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Original Research Article

The Kidney of Diabetic Wistar Rats Administered *Garcinia kola* and *Tetracarpidium conophorum* Extracts: Histological Cum Biochemical Perspectives

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Abstract

This study investigated the histological and biochemical effects of *G. kola* and *T.conophorum* extracts, in diabetic Wistar rats. Phytochemical and LD_{50} of the extracts were determined. Thirty-six (36) adult male Wistar rats weighing 180 – 200 g were divided into 6 groups (n =6). Group 1 served as normal control, group 2 served as diabetic control while groups 3 – 6 were diabetic rats treated with glibenclamide (5 mg/kg body weight (bw)), *G. kola* (548 mg/kg bw), *T. conophorum* (524 mg/kg bw) and combined (*G. kola* and *T. conophorum*) respectively. At the end of the experiment, the animals were anaesthesized and with cardiac puncture, blood was obtained for biochemical studies while the kidneys were harvested for the histological analysis. 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. *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. 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: Kidney of Diabetic Wistar Rats, *Garcinia Kola*, *Tetracarpidium conophorum* Extracts. Streptozotocin, Glibenclamide, Blood.

INTRODUCTION

The word diabetes is derived from Latin and ancient Greek and literally means "a passer through; a siphon". This was based on the traditional belief that in this disease all fluids consumed rapidly pass out through the body as urine, thus, causing polyuria. The word 'mellitus' comes from Latin and means honey-sweet. Mellitus was added to diabetes by Thomas Wills in 1675. Thomas also discovered that the urine of diabetic patients was sweet by tasting it.

Diabetes mellitus affects several organs in the body such as pancreas, liver, testes, kidneys, eyes and so forth (IDF, 2017). The kidney is responsible for the production of urine through which many harmful waste products of metabolism are excreted. It maintains water and electrolytes balance and excrete many drugs or their breakdown products through urine. In diseased conditions, urine can contain glucose (in diabetes mellitus), or proteins (in kidney disease) (Singh, 2002). Chronic high blood glucose concentration together with high blood pressure can cause diabetes nephropathy (Kara, 2014). Hyperglycaemia induces hyperfiltration which causes progressive kidney disease, and morphologic changes in the kidneys that ultimately lead to podocytes damage and loss of filtration surface (Fakhruddin *et al*., 2017).

Its chronic microvascular complications are nephropathy, neuropathy and retinopathy (IDF, 2017), while chronic macrovascular complications are coronary artery disease leading to myocardial infarction, stroke, diabetic encephalopathy, foot ulcer, erectile dysfunction (WHO, 2016; IDF, 2017).

Some medicinal plants have been demonstrated to be useful in the management of diabetes worldwide and have been used empirically as anti-diabetic and antihyperlipidemic remedies. The plants may act on blood glucose in different ways; some of them may have insulin – like substance, some may inhibit insulinase activity, others may cause increase beta cells in the pancreas by activating regeneration of these cells (Gholamali *et al*., 2007). The hypoglycaemic effects of phytochemicals are mainly attributed to reduce intestinal absorption of dietary carbohydrate, modulation of the enzymes involved in glucose metabolism, improvement of β-cells functions and insulin action and stimulation of insulin secretion (Bashar *et al*., 2017). Over 400 to 1,000 plant species having hypoglycaemic activities have been identified in different literatures. However, search for new anti-diabetic plants is still attractive because they contain substances which demonstrate alternative and safe effects in the treatment of diabetes mellitus (Malviya *et al*., 2010; Coman *et al*., 2012). Most of these medicinal plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids etc that are frequently implicated as having anti-diabetic effects (Malviya *et al*., 2010).

Wall nut *Tetracarpidum conophorum* is mainly cultivated for the nuts which are cooked or roasted and eaten as snacks. It produces a bitter taste upon drinking water immediately after eating the nut. This could be as a result of the presence of alkaloid in it (Edem *et al*., 2009). The fruits are greenish with three round seeds in each fruit. The seed testa is hard with white cotyledons. The leaves, bark, and fruit of *T. conophorum* are used medicinally as masticatory, giddiness, thrush, antihelminthic, antidiabetic, antimicrobial, antifungal, toothache, syphilis, dysentery and as antidote to snakebite and as male fertility agent (Amaeze *et al*., 2011; Donatus *et al*., 2014; Aneke *et al*., 2016; and Lepzem and Tongun, 2017).

Bitter kola **(***Garcinia kola)* seeds and bark of the plant have been used for centuries in folk medicine to treat ailments ranging from cough to fever (Adaramoye, 2012; Traci, 2017). A number of studies have shown that walnut and bitter kola have a significant antihyperglycaemic effect (Ofor *et al*., 2013; Donatus *et al*., 2014; Chinwe *et al*., 2015; Ghiravani *et al.*, 2016). Kolaviron has been isolated to be the component of *G. kola* with the hypoglycaemic property in diabetic induced rats (Iwu *et al*., 1990; Adaramoye, 2012).

The kidneys are affected by diabetes mellitus (Dahiru *et al*., 2016; IDF, 2017). 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 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 kidney. Therefore, this study investigates the histological and biochemical alterations in the 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* leaf extracts on the kidney 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 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 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 treatments.
iv. The electrolytes, urea and creatinine of diabetic rats treated with glibenclamide, combination of *G. kola* seed and *T. conophorum* leaf extracts and their single treatments.

This study is justified because it might provide an alternative to the conventional drugs used in the management of kidney alterations as a result of diabetes mellitus. Since these plants are normally eaten, they may not have side effects. These plants are readily available and acceptable by the people who have been using them for different reasons. As such, it may be useful in reduction of the cost involved in the management of diabetes mellitus.

This study provides data on the histological and biochemical alterations in the 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.

EXPERIMENTAL SECTION/MATERIAL AND METHODS

The plants used in this study were collected and extracted, phytochemical screening and mean lethal dose (LD_{50}) of the extracts were performed. Animals were purchased, induced with diabetes and grouped in different cages.

The fresh seeds of *G. kola* and the leaves of *T. conophorum* were weighed separately using a weighing balance,chopped into smaller pieces with a knife, air dried for one week and ground into powder using a Mortar and Pestle. Eight hundred and ninety- eight (898) grammes of dried powdered *G.kola* seed and one hundred and fifty-four (154) grammes of the dried powdered *T. conophorum* leaves were extracted via soxhlet extraction technique on two separate setups. In this method, the apparatus was set up such that the dried powdered *G. kola* seeds and *T. conophorum* leaves were placed in the thimble. The thimble wasthen loaded into the main chamber of the soxhlet extractor. The extraction solvent – absolute ethanol was poured bit by bit through the thimble containing the plants, to the distillation flask. The flask was placed on the heating element. The soxhlet extractor was placed on top the flask and the reflux condenser was atop the extractor (James *et al*., 2014).

During the extraction process, the solvent being the absolute ethanol was heated and the vapour rose up the condenser which cooled the vapour into warm liquid solvent thatdripped back down into the soxhlet extractor chamber containing the thimble and the extraction material (*G. kola* seeds and *T. conophorum* leaf). When the soxhlet chamber was almost filled, it was emptied via the siphon into the distillation flask. This was allowed to repeat for 3 days with each cycle dissolving the non – volatile compounds of the *G. kola* seeds and the *T. conophorum* leaf into the solvent which was concentrated in the flask. Upon completion of the extraction, the solvent was removed using a rotary evaporator.

The extracts were screened of the various metabolites using standard methods of Trease and Evans (1989) and Sofowora (1993). The LD_{50} of the extracts were determined using modified Lorke's (1983) method. The *T. conophorum* extract was given in different doses of 500 mg/kg, 1000 mg/kg, 1500 mg/kg, 2500 mg/kg, 2750 mg/kg, 3000 mg/kg and 5000 mg/kg body weight, while the *G. kola* was given in doses of 500 mg/kg, 1000 mg/kg, 1500 mg/kg, 2000 mg/kg, 2500 mg/kg, 3000 mg/kg, and 5000 mg/kg body weight to mice, three in each group. Forty-two mice were used for the study. The extracts were prepared by dissolving in distilled water and were administered intraperitoneally according to body weight of the mice after overnight fasting.

Thirty six (36) adult male Wistar albino 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 acclimatize 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.

Thirty (30) adult maleWistar 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.

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

Table-1: 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/k
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).			

The extracts, glibenclamide and the distilled water were administered through oral-gastric intubation method using a cannula and asyringe. Each animal in the treatment groups was administered a volume of the extract in accordance with the prescribed dosage for its group for a period of twenty-one (21) days. Group 1 contained normal rats and served as normal control and recieved placebo, 2 mL distilled water per kg body

weight, group 2 had diabetic rats administered 2 mL/kg distilled water and served as the diabetic control, group 3 was treated with glibenclamide 5 mg/kg once daily (Cheng *et al*., 2012; Ogugua and Nwafor, 2017). Group 4 was treated with *G. kola* seed extract 548 mg/kg body weight once a day, group 5 was treated with *T.conophorum* leaf extract 524 mg/kg body weight once daily while group 6 was treated with *G. kola* seed (548 mg/kg) and *T. conophorum* leaf (524 mg/kg) extracts once daily. The extracts dosage represents the medium dose calculated from the LD_{50} (Appendix II).

The weight of the rats was measured with a weighing balance on the first day, then every seventh (7th) day in a week till the end of the twenty-one (21) days of the experiment. Blood samples from the rats in all the groups were collected once a week – on the 7th day, through the tail vein of the rats for the measurement of the fasting blood glucose levels using a glucometer and strips throughout the experiment period. Measurements of the FBG were done after overnight fasting of the rats. At the end of the experiment, the animals were anaesthetized using chloroform. Blood samples from all the groups were collected by cardiac puncture through the right ventricle into plane tubes and centrifuged at 4000 rpm for 15 minutes, from where the serum was obtained for the biochemical analysis of the kidney function test and insulin profile assay. The kidneys were also harvested and processed using routine histological processing techniques for the histological studies.

Determination of Sodium Concentration in Serum**:** This method was performed as described by Agappe diagnostics based on Tietz (1994). Sodium is precipitated as the triple salt, sodium magnesium uranyl acetate, with the excess uranium then being reacted with ferrocyanide to produce a chromophore whose absorbance (colour intensity) varies inversely as the concentration of sodium in the test specimen.

$$\text{Uranyl ions} + Mg^{+} + Na^{+} \longrightarrow \text{Uranyl Mg Na Precipitate} \qquad \text{Equation 3.1}$$

$$\text{Free Uranyl ions} + K_4Fe(CN)_6 \longrightarrow \text{Brown coloured Complex} \qquad \text{Equation 3.2}$$

10µL of serum was drawn into plastic tubes using a micropipette, 1000 µL of sodium R1 (precipitating reagent) was added, shaken vigorously and incubated at room temperature for 5 minutes, then centrifuged at 2000rpm for 2 minutes to obtain a clear supernatant. 20 µL of the supernatant was transferred immediately after centrifugation and 1000 µL of Sodium R2 (colour reagent), mixed well and allowed to stand at room temperature for 5 minutes. The absorbance of the standard and sample against reagent blank was measured.

Determination of Concentration of Potassium in Serum**:** Based on Tietz (1994) as described by Agappe diagnostics, sodium tetraphenylboron produces a colloidal suspension in the presence of potassium. The turbidity of which is proportional to potassium concentration in the range of 2-7 mEg\L.

$$Na^{+}\ \text{Tetraphenylborate} + K^{+} \longrightarrow K^{+}\text{- Tetraphenylborate} + Na^{+} \qquad \text{Equation 3.3}$$

About (25 µL of serum was added to 1000 µL of potassium reagent was mixed and incubated for 5 minutes at room temperature. The absorbance of standard (Abs.S) and test sample (Abs.T) was recorded against distilled water was read at 578 nm within 10 minutes.

Determination of concentration of chloride in serum**:** Chloride ions in the serum react with mercuric thiocyanate to release free thiocyanate ions, which forms a coloured complex with ferric ions with colour intensity proportional to the chloride concentration in sample:

$$6\,Cl^{-} + 3Hg(SCN)_2 \longleftrightarrow 3HgCl_2 + 6(SCN)^{-} \qquad \text{Equation 3.4}$$

$$6(SCN + 2Fe^{3+} \xrightarrow{q11} 2Fe(SCN)_3 \qquad \text{Equation 3.5}$$

About 1000 µL of reagent was added to 10 µL of sample, mixed well and incubated for 1 minute at room temperature. The absorbance of sample and standard was measured against the reagent blank.

Determination of urea concentration**:** Based on Weatherburn (1967), urea is hydrolysed to ammonia and carbon IV oxide in the presence of enzyme urease. The ammonia whose concentration is in proportion to the initial concentration of urea in sample is quantitated photometrically by Berthelot's reaction.

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2 \qquad \text{Equation 3.6}$$

$$NH_3 + \text{hypochloride} + \text{phenol} \longrightarrow \text{indophenol (blue compound)} \qquad \text{Equation 3.7}$$

10 µLserum was added to 1 mL of the reagent and the change in absorbance was recorded at 340 nm for 3 minutes. For standard, 10 µL standard solution of urea (40 %) was added to 1 mL reagent.

Determination of creatinine concentration**:** According to Haeckel (1981), creatinine reacts with picric acid to produce a colored compounds creatinine alkaline picrate. The creatinine concentration is proportional to the change in absorbance.

1 mL of reagent was added to 100 µL of sample, mixed well and absorbance was read at 505 nm after 60 seconds (T1) second reading (T2) taken after 60 seconds from the first one.

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).

Histological Analysis (Staining Techniques)

The kidneys 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 ^{o}C. 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).

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 dipolycystein xylene (DPX) (Dhurba, 2015).

Periodic Acid Schiff (PAS) Staining Procedure: The kidney sections were deparaffinised in xylene and hydrated in distilled water. They were then immersed in periodic acid solution for 10 minutes and rinsed in running tap water for 3 minutes. Slides were now taken to Schiff's solution for 20 minutes and then rinsed in running tap water for 10 minutes. Tissue sections were dehydrated in graded alcohols, cleared in xylene and mounted in synthetic resin (Kiernan, 1990).

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

Ethical permit was obtained from the Faculty Animal Care and Use Committee (FACAUC) of the Faculty of Basic Medical Sciences, before the commencement of the research work with the rats.

RESULTS AND DISCUSSION

RESULTS

The extraction of 898 g of the dried powdered *G. kola* seeds yielded 120.2 g of the dried extract, while 154 g of the dried powder of the *T. conophorum* leaf yielded 33.9 g. The two extracts were preserved in the refrigerator at 4 ^{o}C to reduce microbial activities until it was used for the treatment.

The phytochemicals found in the ethanolic extract of *G. kola* seed are alkaloids, flavonoid, saponins, tannin, and cardiac glycosides such as terpenoids, steroids and cardenolides as shown in Table 2.

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

The ethanolic leaf extract of *T. conophorum* was found to contain alkaloids, flavonoid, saponin, tannin, and cardiac glycosides such as terpenoids, steroids and cardenolides as shown in Table 3.

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

Upon the administration of the *G. kola* seeds ethanolic extract in its different doses, mice administered 3,000 mg/kg of body weight (bw) and above showed signs of toxicity and later died, while those given 2,500 mg/kg bw and below all survived. Thus, the lowest dose that caused mortality was taken to be 3,000 mg/kg bw and the highest dose that did not cause mortality was taken to be 2,500 mg/kg. The LD_{50} of the *G. kola* seeds extract was then calculated to be 2,738.6 mg/kg bw. In the case of *T. conophorum*, the lowest dose that produced mortality was 2,750 mg/kg bw while the highest dose that did not cause mortality was 2,500 mg/kg. Thus, the LD_{50} of *T.conophorum* leaf extract was determined to be 2,622.02 mg/kg bw.

Haematoxylin and Eosin (H&E) section of the kidney of the normal control (group 1) shows normal glomerulus, urinary space and tubules (Figure 1). A section of the diabetic control (group 2) reveals morphological changes such as atrophied glomerulus, dilated and distorted urinary space, distorted tubules, haemorrhagic pool and splitting of the glomerulus (Figure 2). A section of the kidney treated with glibenclamide shows hypertrophied glomerulus, narrowed and discontinued urinary space, distorted tubules and haemorrhagic pool (Figure 3). Sections of the kidney treated with the *G. kola* seed, *T.conophorum* leaf and the combination of *G. kola* and *T. conophorum* extracts in groups 4 to 6 respectively, show amelioration of these pathologic alterations to levels comparable with the normal control except the mildly distorted tubules that were still present (Figures 4 to 5 respectively).

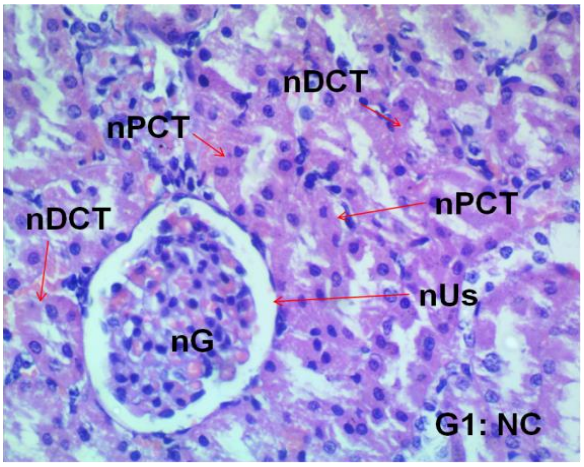

Fig-1: Photomicrograph of a cross-section of the kidney of normal control (group 1) indicating normal glomerulus (nG), normal urinary space (nUs), normal distal convoluted tubule (nDCT) and normal proximal convoluted tubule (nPCT). H&E. X 400.
Source: Field data (2019).

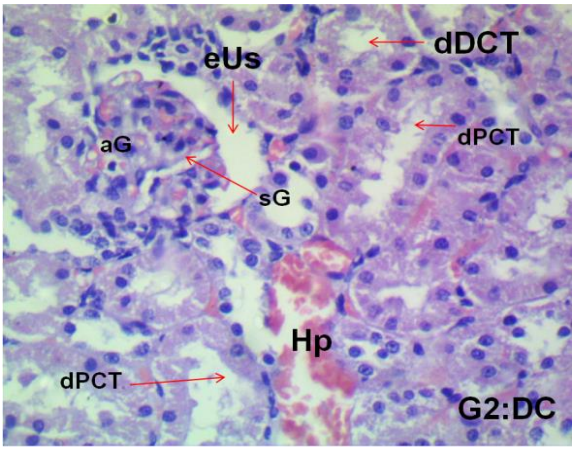

Fig-2: Photomicrograph of a cross-section of the kidney of diabetic control (group 2) indicating atrophied glomerulus (aG), expanded urinary space (eUs), splittedglomerulus (sG), haemorrhagic pool (Hp), distorted proximal

convoluted tubule (dPCT) and distorted distal convoluted tubule (dDCT). H&E. X 400.
Source: Field data (201).

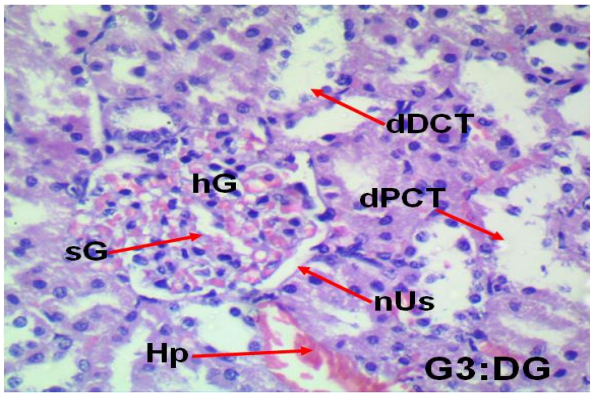

Fig-3: Photomicrograph of a cross-section of thekidneyof diabetic rat treated with glibenclamide 5 mg/kg body weight (group 3) indicating hypertrophied glomerulus (hG), narrowed and distorted urinary space (nUs), splitting of glomerulus (sG), haemorrhagic pool (Hp), distorted proximal convoluted tubule (dPCT), and distorted distal convoluted tubule (dDCT). H&E. X 400.
Source: Field data (2019).

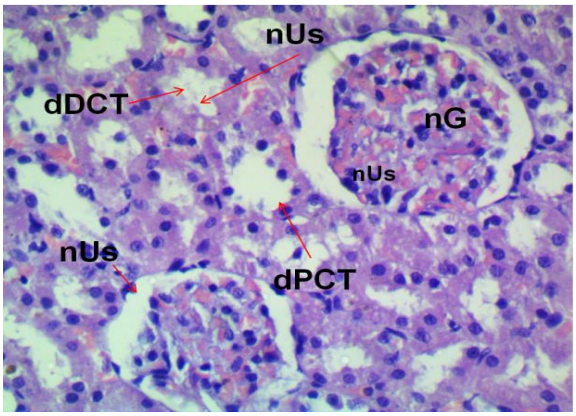

Fig-4: Photomicrograph of a cross-section of the kidney of diabetic rat treated with *G. kola* 548 mg/kg body weight (group 4) indicating normal urinary space (nUs), normal glomerulus (nG), improved distal convoluted tubule (iDCT), mildly distorted distal convoluted tubule (dDCT) and proximal convoluted tubule (dPCT). H&E. X 400.
Source: Field data (2019).

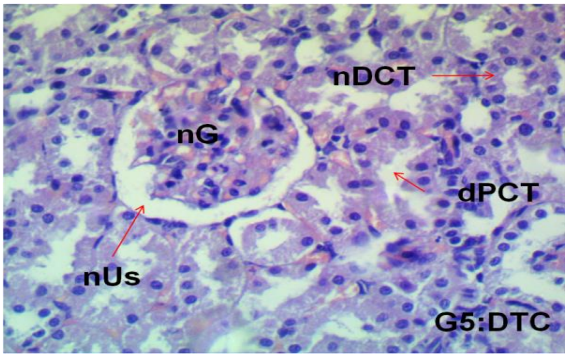

Fig-5: Photomicrograph of a cross-section of the kidney of diabetic rat treated with *T. conophorum* (524 mg/kg)

extract (group 5) indicating normal glomerulus (nG), normal urinary space (nUs), normal distal convoluted tubule (nDCT) and mildly distorted proximal convoluted tubule (dPCT). H&E. X 400.
Source: Field data (2019).

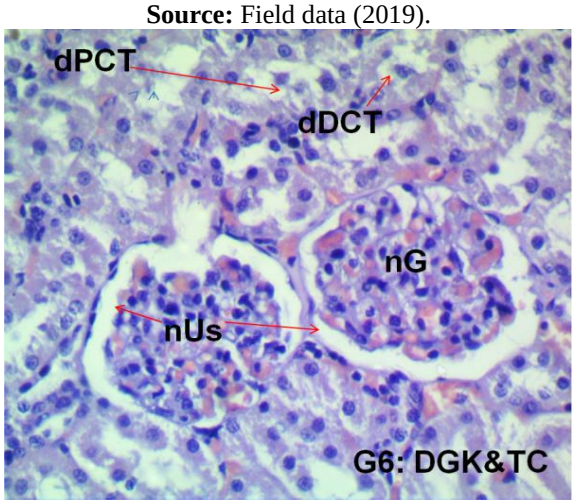

Fig-6: Photomicrograph of a cross-section of the kidney of diabetic rat treated with *G. kola* (548 mg/kg) and*T. Conophorum* (524 mg/kg) extracts (group 6) indicating normal glomerulus (nG), normal urinary space (nUs), mildly distorted proximal convoluted tubule (dPCT) and mildly distorted distal convoluted tubule (dDCT). H&E. X 400.
Source: Field data (2019).

Periodic Acid Schiff (PAS) section of the kidney of the normal control (group 1) shows moderate glycogen expression in the glomerulus and tubules (Figure 7). The diabetic control (group 2) demonstrates high glycogen expression (Figure 8). The section of the kidney treated with glibenclamide (group 3) shows high glycogen expression (Figure 9), while the sections treated with *G. kola* seed (group 4), *T. conophorum* leaf (group 5) and the combination of *G. kola* seed and *T. conophorum* leaf extracts (group 6) show moderate glycogen expression respectively (Figures 10 to 12, respectively).

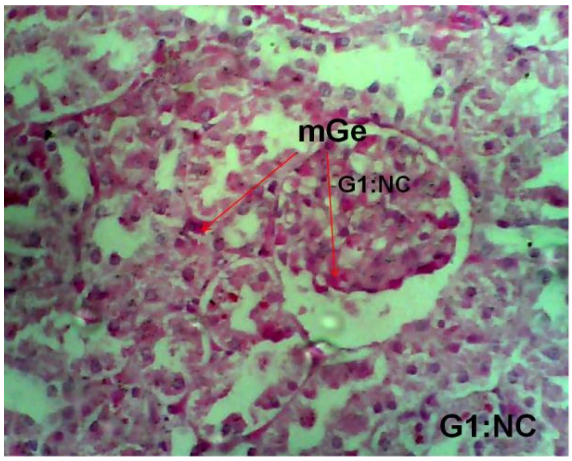

Fig-7: Photomicrograph of a cross-section of the kidney of normal control rat (group 1) indicating moderate glycogen expression (mGe). PAS. X 400.

Source: Field data (2019).

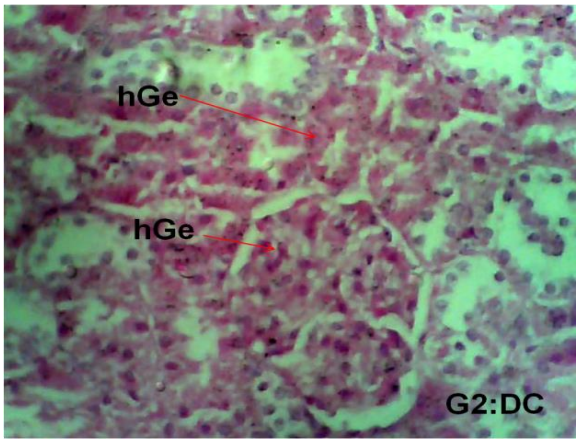

Fig-8: Photomicrograph of a cross-section of the kidney of diabetic control rat (group 2) indicating high glycogen expression (hGe). PAS. X 400.
Source: Field data (2019).

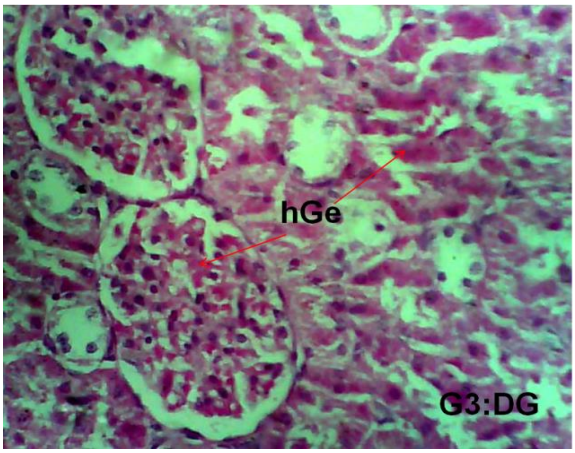

Fig-9: Photomicrograph of a cross-section of thekidney of diabetic rat treated with glibenclamide 5 mg/kg body weight (group 3) indicating high glycogen expression (hGe). PAS. X 400.
Source: Field data (2019).

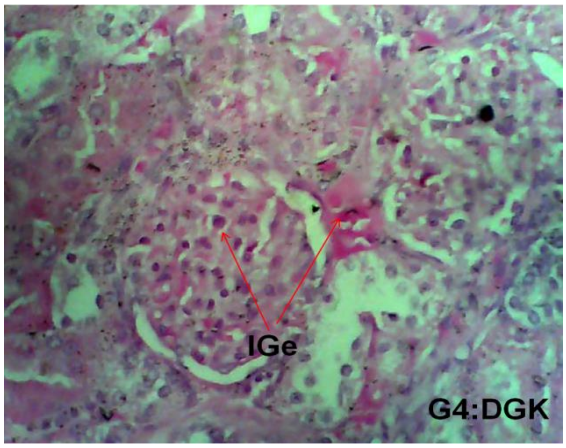

Fig-10: Photomicrograph of a cross-section of the kidney of diabetic rat treated with *G. kola* 548 mg/kg body weight (group 4) extract indicating low glycogen expression (lGe). PAS. X 400.

Source: Field data (2019).

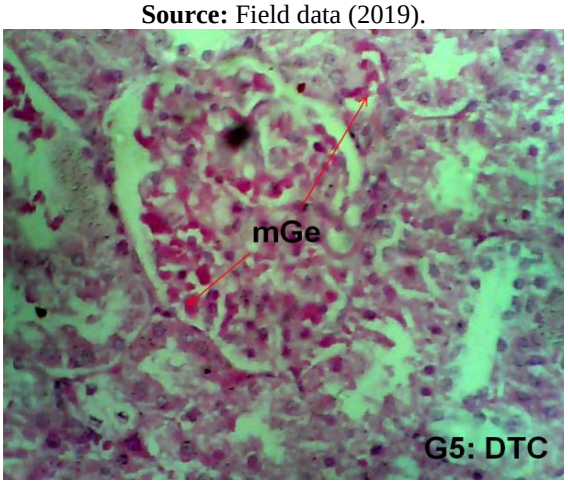

Fig-11: Photomicrograph of a cross-section of the kidney of diabetic rat treated with *T. conophorum* extract 524 mg/kg body weight (group 5) indicating moderate glycogen expression (mGe). PAS. X 400.
Source: Field data (2019).

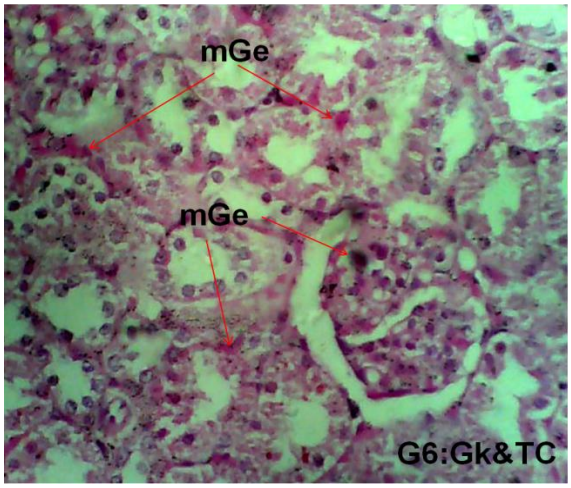

Fig-12: Photomicrograph of a cross-section of the kidney of diabetic rat treated with *G. kola* 548 mg/kg body weight and *T. conophorum* 524 mg/kg (group 6) indicating moderate glycogen expression (mGe). PAS. X 400.
Source: Field data (2019).

Effect of Glibenclamide, *G. kola* Seed Extract, *T. conophorum* Leaf Extract and Combined Extracts on the Serum Electrolytes, Urea, Creatinine and Insulin. The serum assay for some electrolytes, urea, creatinine and insulin revealed a significant ($p < 0.05$) reduction in sodium, chloride, creatinine and insulin but increase in urea, and an insignificant reduction in potassium in the diabetic control (group 2) compared with the normal control (group 1). Treatment with glibenclamide (5 mg/kg, group 3) had no significant effect on the electrolytes, urea and creatinine but significantly increased the insulin level above all the groups. *G. kola* seed (548 mk/kg, group 4) significantly ($p < 0.05$) reduced the urea, and increased the creatinine, chloride and insulin levels; *T. conophorum* leaf (524 mg/kg, group 5) increased the sodium, chloride, insulin and

reduced the urea levels; and the combined (group 6) reduced the urea and increased the sodium and insulin levels significantly as shown in Table 4).

Table-4: Effect of glibenclamide, *G. kola* seed extract *T. conophorum* leaf extract and combined extracts on the serum electrolytes, urea, creatinine and insulin

Group/ Electrolytes	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mg/dl)	Urea (mg/dl)	Creatinine (mEq/L)	Insulin (µIU/ml)
1	80.71 ± 2.65	9.24 ± 0.42	123.98 ± 0.47	5.09 ± 0.27	0.42 ± 0.00	5.90 ± 0.13
2	66.42 ± 5.00	8.57 ± 0.58	118.20 ± 1.45	14.86 ± 2.57	0.38 ± 0.02	2.46 ± 0.23
3	75.03 ± 3.19	8.75 ± 0.82	126.15 ± 3.50	15.26 ± 0.62	0.41 ± 0.01	7.27 ± 0.34
4	73.67 ± 2.81	7.50 ± 0.53	129.56 ± 2.37	5.64 ± 2.20	0.49 ± 0.04	4.42 ± 0.76
5	79.28 ± 3.00	8.63 ± 0.71	129.09 ± 0.60	9.32 ± 2.47	0.40 ± 0.01	3.81 ± 0.12
6	84.37 ± 1.11	8.32 ± 1.27	121.94 ± 3.83	8.77 ± 0.71	0.38 ± 0.01	3.69 ± 0.08
F. value: 3.96	0.55	3.25	6.24	3.99	22.99	
P. value: 0.009	0.735	0.022	0.0001	0.009	0.001	

Values expressed as mean ± standard error of mean (SEM).

Source: Field data (2019).

DISCUSSION

The kidneys regulate the solute concentration of the blood (Eroschenko, 2008). Hyperglycaemia induces hyperfiltration which causes progressive kidney disease and morphologic alterations in the kidney that may result in podocyte damage and loss of filtration surface (Fakhruddin *et al*., 2017). Diabetes nephropathy (DN) is one of the complications of diabetes mellitus (Lukacinova, 2008; Wang *et al*., 2011). Glomerular dysfunction contributes critically to the development of DN, but deterioration of renal function also correlates with tubular alterations (Gatica *et al*., 2015). The major histopathological changes in diabetes nephropathy are mesangial expansion, glomerular basement membrane thickening, glomerular sclerosis and splitting of Bowman's capsule (Fowler, 2008; Tervaert *et al*., 2010; Batuman, 2018). Others include hypercellularity, failure of capsular space, hylination of urinary spaces, nephrotoxic necrosis, arteriolar thickening, and tubular dilation and atrophy (Prabhakar *et al*., 2007; Mouna *et al*., 2010; Teoh *et al*., 2010).

In this study, ip. injection of STZ (45 mg/kg) caused atrophy of the glomerulus, expanded urinary space, distorted tubules, haemorrhage and splitting of the glomerulus in the H&E section of the diabetic control (group 2) shown in Figure 2. Figure 1 shows normal kidney structure in the normal control (group 1). These pathologic changes marked the development of DN (Prabhakar *et al*., 2007; Fowler, 2008; Mouna *et al*., 2010; Tervaert *et al*., 2010; Reddy *et al*., 2019). Treatment with *G. kola* (548 mg/kg bw), *T. conophorum* (524 mg/kg bw) and the combined for 21 days attenuated most of the alterations caused by the hyperglycaemia. The extracts normalised the glomerulus, urinary space, and haemorrhage and improved the tubular distortions to levels comparable to the normal control as shown in Figures 4 – 6. However, moderate distortions of the tubules were still observed in the extracts treatment groups. Whereas, the glibenclamide could not reverse these pathological changes and as such the distorted tubules, hypertrophied glomerulus, obliterated urinary space and haemorrhagic pool were still observed in the glibenclamide treated group (group 3) as shown in Figure 3. This result is in agreement with Adedara *et al*. (2015) and Ajilore and Adesokan (2018) who reported that *G. kola* seed and *T. conophorum* seeds significantly improved the histoarchitecture of the kidney of diabetic rats. The beneficial effects of these extracts on diabetic-induced kidney injury may be attributed to the phytoconstituents they possess. Metabolites like kolaviron has inhibitory action on oxidative stress, interleukin (IL)-1β production and apoptosis, leading to restoration of the kidney structure (Ayepola and Oguntibeju, 2014).

Sections of the kidney stained with Periodic Acid Schiff (PAS) demonstrated high glycogen expression in the diabetic control (group 2) as shown in Figure 8, when compared with the normal control (group 1) which demonstrated fairly low glycogen expression as shown in Figure 7. Treatment with G. kola seed extract (group 4) reduced the glycogen expression to a low level as shown in Figure 10. While *T. conophorum* leaf extract (group 5) and the combined (group 6) demonstrated moderate glycogen expressions as shown in Figures 11 and 12 respectively. The

glibenclamide group (group 3) demonstrated high glycogen expression. Accumulation of glycogen in the kidney in sever hyperglycaemia has been reported (Nannipieri *et al.,* 2001; Moshen *et al*., 2015; Glastras *et al.,* 2016; Reddy *et al*., 2019). The accumulation of glycogen in the kidney tubules in both humans and rats diabetic nephropathy have been reported (Kang *et al.,* 2005; Gatica *et al*., 2015). The triggering mechanisms for this accumulation are not fully understood. However, over-expression and activation of muscle glycogen synthase (MGS) has been implicated (Cammisotto *et al*., 2008; Gatica *et al*., 2015). The reduction of the glycogen expression in the extracts treated groups may be attributed to the amelioration of the kidney histological alterations by the extracts. Also, the metabolites possessed by the extracts might have inhibited the over-expression and activation of glycogen synthase. The result of the PAS corroborates with the H&E result indicating that G. kola seed, T. conophorum leaf and their combined treatment possess renoprotective potentials at these doses since they were able to ameliorate the pathological alterations in the kidney caused by the STZ-induced diabetes.

STZ is the most frequently used chemical for the induction of experimental diabetes (Federiuk *et al*., 2004). In this study, a single ip injection of 45 mg/kg bw of STZ was used to induce type 1diabetes mellitus (Mehmet *et al*., 2008; Arikawe *et al*., 2012; Haseeb and Mohammad, 2012). Lower dose of STZ produced partial destruction of β – cells while the rats become permanently diabetic (Aybar *et al*., 2001) but do not require insulin for survival since the β – cells are not completely destroyed (Hayden and Tyagi, 2002). From Table 4.3, it was observed that at baseline (BL), groups 2 to 6 administered with 45 mg/kg bw of STZ had a significantly ($p < 0.05$) higher FBG compared with group 1 (normal control). Administration of *G. kola* seed extract 548 mg/kg, *T. conophorum* leaf extract 524 mg/kg, their combination (*G. kola* seed extract 548 mg/kg and *T. conophorum* leaf extract 524 mg/kg), and glibenclamide (5 mg/kg) significantly ($p < 0.05$) reduced the mean FBG when compared with the diabetic control (group 2). At week 1 of treatment, groups 4 and 5 significantly ($p < 0.05$) reduced the FBG to values comparable to the normal rats, while at week 2 all the treatment groups 3 to 6 significantly ($p < 0.05$) reduced the FBG comparable to the normal rats but at week 3, the mean FBG of group 6was significantly higher than group 1, 3 and 5. This findings was in tandem with Azu *et al*. (2005); Adaramoye (2012); Udenze *et al*. (2012) and Adedara *et al*. (2015) who all reported the hypoglycaemic activities of *G. kola* seed; and Donatus *et al*.(2014); Aneke *et al*. (2016) and Lepzem and Tongun (2017) who reported that *T. conophorum* leaf extract reduced blood glucose compared with glibenclamide.This result showes that the co-administration of *G. kola* seed and *T. conophorum* leaf extracts did not reduce FBG level better than their individual administrations or glibenclamide, but that the single administration of *G. Kola* seed or *T. Conophorum* leaf reduced FBG equally and as glibenclamide.

The biochemical assay of some electrolytes to analyse the kidney function test is shown in Table 4.4. The assay revealed a significant decrease in sodium, chloride, creatinine and insulin but an increase in urea, and an insignificant decrease in potassium in the diabetic control (group 2) compared with group 1. Upon treatment with glibenclamide, *G. kola* seed, *T. conophorum* leaf and combined extracts, it was found that the combined extracts and *T. conophorum* leaf significantly ($p < 0.05$) raised the serum sodium and lowered the urea levels to mean values compared with the normal rats (group 1). *T. conophorum* leaf also increased the chloride level significantly. *G. kola* seed significantly ($p < 0.05$) reduced the serum urea, and raised the creatinine and chloride levels towards normal. The combined treatment demonstrated no significant effect on the creatinine level. Decrease in serum electrolytes following a single ip administration of STZ had been reported (Prohp and Onoagbe, 2014). Haseeb and Mohammad (2012) reported a significant increase in serum urea and creatinine, an insignificant difference in electrolytes but a significant decrease in serum sodium following a single ip administration of 45 mg/kg body weight of STZ in Wistar rats. Kang *et al*. (2006) reported a significant increase in serum urea but insignificant change in creatinine in STZ induced diabetic rats. The result of this present study was in consonance with the above reports.

Significant increase or decrease in electrolytes, urea and creatinine is a pointer to compromised renal function. The decrease in the electrolytes and increase in urea observed in this study may be due to compromised renal function (Ikpi *et al*., 2009). Compromised kidney function may cause dehydration through glucose osmotic diuresis elicited by glycosuria which is accompanied with severe loss of electrolytes including sodium, potassium, calcium, chloride and phosphates (Eteng *et al*., 2008). The result of the biochemical assay in this study indicates that glibenclamide could not significantly improve the effect of STZ on sodium, chloride and potassium. It was able to insignificantly increase the creatinine level towards normal, but exhibited no responds to the serum urea to the extent that the urea level went further higher than in the diabetic control group.

CONCLUSION

It is concluded that the combined treatment of *G. kola* seed (548 mg/kg) and *T. conophorum* leaf (524 mg/kg) as well as their single treatments for a period of 21 days showed hypoglycaemic and nephroprotective effects.

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