)

Group	BL (mmol/L)	Week 1 (mmol/L)	Week 2 (mmol/L)	Week 3 (mmol/L)
1	6.08 ± 0.42	6.14 ± 0.47	5.96 ± 0.44	5.60 ± 0.17
2	20.92 ± 2.98	27.22 ± 2.00	23.72 ± 3.10	24.46 ± 1.59
3	22.10 ± 1.65	16.54 ± 3.47	6.30 ± 1.53	4.48 ± 0.29
4	22.04 ± 4.06	8.36 ± 2.70	7.03 ± 1.92	7.40 ± 2.41
5	14.26 ± 2.38	8.46 ± 2.65	4.90 ± 0.39	5.98 ± 0.57
6	17.54 ± 1.72	17.26 ± 2.71	7.36 ± 1.34	11.58 ± 2.11
F. value:	6.29	9.99	17.24	25.02
P. value:	0.0001	0.0001	0.0001	0.0001

Key: BL = Base line (3rd day following diabetes induction). Values are expressed as mean ± Standard error of mean (SEM).
Source: Field data (2019).

4. DISCUSSION

The pancreas plays an important role in the homeostasis of glycaemia in the body. It does this through the secretion of two important hormones – insulin and glucagon (Barbara *et al*., 2006; Saladin, 2012). Insulin insufficiency or resistance is known to cause diabetes mellitus (Saladin, 2012). The β – cells of the islet of Langerhans are responsible for the production of insulin in the pancreas. The i.p. administration of a single dose of STZ (45 mg/kg bw) might have caused a significant but incomplete destruction of the β – cells resulting to diabetes mellitus as indicated by the insulin assay.

The H&E section from the diabetic control (group 2) shows marked pathological vacuolisation and atrophied pancreatic nuclei as shown in Figure 4.2, compared with the normal control (group 1) which shows normal islet morphology (Figure 4.1). Following the 21 days of treatment, it was found that glibenclamide was able to normalise the islet nuclei but could not correct the vacuolisation (Figure 4.3). *G. kola* seed (548 mg/kg) restored normal pancreatic nuclei but still had mild vacuolisation (Figure 4.4). *T. conophorum* leaf (524 mg/kg) restored pancreatic nuclei and and ameliorated the vacuolisation (Figure 4.5). The combined treatment ameliorated the pathological altrations as observed in (Figure 4.6). Diabetes mellitus have been reported to cause histological alteration such as vacuolisation, disturbance of islet shape and acini arrangement, pyknotic nuclei,

lymphocytes infiltration and degranulation of β – cells in the pancreas in rats (Adib, 2000; El-Desouki *et al*., 2007; Azza, 2009). This result is in agreement with Ahmed *et al.* (2010), Gupta *at el*. (2016) and Prakash *et al.* (2017) who reported that glibenclamide prevented the destruction of β- cells and increase the number of β-cells and size. The result is also in tandem with Lepzem and Tongun (2017). The amelioration of the pathological changes in the pancreas by the plants extracts may be due to the presence of the phytoconstituents. Secondary metabolites like flavonoids, alkaloids and saponnins are antioxidants and free radical scavengers which have cleansing effects and enhance functions of organs (Kevin, 2013; Mazi *et al*., 2013; Akomolafe *et al*., 2017; Salau *et al*., 2017).

The immunochemical study of the pancreas using insulin – antibody revealed that the normal control (group 1) Figure 4.7 demonstrated high insulin expression compared with the diabetic control (group 2) Figure 4.8, which had very weak insulin expression. Treatment with glibenclamide 5 mg/kg (group 3), Figure 4.9, shows a strong insulin expression while *G. Kola* seed 548 mg/kg (group 4) Figure 4.10, *T. conophorum* leaf 524 mg/kg (group 5) Figure 4.11 and the combined (group 6) Figure 4.12, had moderate insulin expression. The result of the immunohistochemical study correlates positively with the findings of the H&E. The attenuation of the islet pathological alterations by the plants extracts and the glibenclamide might have led to the corresponding insulin expressions. It also agrees with the biochemical results which demonstrated that among the treatment groups, glibenclamide had the highest serum insulin level, followed by *G. kola* seed, *T. conophorum* leaf and the combined. However, the glibenclamide treatedgroupdid not correlate with the normal control which demonstrated the highest insulin expression, but had a lower insulin levels in the serum. This may be due to the fact that though the number of β – cells and size of islet have been reduced in the glibenclamide group by the STZ, the glibenclamide was able to stimulate the residual and newly regenerated β – cells to secrete insulin even more than the normal control group irrespective of the size of the islet that might be responsible for the insulin expression in the normal control.

The hypoglycaemic properties exhibited by *G. kola* seed and *T. conophorum* leaf may be attributed to the presence of the phytochemicals. Flavonoids are antioxidants and free radical scavenger (Mazi *et al*., 2013; Akomolafe *et al*., 2017).Saponins have cleansing effect which enhances liver and gall bladder functions (Kevin, 2013). Phytochemical such as contained in *G. kola* and *T. conophorum* have been said to have anti-diabetic effects through different mechanisms (Malviya *et al*., 2010). These could be through reduction of intestinal absorption of carbohydrate, enzymes modulation, improvement of β - cells and insulin functions, and stimulation of insulin secretion (Bashar *et al*., 2017).

The result of the insulin profile shown in Table 4.4 indicates that STZ caused a significant ($p < 0.05$) reduction in the serum insulin level. This might be due to the partial destruction of the β - cells by STZ through its different mechanisms (Ozturk *et al*., 1996; Ho *et al*., 2000; Szkudelski, 2001; Mythili *et al*., 2004). It was observed that glibenclamide significantly ($p < 0.05$) increase serum insulin even higher than the normal rats. *G. kola* seed, *T. conophorum* leaf and their co-administration significantly ($p < 0.05$) increased the insulin level compared with the diabetic control (group 2) but not up to the normal rats. This implies that glibenclamide was a better insulinotropic agent than the extracts. Again, that the hypoglycaemic activities of *G. kola* seed*, T. conophorum* leaf, and the co-administration might be attributed to other mechanisms in addition to the stimulation of insulin secretion (Malviya *et al*., 2010; Bashar *et al*., 2017). Therefore, the improvement of the kidney function and amelioration of renal damages by *G. kola* seed, *T. conophorum* leaf, and their combination is an added advantage of these extracts over glibenclamide in the management of diabetes mellitus.

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