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Effects of Four Herbal Plants on Kidney Histomorphology in STZ-induced Diabetic Wistar Rats

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Abstract

The aim of this study is to investigate the effect of four herbal extract and their efficacy on the histomorphometry of the kidney in STZ induced diabetic rats with a view to understanding their antidiabetic properties.

Forty- two healthy adult Wistar rats (*Rattus novergicus*) with an average weight of 153.4 g were randomly divided into seven groups (n=6). STZ (65 mg/kg) dissolved in citrate buffer was administered intraperitoneally to animals in groups (B-G) while animals in group A received equivalent volume of citrate buffer. Plant extracts (100 mg/kg) were administered daily (orally) to animals in groups C-F and glimepiride (anti-diabetic drug) to animals in group G for fourteen days. After the expiration of the study the animals were sacrificed and the kidneys were excised, fixed in 10% formol saline for histology and morphometric analysis.

The glomeruli of the diabetic group were atrophied which is validated by significant decrease in its density, shrinkage and increased bowman's space. These observations were also characterized by diminished cellular proliferation, decreased cellular volume and ischemia. The histology and morphometric analysis revealed that the kidney in the group treated with *Psidium guajava* shows a better histoarchitectural outline of all the four plant extracts used.

This present study therefore suggests that *Psidium guajava* could be a better alternative therapy in ameliorating diabetic-associated disorders of the kidney.

Keywords: Diabetes; *Psidium guajava; Veronia amygdalina; Ficus mucuso; Citrullus colocynthis;* Kidney

Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia (high blood glucose level) which results from defects in insulin secretion, insulin action or both [1]. The chronic hyperglycaemia of DM is associated with long-term damage, dysfunction and failure of various body structures and organs especially the eyes, nerves, heart, blood vessels and also the kidney [1]. Existing therapy for DM are known to provide good glycaemic control, but are believed to do little in regards to the complications to various organs. Besides, these anti diabetic drugs are associated with mild to moderate side effects [2]. In view of this, the present study has investigated the effects of some common plants traditionally used in herbal management of diabetes amongst the Yorubas of Ile-Ife, Nigeria, on the histomorphology of the kidney in STZ-induced Wistar rats.

The herbal plants used for this study were leaves of *Veronia amygdalina*, shaft of *Citrullus colocynthis* seed, leaves of Psidium *guajava*, and leaves of *Ficus mucuso* (SPP).

Veronia amygdalina (VA) commonly called bitter leaf belongs to the family *Asteraceae.* It has petiolate leaves of about 6 mm diameter and elliptic shape. The leaves are green with a characteristic odour and a bitter taste [3]. It is called 'Ewuro' by the Yorubas of Nigeria. The leaves have been used in traditional folk medicine as anthelmintics, antimalarial, antimicrobial anticancer and as a laxative herb [4]. Phytochemical substances in VA include oxalates, phylates and tannins [5,6], and also flavonoids [7,8].

Citrullus colocynthis (CC) popularly known as 'bitter apple', 'colosynth', and 'vine-of-Sodom' is a tropical plant belonging to the family *Cucurbitaceae* [9]. It is also commonly referred to as 'egusi' amongst the Yorubas of Nigeria. In the traditional medicine, it has been used in treatment of constipation [10], diabetes [11] oedema, fever, jaundice leukaemia, bacterial infections, cancer and used as an abortifacient [12].

Psidium guajava (PG) is a semi deciduous tropical tree commonly known as 'guava' and belongs to the family *Myrtaceae*. Phytochemical constituent have been shown to include Vitamin C, B1, B2, and B6, free sugars [13]. Guava fruits have been shown to have antioxidant properties [14]. The fruits have been shown to possess hypoglyceamic effects in diabetic mice and human volunteers [13]. Studies have indicated the presence of various flavonoids, terpenoids and their glycosides [15,16], and these compounds have been shown to be antidiabetic [17,18].

Ficus mucuso (FM) belongs to the family Moraceea. The *Ficus* genus has wide distribution and is used traditionally as medicine, vegetable, food, fodder and fuel wood [19]. Phytochemical analyses of FM have revealed the presence of monoterpenoids and flavonoids [20].

Materials and Methods

Animal management

Forty- two healthy adult Wistar rats (*Rattus novergicus*) with an

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average weight of 153.4 g were procured from the animal house of College of Health Sciences, Obafemi Awolowo University, Ile - Ife, Osun State. The animals were kept under standard laboratory condition of good lighting, moderate temperature, and adequate ventilation in a hygienic environment. They were feed on standard rat chow containing proteins, carbohydrate, fats, vitamins and minerals. The animals were placed under standard laboratory protocols as stipulated by the Institutional Animal Care and Use Committee (IACUC, 2010).

Animal grouping and treatment

The animals were randomly divided into seven groups of 6 animals each

- Group A - control normal rats administered with equivalent volume of citrate buffer.
- Group B - experimentally-induced diabetic rats were administered with single intraperitoneal injection of streptozotocin (65 mg/kg),
- Group C - experimentally-induced diabetic rats (65 mg/kg) treated with aqueous extract of VA leaves (100 mg/kg) orally, dissolved in normal saline for 14 days,
- Group D - experimentally-induced diabetic rats (65 mg/kg) treated with aqueous extract of shaft of CC seeds (100 mg/kg) orally, dissolved in normal saline for 14 days,
- Group E - experimentally-induced diabetic rats (65 mg/kg) treated with aqueous extract of PG (100 mg/kg) orally, dissolved in normal saline for 14 days,
- Group F - experimentally-induced diabetic rats (65 mg/kg) treated with aqueous extract of FM (100 mg/kg) orally, dissolved in normal saline for 14 days,
- Group G - experimentally-induced diabetic rats (65 mg/kg) treated with a standard antidiabetic drug (2 mg/kg of glimepiride) orally, dissolved in normal saline for 14 days.

Plants materials

Preparation of extracts: The plant leaves were procured from a local market in Ile-Ife metropolis in Osun state, Nigeria. The leaves were taken to the herbarium in the Department of Botany, Obafemi Awolowo University, Nigeria, to confirm identification. The leaves and shaft of the plants were air dried and powdered in a warring blender. The extraction process of the plant leaves of VA (425 g), PG (970 g), FM (370 g) and shaft of CC (615 g) were prepared by dissolving it in 2.9 L, 3.19 L, 3.5 L and 2.2 L respectively for 72 hr with intermittent shaking. Thereafter, the solution was filtered using a filter paper. The filtrate was then concentrated *in vacuo* at 35°C using a rotator vacuum evaporator (Buchi Rotavapor, R110 Schweiz). The extracts were oven dried at 37°C, and the respective percentage yield (3.00 g, 2.65 g, 5.34 g and 1.76 g) were stored until ready to use. The aliquot portion of each of the extracts were weighed and dissolved in normal saline for use on each day of the experiment.

Induction of diabetes

Diabetes mellitus was experimentally-induced in groups B, C, D, E, F, and G by a single intraperitoneal injection of 65 mg/kg body weight of streptozotocin (Tocris Bioscience, UK) dissolved in 0.1 M sodium citrate buffer (pH 6.3) [21]. Diabetes was confirmed in animals 48 hours after induction, by determining fasting blood glucose level using a digital glucometer (Accu-chek® Advantage, Roche Diagnostic, Germany) consisting of a digital meter and the test strips using blood samples obtained from the tail vein of the rats. The animals were stabilized for twenty eight days before the commencement of extract and glimepiride administration. The fasting blood glucose was subsequently monitored throughout the experimental period. Animals in group A were given equal volume of citrate buffer used in dissolving streptozotocin intraperitoneally.

Method of administration of extracts

The animals were fed orally using orogastric tube. The animals were held with a glove with the left hand such that the neck region was held by the fingers to still the neck while being fed. Treatment was done at 07.00 hour every day before the animals were fed over a period of two weeks (14 days).

Sacrifice and specimen collection

The animals were sacrificed by cervical dislocation 24 hours after the expiration of research. The kidneys were excised following midline-abdominal incision. The kidney which is reddish-brown organ situated posteriorly behind the peritoneum on each side of the vertebral column were excised and weighed.

Histological evaluation

The harvested kidneys were fixed in 10% formal saline for a minimum of 48 hours and process routinely for paraffin embedding. Serial sections were obtained at 5 µm from a rotary microtome (Bright B5040, Huntington England) and stained using routine haematoxylin and eosin method. Stained sections were viewed under a LEICA digital microscope (DM 750) and photomicropgraphs were taken with the aid of an attached camera (Leica ICC50).

Histomorphometric analysis

The stained sections were subjected to morphometric analysis recommended by World

Health Organization W.H.O. [22]. which included: dividing the eye piece occulometer into two 100 small divisions, the stage micrometer scale was made up to 1 mm divided into 0.1 mm divisions and each 0.1 mm was divided into 0.01 mm, the eye piece scale (occulometer) was inserted into the eye piece of the microscope by removing the superior lens thus placing the scale on the field stop, the stage micrometer was also placed on the stage of the microscope, the stage scale was focused by the low power objective lens (x4), the stage and the eye piece scales were adjusted until there was a parallel point between the two scales, the number of the eye piece divisions and its corresponding stage measurements was noted; (if 70 occulometer divisions equal to 14 µm, all the objective lens were thus calibrated). Calibration was needed for each microscope use. The occulometer fixed into the Olympus Microscope was then focused through stained sections of the tissue to allow for the measurement of the parameters.

Statistical analysis

Data were expressed as mean ± SEM. Data were analysed using One-way ANOVA, followed by Student Newman-Keuls (SNK) test for multiple comparisons. Significant difference was taken as $p<0.05$.

Results

Effects of extracts on relative weight of kidney

As shown in Table 1, the relative weights of the kidney was significantly reduced in all groups (B-G) compared to control group (A).

Effects of extracts on kidney histology

As shown in Figure 1, Control animals showed normal kidney histology. The glomeruli were well demonstrated with normal bowman space. The renal tubules filling the bulk of the kidney parenchyma were clearly observed. Diabetic kidney, in Group B, showed atrophy of the glomeruli and the tubules were fairly

Groups	Absolute weight of kidney (g)	Relative weight of kidney (%)
A: Control	1.05 ± 0.09^{a}	1.07 ± 0.05^{d}
B: Diabetic	1.14 ± 0.04^{a}	0.85 ± 0.05^{bc}
C: Diabetic+VA extract	1.69 ± 0.08^{c}	0.73 ± 0.02^{ab}
D: Diabetic+CC extract	1.65 ± 0.05^{c}	0.70 ± 0.03^{a}
E: Diabetic+PG extract	1.40 ± 0.11^{b}	0.82 ± 0.02^{abc}
F: Diabetic+FM extract	1.70 ± 0.09^{c}	0.78 ± 0.04^{abc}
G: Diabetic+Glimepiride	1.65 ± 0.05^{c}	0.72 ± 0.02^{a}

Values are given as Mean ± SEM in each group.
a, b, c, d, ab, bc, cd, abc within column signifies that means with different letters differs significantly at p<0.05 while means with the same letters does not differ significantly at p<0.05 (using one way ANOVA with SNK).

Table 1: Absolute/relative weights of the kidney.

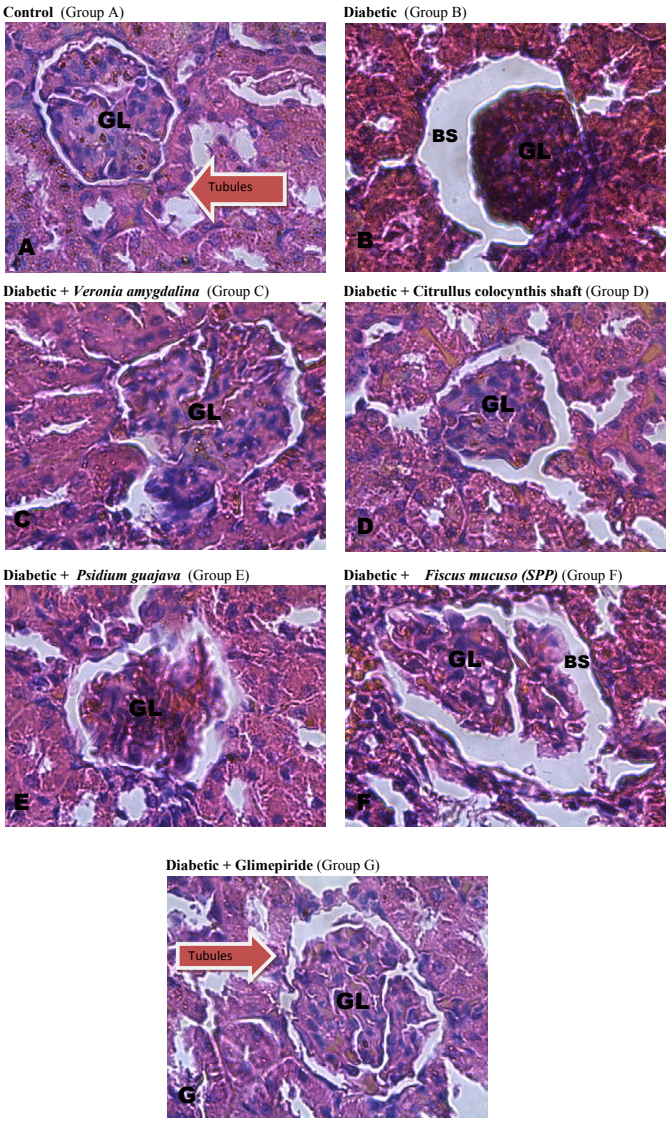

Figure 1: Photomicrographs of kidney of Groups A, B, C, D, E, F, and G. (H&E x400). GL: glomerulus. There was atrophy of the glomerulus and the tubules are fairly preserved in the diabetic animals (Figure 1B). The extract treated groups (Figures 1C-1F) reveals renal tissue regenerations which shows a remarkable reversible cellular injury as compared with group B.

Groups	Glomerular density (glomeruli/mm^2)
A: Control	17.60 ± 1.21^a
B: Diabetic	9.80 ± 1.36^b
C: Diabetic+VA extract	13.20 ± 1.36^b
D: Diabetic+CC extract	21.40 ± 1.03^a
E: Diabetic+PG extract	24.60 ± 1.47^{ac}
F: Diabetic+FM extract	18.80 ± 1.46^a
G: Diabetic+Glimepiride	20.00 ± 1.58^a

Values are given as Mean ± SEM in each group.
a, b, ac within row signifies that means with different letters differs significantly at $p<0.05$ while means with the same letters does not differ significantly at $p<0.05$ (using one way ANOVA with SNK).

Table 2: Glomerular density.

preserved. Administration of the extracts and antidiabetic drug improves cellular regeneration which is quite prominent in Groups C and G.

Effects of extracts on histomorphometric glomerular density

As shown in Table 2, there was significant decrease ($p<0.05$) in glomerular density of diabetic animals (Group B) compared to control, extracts (Groups D, E, F) and drug treated groups. Group C (treated with VA extract) showed no significant improvement ($p<0.05$) in glomerular density while group E showed significant increase in density compared to control.

Discussion

Long-term damage, dysfunction and failure of the kidneys are a major complication of diabetes mellitus [1]. Disorders of the kidneys are serious secondary consequence of diabetes, resulting in end stage-renal diseases. Increased glucose levels in the blood have been shown to lead to oxidative stress, which is considered as one of the causative factor for diabetes-associated kidney disorders [23]. STZ-induced diabetic rodents are seen to develop kidney disorders similar to the early stage of human diabetic-associated disorders of the kidney [24]. Renal hypertrophy has been reported in diabetes [23].

Also diabetic nephropathy has been known to cause renal failure thus leading to mortality and morbidity.

However the histological and histomorphometric evaluation of the present study shows atrophy rather than hypertrophy in the glomeruli of diabetic animals which is validated by significant decrease in its density and shrinkage. These observations were also characterized by diminished cellular proliferation, decreased cellular volume and ischemia.

The primary function of the glomeruli is to assist in the production of ultrafiltrate of the plasma such as $Na^{+,}$ water and urea for further processing by the renal tubules thus playing a vital role in the maintenance of fluid and electrolyte homeostasis.

Administration of the extracts improves the histoarchitecture of the kidney and by extension restores its functionality. The groups administered with PG extract demonstrated a distinct regenerative capacity over the other three extract. This was closely followed by the group administered with FM extract.

Previous studies have reported some similar histopathological findings [24,25]. The plant extracts used for the study, are common herbal plant used traditionally in the management of diabetes, amongst the Yorubas of Ile-Ife, Nigeria. Three of these plants – VA, CC, and PG; have been reported to possess anti-diabetic properties [3,9,13]. The four medicinal plants used in this study are well known for their antioxidant properties which are due to their high level content of flavonoids [7,8,11,15,20]. The present study has provided useful information in the management of kidney related disorders resulting from diabetes.

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