Abstract

The preliminary phytochemical studies of Alafia barteri extract revealed the presence of reducing sugar, steroids, glycosides, flavonoids and anthraquinones. This research work was carried out to determine some effects of aqueous extract of Alafia barteri on the histology of kidney, blood glucose level, and average body weight of diabetic Wistar rats.

Twenty (20) Wistar rats weighing between 75kg -120kg were divided into (4) groups of five (5) animals. Group 1 was made of normoglycaemic animals that received 2m/s of water. Group 2 consisted five normoglycaemic rats that received 400mg/kg of Alafia barteri aqueous extract. Group 3 were diabetic (hyperglycaemic) animals that received 2m/s of distilled water. Group 4 were diabetic animals that received 400mg/kg of Alafia barteri extract. The administration was done for 6 weeks, animals were euthanized by cervical dislocation. The kidneys were removed, weight and fixed in 10% formolsaline for H&E and Masson Trichrome staining.

There was significant increase in the body weight of the animals in group 2 which were administered 400mg/kg aqueous extract of Alafia barteri compared to group 1 which were administered 2m/s of water. Histological findings showed that administration of aqueous extract of Alafia barteri has a positive effect and maintained the histological architecture of the kidney. However, significant reduction in the blood glucose level in diabetic Wistar rat treated with 400mg/kg of Alafia barteri aqueous extract was observed.

In conclusion, this study showed that Alafia barteri aqueous extracts maintained renal microarchitecture and blood glucose level of a diabetic Wistar rats.

Keywords: Hyperglycemia, Alafia Barteri, Kidney, Diabetes and Wistar rats

Background

The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1]. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs.

There are three main types of Diabetes mellitus. There are type 1, which is insulin dependent and accounts for about 10% of Diabetes well cases. Type 1 is usually as a result of end organ receptor resistance. It is common among adult, it accounts for about 90% of all Diabetes cases. The third one is gestational diabetes which is among pregnant women. It usually disappear after delivery, but some cases may eventually result into type 2 Diabetes mellitus aside these there is still some cases with a definite cases like Diabetes as a result of drugs, parereatic disease (parerentitis) [2,1].

Type 1 cases are usually treated with insulin, type 2 with anti-hyperglycaemic drugs and insulin. Due to complications orthodox drugs of emphasis have been shifted to the use of herbal products in the treatment of Diabetes mellitus [3].

Diabetes mellitus present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss [4] In its most severe forms, ketoacidosis or a non–ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. Often symptoms are not severe, or absent, and consequently hyperglycaemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made [1,4]. The long–term effects of Diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy.
with risk of foot ulcers, amputation, Charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease [5].

Alafia barteri is a high climbing, scandent shrub with small white or pink flowers; it is reportedly used in the treatment of sickle cell anaemia, toothache, rheumatism, fever, and inflammation [6]. Leaf infusion and root decoctions are used in Nigeria and other African countries as a remedy for malaria (Olowokudejo et al., 2008). Phytochemical constituents of the ethanol extract of leaf and root showed the presence of total polyphenols, flavonoids, tannins, saponins, alkaloids and terpenoids (Lasisi et al., 2012). Hamid and Aiyelaagbe (2011) reported the presence of reducing sugars, glycosides, flavonoids and anthraquinones for all the extracts. Steroids from the ethylacetate and methanol extract. Only ethanol extract contained saponins. Adekunle and Okoli (2002) [2] reported the antifungal properties of the ethanol and aqueous leaf extracts, while the antibacterial and antifungal properties of the hexane, ethylacetate and methanol extracts of the stem was investigated by Hamid and Aiyelaagbe (2011). Methanol extract showed inhibitory activity against Escherichia coli and Pseudomonas aeruginosa at 25 to 200 mg/ml, while the hexane and ethylacetate extracts showed inhibition against E. coli and P. aeruginosa at varied concentrations. The brine shrimp (Artemiasalina) lethality assay for possible cytotoxicity was moderate. However, the leaf was higher in cytotoxicity as compared to the root fractions (Lasisi et al., 2012).

The urinary organs consist of the right and left kidneys and urethers, the urinary bladder and the urethra. These organs are responsible for the production, storage, and passing of urine. Many harmful products (that results from the metabolism) are removed from blood through urine. These include urea and creatinine that are end products of protein metabolism (Rohtak, 2006, Inderbir Singh, 2007). Each kidney has a concave medial border, the hilum through where nerves enter, blood and lymph vessels enter and exit. The functional unit of the kidney is nephron. There are 1 to 1.4 million nephrons per kidney. Nephron consists of several discrete structures that are essentially consist of a filtration apparatus connected with a series of tubules. The tubule system of the nephron facilitates the recovery of the nutrients, the excretion of waste, and the maintenance of osmotic pressure (Inderbir, Singh, 2007). Histologically the kidney has an outer cortex and an inner medulla. The medulla consists of 8-15 conical structures called renal pyramids plus the cortical tissues at its base and along its side constitutes a rena lobe. The major divisions of each nephrons are: renal corpuscles, proximal convoluted tubules, thin and thick limbs of nephron loop, distal convoluted tubules and collecting tubules (Inderbir, Singh, 2007). Several collecting tubules of nephrons from collecting ducts which carry urine to the calyces and the ureter. Cortical nephrons are located almost completely in the cortex while juxtamedullary nephrons are located close to the medulla having long loops in the medulla. There is traditional claim of anti-hyperglycaemic effect of Alafia barteri, kidney is one of the target organ affected by chronic hyperglycaemic. This study was carried out to add to the information in the literature on the effects of the plant extract on the kidney of diabetic Wistar rats.

**Aims**

The aim of this study is to painstakingly assess the effects of Alafia barteri on the diabetic Wistar rats by studying the histology of the kidney.

**Materials and Methods**

**Plant**

Alafia barteri was obtained in Ogbomoso, Oyo State, Nigeria. The leaf was identified with Voucher Number LHO 407 and authenticated at the Department of Pure and Applied Biology of Ladoke Akintola University, Ogbomoso, Oyo State.

**Extraction Procedure**

The extraction of Alafia barteri leaf was carried out at the Laboratory of Chemistry Department of University of Ilorin, Kwara State. The extraction procedure started by air drying the Alafia barteri at room temperature for five days. After which it was minced with mortar and pestle into it powdery form. At the laboratory, the powdery form of Alafia barteri leaf was immersed in water for two days for the soluble active ingredient to dissolve in it, and then it was filtered and the filtrate was transferred into the extractor for another three days till the extract is concentrated and the temperature of the extractor was regulated to 37°C so that the active ingredient will be preserved. It was then collected and stored in a refrigerator for preservation.

**Experimental Animal**

Twenty (20) Wistar rats weighing between 75g-120g were purchased from a laboratory in Osogbo. They were brought to the Animal House of the Faculty of Basic Medical Sciences, Ogbomoso. They were acclimatized for two weeks. The animals had free access to fat pellets and water.

**Induction of Hyperglycaemia**

Hyperglycaemia was induced in 10 rats overnight-fasted randomly selected rats by a single intraperitoneal administration of allosan at 120 mg/kg bw (Lal, Korner and Mastsuo 2000). Allosan was dissolved in citrate buffer (0.1m, pH 4.5) just prior to injection. Hyperglycaemia was allowed to develop for 72hours (Lenzen, 2008). Animals with Fasting Blood Glucose ≥ 250 mg/dl were considered hyperglycemic (Tende et .al., 2011) and were included in this study. Control animals (n= 5) received a single intraperitoneal injection of 0.1M citrate buffer (1ml/kg bw; pH 4.5)

**Experimental Design**

Twenty Wistar (20) rats were divided into four (4) groups of five animals each. Group 1 was made of five norglucemic animals that received 2m/s of distilled water; Group 2 consisted five norglucemic rats that received 400mg/kg of Alafia barteri extract, Group 3 were diabetic (hyperglycaemic) animals that received 2m/s of distilled water, Group 4 were
diabetic animals that received 400mg/kg of Alafia barteri extract.

**Administration**

All the animals were given feeds and water. The animals were treated daily through oral route of administration at around 9:00am for 42 days. The animals were weighed at alternate days. The blood glucose of the animals was measured every week.

**Collection of Organs**

At the end of the third week the animals were sacrificed by cervical dislocation. The abdomen accessed and the kidney was removed weighed and fixed in 10% formol saline for histological staining. Blood was also collected from the animals for Renal Function Test.

**Histological Techniques**

The harvested kidneys were fixed immediately in 10% formol saline. The purpose of fixation is to preserve tissues permanently in as life-like a state as possible (Weiss et al., 2010). The kidneys were fully immersed into the 10% formol saline as soon as possible after the removal of the kidneys to prevent autolysis.

**Body weight, Feed weight and Photomicrograph**

The body weight was measured weekly i.e. once every week. The feed weight was measured on a daily basis and the photomicrograph of the slides was taken at the histopathology department, University of Ilorin teaching hospital, Ilorin.

**Statistical Analysis**

Data were analyzed using SPSS. Data were expressed as mean +/- standard error of the mean (Mean ± SEM). Mean values were compared using one way analysis of variance (ANOVA). P value less than 0.05 (P<0.05) were taken to be statistician significant. All graph were drawn with SPSS.

**Results**

The results shows that the average glucose level of the rats in control and the animals treated with the Alafia barteri leaf aqueous extracts are insignificant in the differences. However, surge increases in the blood glucose was observed in the animal following treatment with the 120 mg/kg bw dose of allosan. A significant reduction in the blood glucose was observed in the 5th and 6th week following administration of Alafia barteri leaf aqueous extracts as shown in the figure 1 and 2.

Figure 3 and 4 showed significant reduction in the body weight.

**Figure 1:** Mean plot of glucose level of animal.

**Figure 2:** Graph and Error bar of Blood Glucose of Rat.
weight of the untreated diabetic animals, however, significant increases was noticed in the diabetic animals treated with the Alafia barteri leaf aqueous extracts and the animals that were treated only with the Alafia barteri leaf aqueous extracts.

Histo-architectural observation revealed clearly, loss of the bowman’s capsule, the renal tubules and characteristically distal and proximal convoluted tubules as observed in plate 1 C and 1D, relative to the control animals both positive and the he negative control showed histo-renal integrity to be maintained. Presences of the bowman’s capsule, Plates 3C and 4C showed Trichrome stained renal tissue of untreated diabetic animals. Tubule with mild haemorrage, degenerating glomeruli and consequent loss of glumerili. Plate 3A and 3B revealed basic features of histology of the kidney, with intact bowman’s capsule and glumeruli demonstration.

![Figure 3: Mean plot of weight of animal.](image)

![Figure 4: Graph and Error Bar of Mean Weight of Rat.](image)

![Figure 5: Graphical Illustration of Relative Weight of Animal.](image)
Plate 1A. (x100) H&E staining of group 1 showing:
- normal renal tubule (white arrow),
- glomeruli (red arrow),
- Bowman's space (black arrow).

Plate 1B. (x100) H&E staining of group 2 showing:
- normal glomeruli (red arrow),
- renal tubule (white arrow),
- interstitium appear normal (slender dark arrow).

Plate 1C. (x100) H&E staining of group 3 showing:
- glomerulosclerosis (second black arrow),
- dilated tubule (red arrow),
- juxtaglomerular cells (first black arrow).

Plate 1D. (x100) H&E staining of group 4 showing:
- glomeruli recovering from glomerulosclerosis (red and black arrow).

Plate 2A. (x400) H&E staining of group 1 showing:
- normal glomeruli (red arrow),
- convoluted tubule (black arrow),
- podocyte (white arrow).

Plate 2B. (x400) H&E staining of group 2 showing:
- Bowman's capsule (red arrow),
- tubules (black arrow).

Plate 2C. (x400) H&E staining of group 3 showing:
- thickened Bowman's capsule (black arrow),
- diffuse intercapillary glomerulosclerosis (red arrow).

Plate 2D. (x400) H&E staining of group 4 showing:
- recovering glomerulosclerosis (black arrow),
- renal tubule (red arrow).
Plate 3A. (x100) Trichrome staining of group 1 showing:
- glomeruli (red arrow),
- tubules (black arrow).

Plate 3B. (x100) Trichrome staining of group 2 showing:
- glomeruli (white arrow),
- tubules (black arrow).

Plate 3C. (x100) Trichrome staining of group 3 showing:
- renal tubules (red arrow),
- tubule with mild haemorrhage (white arrow),
- degenerating glomeruli (black arrow).

Plate 3D. (x100) Trichrome staining of group 4 showing:
- recovering glomeruli (white arrow),
- renal tubule (red arrow).

Plate 4A. (x400) Trichrome staining of group 1 showing:
- renal tubules (red arrow),
- interstitium shows very mild haemorrhage (black arrow).

Plate 4B. (x400) Trichrome staining of group 2 showing:
- proximal convoluted tubule (black arrow),
- glomeruli (red arrow).

Plate 4C. (x400) Trichrome staining of group 3 showing:
- degenerating glomeruli with mild haemorrhage (black arrow).

Plate 4D. (x400) Trichrome staining of group 4 showing:
- bowman's space (red arrow),
- recovering glomerulosclerosis (black arrow).
Discussion

In the study, the blood glucose of the group 1 and 2 fluctuated throughout the experimental period but there was no period it went above normal. Diabetes mellitus is a group of metabolic diseases characterized by elevated blood glucose levels (hyperglycemia) resulting from defects in insulin secretion, insulin action or both [7]. The blood glucose of these two groups remained normal throughout (Figure 1). This showed that water and Alafia barteri, have no effect on a normoglycaemic state. The blood glucose of the animals in group 3 remained normal before the induction of diabetes, but started increasing from the week 1 and remained high up to the end of the experimental period (Figure 2). Several studied had shown that distilled water had no effect on a diabetic state [8-11] in agreement with this study. However, animals that were induced to be diabetic in group 4, showed significant reduction in the blood glucose. There was a significant drop at the end of the 6th week. This showed that Alafia barteri has some antihyperglycaemic effect. Some antihyperglycaemic effects of Alafia barteri had been documented, which is in agreement with this study [2].

The weight parameters of animals in group 1 remained normal throughout the experimental period. Group 2 animals showed a significant increase in their weight which indicates that Alafia barteri leaf extract has an effect on the body weight. Group 3 and 4 animals showed a decrease in their body weight two weeks after the induction of diabetic this is in accordance with the work of [12]. The histological findings of the kidney showed that the glomeruli, distal convoluted tubules were normal in group 1 and 2 animals (Plates 1A, 1B, 2A and 2B). This showed that water and Alafia barteri, had no adverse effect on the histology of the kidney. In diabetic untreated (group 3) animals, there was glomerulo-sclerosis and distortion of both proximal and distal convoluted tubules. There was general distortion of cytoarchitecture of the kidney (Plate 3C and 4C). This showed that diabetic state had serious adverse effect on the histology of the kidney. This is in agreement with various studies in the literature that said that diabetic state caused nephropathy (Adeeyo et al; 2013). In other studies by [5] it was discovered that diabetes mellitus is associated with glomerulosclerosis which is also in agreement with this study. In group 4 animals, (Plates 3D and 4D), the diabetic animals were treated with aqueous extract of Alafia barteri. The plates showed some regeneration of glomeruli and organization of the cytoarchitecture of the kidney. This meant that Alafia barteri extract some protective effect on the kidney of diabetic animals. This could be explained by the antihyperglycaemic action possessed by Alafia barteri. Since the aqueous extract of the plant was able to bring about antihyperglycaemic effect, then it should be able to prevent nephropathy that is usually associated with diabetic state. Some plant like Allom cepa which had antihyperglycaemic effect was able to prevent nephropathy in diabetic animals as reported by [5]. This finding is in agreement with the present study.

Conclusion

The result of this study showed that Alafia barteri leaf extract had a significant effect on the weight of the animals, exert reduction in the blood glucose of the diabetic animals and maintained histo-renal architecture.

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