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Combined Effects of Aqueous Extracts of *Tetracarpidium Conophorum* (Walnuts) and *Vernonia Amygdalina (Bitter leaves)* on the Pancreas and Kidney of Alloxan Induced Diabetic Wistar Rats

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ABSTRACT: This study evaluated the effect of combined treatment of aqueous extracts of *Tetracarpidium conophorum* (TCE) nuts and *Vernonia amygdalina* (VAE) leaves on some biochemical parameters such as amylase activity, urea and creatinine in alloxan induced diabetic Wistar rats. Forty two (42) Wistar rats with weight range of 125-275g were grouped into 6 groups of 7 rats in each group. The first group served as the normal control while the remaining five groups were induced with diabetes using alloxan at 120 mg/kg body weight. Group two served as diabetic control and the remaining groups were treated with 500 mg/kg TCE, 500 mg/kg VAE, combined extract of 500 mg/kg TCE and 500 mg/kg VAE and 7.69 mg/kg metformin respectively. The rats were treated orally once daily for 28 days. Three rats per group were sacrificed on the 14th and 28th day of treatment. The plasma levels of glucose, amylase, urea and creatinine were measured. The result showed that there was a reduction ($p<0.05$) in the blood glucose level of all the treated groups compared to the diabetic control. The results also showed that urea and creatinine were ($p<0.05$) decreased in all treated groups compared to the untreated diabetic group. The histology of the pancreas of treated groups showed that the plant extracts ameliorated the effect of alloxan on the organ. In conclusion, the combined aqueous extracts of *Tetracarpidium conophorum* nuts and *Vernonia amygdalina* leaves reduced the level of damage to the kidney and pancreas when administered to diabetic rats at the dosage used in this study. © JASEM

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***Keywords*:** Creatinine, Diabetes, Kidney, Pancreas, *Tetracarpidium conphorum*, Urea, *Vernonia amygdalina*

Diabetes mellitus is a chronic condition that occurs when the body cannot produce enough or effectively use insulin (Chineye, *et al*., 2011). Insulin is a hormone produced by the pancreas that allows glucose (and other nutrients) from food to enter into the cells of the body where it is converted into energy required by tissue and muscle to function.
Diabetes mellitus is one of the leading causes of death in developed countries today and it is technically defined as a metabolic disorder that is characterized by chronic hyperglycemia, absolute or relative lack of disturbance of carbohydrate, fat and protein metabolism (Adeboye, *et al*., 2013).

The symptoms of diabetes include weight loss, polydisia (increased thirst), polyuria, (frequent urination), polyphagia (increased hunger), and lassitude and blurred vision with prusitus vulvae and in extreme situation could lead to death.

Epidemiological studies have shown that approximately 5% of world population or estimates of 194 million people have diabetes mellitus (Donatus, *et al*., 2014). Modern drugs, including insulin and other biochemical agents e.g tolbutamide, phenformin, troglitazone, rosigliitazone and repaglinide control blood glucose level only when they are regularly administered, but these treatments are tedious and have several undesirable side effects and fail to significantly alter the course of diabetic complications (Upadhyay, *et al*., 1996).

It is therefore imperative for a biomedical research on development of hypoglycaemic agent for the control of the disease from natural sources to be instituted. Some plant products used by the population as antidiabetic remedies are edible plants which have added further interest in their study because of their dual role as food and medicine for the management of diabetes.

Some medicinal plants have been associated with the management and control of diabetes. There are more than 800 plant species with hypoglycaemic activity (Donatus, *et al*., 2014).

The African walnut, botanically called *Tetracarpidium conophorum* is known as “Ukpa” in Igbo and “Awusa or “Asala” in Yoruba. It is an economic plant widely cultivated for the production of nuts used as delicacies (Edem, *et al*., 2009). Apart from consuming as snacks, some studies on the plants have revealed that there is good nutritive value in the nuts (Akpuaka and Nwankwo, 2000).

The nuts have been shown to cure male infertility problems and the leaves are used for the treatment of dysentery (Anosike, *et al*., 2015). The husk has anti-

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microbial activity and it is also useful for its anti-proliferative activity (Okon and Atai, 2014). Anosike, *et al*., (2015) reported that the nuts of *T. conophorum* possess antioxidant activity.

Vernonia amygdalina is commonly called bitter leaf because of the characteristics aroma and astrigent bitter taste of the leaf. The plant is widely distributed in West coast of Africa where it grows wild and as a domestic plant (Farombi, 2013). The aqueous leaf extract has been shown to possess hypolipidemic effects in diabetic and non-diabetic rats. Its protective role on kidney and liver of alloxan-induced diabetic rat has also been reported (Atangwho, *et al*., 2007).

Urea is synthesized in the body of many organisms as part of the urea cycle, either from the oxidation of amino acids or from ammonia. Amino acids from ingested food that are not used for the synthesis of proteins and other biological substances are oxidized by the body, yielding urea and carbon dioxide, as an alternative source of energy generation (Sakami, *et al*., 1963). The oxidation pathway starts with the removal of the amino group by a transaminase; the amino group is then fed into the urea cycle. Creatinine is a protein breakdown product and its level is a reflection of the bodies muscle mass. Creatinine is a metabolite of creatine. Creatinine production is endogenous and is determined by muscle mass. Unlike urea, creatinine concentration is almost independent of diet. It increases in renal failure (Okoye and Nyimone, 2015; Wootton and Freeman, 1982).

The antidiabetic activities of *Tetracarpidium conophorum* nuts and *Vernonia amygdalina leaves* have been reported. In a recent report, the chemical components thought to have exerted the antidiabetic action were compared (Atangwho, *et al*., 2009). Although extracts from these plants have individually demonstrated antidiabetic action, recent studies show that antidiabetic efficacy of the extract is enhanced when given in combination (Ebong, *et al*., 2008). In this present study, the effects of combined aqueous extracts of *Tetracaridium conophurm* nuts and *Vernonia amygdalina* leaves on some biochemical parameters such as urea and creatinine of alloxan induced diabetic rats were investigated.

The objective of this research was to investigate the effect of the combined aqueous extracts of *Tetracarpidium conophorum* nuts and *Vernonia amygdalina* leaves on blood glucose, serum, amylase and renal function using Wistar rats as experimental model.

MATERIALS AND METHODS

Chemicals and Reagents: They include the following: Metformin (Merck Serono Ltd. U.K), chloroform (BDH chemicals Ltd.), formalin 10%, alloxan (Qualkems Lab. Reagents), Biochemical reagent kits (MINDRAY) and finisher feed (Top Feed Ltd.).

Experimental Animals: Forty two (42) albino rats of both sexes weighing between 125g and 275g were used for the experiment. They were purchased and housed at the Biochemistry Departmental Animal House at Choba Campus, University of Port Harcourt. They were left for one week to acclimatize to the laboratory conditions during which they were fed with normal feed (Top feeds- grower´s mash) and clean water. The animals were marked for easy identification. Three (3) rats were used for pilot studies to ascertain that the rats could be made diabetic by alloxan treatment at the dose level used (120 mg/kg).

Plant Materials: Collection of Plant/ Identification: The leaves of *Vernonia amygdalina* and the nuts of *Tetracarpidium conophorum* were purchased from Rumuokoro Market in Obio/Akpor Local Government Area, Rivers State. The plant samples were identified at the Herbarium of the Plant Science and Biotechnology Department, University of Port Harcourt, Rivers State, Nigeria.

Preparation of Extract: Tetracarpidium Conophorum Extract: The nuts were boiled for one hour –thirty minutes and air dried for about 30 minutes. The nuts were removed from the shell and ground to coarse powder form using a home grinder /blender. Five grams of the powdered nut was soaked in 50 ml of distilled water for 24 hours after which it was sieved using a muslin cloth and afterwards filtered through Whatmann Filter Paper. The filtrate was kept in the refrigerator until usage.

Vernonia Amygdalina Extract: The leaves of *Vernonia amygdalina* were washed and shade dried at room temperature for seven (7) days, after which the leaf powder was prepared using a home grinder. Powdered *V. amygdalina* leaves weighing 5 g was soaked in 50 ml of distilled water for 24 hours, after which it was sieved using a muslin cloth and afterwards filtered through a Whatmann Filter Paper. The filtrate was kept in a corked container in the refrigerator until usage

Administration of Alloxan: One gramme (1g) of Alloxan was dissolved in 20 ml of distilled water from which a single dose of 120 mg/kg body weight

was administered intra-peritoneally to the rats. Diabetes was confirmed by ascertaining the glucose concentration in the blood of the rats 2-3 days following alloxan injection using a glucometer and was found to have increased by three to four times the normal value.

Experimental Design: The acclimatized animals were sorted into six groups. The diabetic rats were treated with *Tetracarpidium conophorum* extract only, *Vernonia amygdalina* extract only, combined extract of *T. conophorum* and *V. amygdalina* and Metformin a standard antidiabetic drug as shown in the table following

:

Grouping of the Experimental Animals

Groups	Title	Treatment
1	Negative control	The animals in this group are non- diabetic and were given distilled water and normal feed throughout the course of this study.
2	Positive control	The animals in this group were induced with diabetes but were not treated with metformin or the extracts
3	Diabetic rats	The animals in this group were treated with 500 mg/kg *T. conophorum* extract only.
4	Diabetic rats	The animals in this group were treated with 500 mg/kg *V. amygdalina* extract only.
5	Diabetic rats	The animals in this group were treated with 500 mg/kg T. conophorum and 500 mg/kg *V. amygdalina* Extract.
6	Diabetic rats	The animals in this group were treated with 7. 69 mg/kg Metformin only.

The dosage of metformin was obtained by using the standard dose of an average adult which is 500 mg/65kg body weight. So if 500 mg is for 65 kg, therefore 7.69 mg will be for per kg body weight. Osinubi, *et al*. (2007) reported the use of 500 mg/kg body weight of *V. amygdalina* leaves and Okon and Atai (2014) reported the use of 500 mg/kg of *T.conophorum* nuts.

Method of Blood and Organ Collection: The blood samples used to check for glucose level were collected from the tip of the tail of the rats and the diabetic rats tested diabetes positive using glucometer. Three (3) rats from each of the group were sacrificed on the 14^{th} and 28^{th} day of treatment. The animals were anaesthetized using cotton wool soaked in chloroform in a desiccator. The anaesthetized animals were placed on a dissecting slab, the blood sample were collected from the jugular vein with lithium –heparin bottles for chemistry tests. The blood samples were then taken to the laboratory for various analyses. The biggest lobe of the liver and the pancreas were cut off with surgical blade and placed in a sample bottle containing 10% formal- saline solution for histological examination.

Plasma Creatinine Estimation: Method: Modified Jaffé method according to Bartels and Bohmer (1971) was used to determine the level of creatinine in the samples. Mindray test kits was used for the analysis.
Reaction Principle

Creatinine + Picric acid ↔ Creatinine-Picric acid complex

At an alkaline solution, creatinine combines with picric acid to form an orange-red colored complex. The absorbance increase is directly proportional to the concentration of creatinine.

Procedure: Two test tubes labeled T1 (reagent blank) and T2 (test sample) were set up. T1 contained 180 µL of reagent (R1) and 18 µL of distilled water, while T2 contained 180 µL of reagent (R1) and 18 µL of test sample. The contents of each tube were mixed thoroughly at 37℃ for 1 min. After incubating, 180 µL of the second reagent (R 2) was added to both test tubes. The content of the tube was mixed thoroughly, incubated at 37℃ for 30 seconds and the absorbance read at 492 nm wavelength 2 minutes later.
Calculation

ΔA = [ΔA sample]- [ΔA blank]; Conc. of creatinine = [change in absorbance of sample] – [change in absorbance of blank]., The result is expressed in mmol/L.

Plasma Urea Estimation: Method
Urease-glutamate dehydrogenase -UV method according to Berthelot's method (Weatherburn, 1967) was used to determine the level of Urea in the samples. Mindray test kits was used for the analysis.
Reaction Principle

$$Urea + H_2O_2 \leftrightarrow 2NH_4^+ + CO_3^{2-}$$

$$\alpha\text{-Oxoglutarate} + NH_4^+ + NADH \leftrightarrow \text{L-Glutamate} + NAD^+ + H_2O$$

Urea is hydrolyzed by urease, and one of the products, ammonia, oxidises NADH to NAD^+ catalysed by glutamate dehydrogenase (GLDH). The absorbance decrease is directly proportional to the concentration of urea.

Procedure
Two test tubes labeled T1 (reagent blank) and T2 (test sample) were set up. T1 contained 1000 µL of reagent (R1) and 10 µL of distilled water, while T2 contained 1000 µL of reagent (R1) and 10 µL of test sample. The contents of each tube were mixed and incubated at 37℃ for 2 min. After incubating, 250 µL of the second reagent (R2) was added to both test tubes. The contents of each tube was incubated again for 30 seconds at 37℃, the absorbance was read after 2 minutes at a wavelength of 546 nm.
Calculation

ΔA = [ΔA sample]- [ΔA blank],Conc. of urea = [change in absorbance of sample] – [change in absorbance of blank]., The result is expressed in mmol/L.

Assay of amylase activity in the serum. Method: Enzymatic method for estimating amylase activity by Rauscher and Buelow (Rauscher, *et al.,* 1982; Rauscher, *et al.,* 1985) was used to determine the plasma amylase concentration in the samples. This method is in accordance with the continuous monitoring of recommendations on the IFCC (International Federation of Clinical Chemistry). Mindray test kit was used for the analysis.
Reaction Principle

4,6-Benzylidene-G_7PNP → Benz-G $_{(7-n)}$ + PNP-Oligosaccharide

PNP-Oligosaccharide → Glucose + PNP-Glucoside; PNP-Glucoside → p-Nitrophenol + Glucose; (PNP= p-Nitrophenol; G= α-Glucose)

In the assay reaction, the substrate 4, 6- Benzylidene-(G7)-1, 4-nitrophenyl-(G1) –α, D-maltoheptaoside (EPS-G7) is cleaved by α-amylases and Glucoamylase and subsequent hydrolysis of all the degradation products to p-Nitrophenol with the aid of α-glucosidase (100% chromophore liberation). The increase in absorbance of the p-nitrophenol formed at 405 nm is directly proportional to the activity of α-amylases in the sample.
Procedure

Two test tubes labeled T_1 (blank) and T_2 (test sample) were set up. The reagent was incubated for 2-3 minutes at 37℃. T_1 contained only distilled water which serves as the blank, while T_2 contained 500 µL of reagent and 10 µL of test sample and mixed. The test tube content was emptied into a measuring cuvette and the absorbance read after 1,2,3,4, and 5 minutes at a wavelength of 405 nm.

Calculation: Estimate the ΔA/min. for every reading and find the mean value. Conc. of amylase = $(\Delta A_{405nm}$ /min.) x 3178; $(\Delta A_{405nm}$ /min.) = change in absorbance per minute and the result is expressed in U/L.

Histopathological Analysis: The pancreas were cut off and placed in a sample holder containing 10% formal saline. The tissues were placed in increasing strengths of ethanol for dehydration. The tissues were first placed in 70% ethanol for $1^{1}/_{2}$ hr. The tissues were then transferred to 95% ethanol for $1^{1}/_{2}$ hr. They were then transferred to another bath of 95% ethanol for $1^{1}/_{2}$ hr. The tissues were then transferred to three baths of absolute ethanol, the tissues lasting in each bath for $1^{1}/_{2}$ hr. The tissues were cleared in xylene (2 baths for 1 hour each). The tissues were infiltrated with 2 baths of molten paraffin wax for 2 hours. They were then embedded in paraffin wax using embedding rings. Tissue blocks were placed at 4°C for 15 minutes to solidify. Five micrometer sections of the tissues were cut using microtome. Cut sections were placed in a 45°C water bath and placed on a slide. Slides were allowed to dry in a 37°C oven overnight before staining.

The tissues were stained using Haematoxylin and Eosin stains. The tissues for staining were dewaxed using xylene and hydrated using decreasing strengths of alcohol (absolute, 95%, 70%, 50%). The tissues were then immersed in water for complete hydration. The tissues were stained with haematoxylin for 20 minutes. The tissues were washed thoroughly in running tap water. The tissues were differentiated in acid-alcohol until only the cell nuclei retained the stain. The tissues were then blued in Scotts tap water substitute for 1 minutes followed by running tap water. The tissues were counterstained in Eosin for 2 minutes. The tissues were washed in running water until excess eosin is removed. The tissues were dehydrated in increasing strengths of alcohol (50%, 70%, 95% and absolute). There were then cleared in xylene and mounted in DPX ready for microscopic examination (Young, 2006).

Statistical Analysis: All data were subjected to statistical analysis. Values are reported as mean ± standard error of mean (SEM) while one way ANOVA was used to test for differences between treatment groups. The results were considered

significant at p-values of less than 0.05, that is, at 95% confidence level (p<0.05).

RESULTS AND DISCUSSION

From the glucose result represented in figure 1, it was observed that there was a significant (p<0.05) increase on the glucose level of the diabetic group compared to other treated groups which showed a significant (p< 0.05) decrease in glucose level as treatment progressed. Biochemical parameters such as glucose level, amylase level, urea and creatine were analyzed to determine their concentration in the various samples. The results obtained are shown in figures 1 to 4.

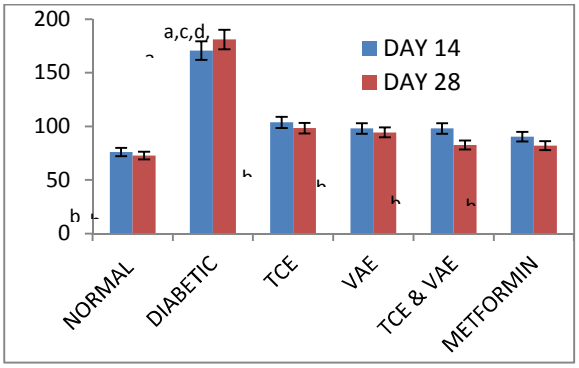

Fig 1: The effect of combined aqueous extracts of *Tetracarpidium conophorum* nuts and *Vernonia amygdalina* leaves on glucose level of alloxan – induced diabetic rats**. KEY: NORMAL=** Non diabetic; **DIABETIC=** Diabetic & untreated; **TCE=** Treated with 500 mg/kg *Tetracarpidium conophorum* extract; **VAE=** Treated with 500 mg/kg *Vernonia amygdalina* extract; **TCE & VAE=** Treated with 500 mg/kg *Tetracarpidium conophorum* and 500 mg/kg *Vernonia amygdalina* extract. **METFORMIN=** Treated with 7.69 mg/kg metformin.

On day 28, significant difference (p<0.05) was observed when diabetic group was compared with the treated groups. Thus, there was a decrease in the blood glucose level in all the treated groups. Also there was no significant difference when the group treated with the combined extracts of *Tetracarpidium conophorum* and *Vernonia amygdalina* were compared with the group treated with metformin a standard drug both on day 14 and day 28 of treatments.

As shown in figure 3, it was observed that there was a significant increase (p<0.05) on the creatinine level of the diabetic untreated group on day 14 when compared with other treated groups which showed a significant (p<0.05) decrease. On day 28, the groups treated with *Vernonia amygdalina* extract and metformin showed a significant (p<0.05) decrease when compared to the group treated with *Tetracarpidiun conophorum* extract.

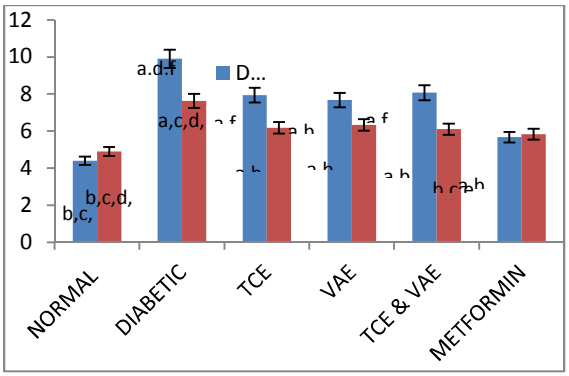

Fig 2: The effect of combined aqueous extracts of *Tetracarpidium conophorum* nuts and *Vernonia amygdalina* leaves on plasma urea level of alloxan induced diabetic wistar rats. **KEY: NORMAL=** Non diabetic; **DIABETIC=** Diabetic & untreated; **TCE=** Treated with 500 mg/kg *Tetracarpidium conophorum* extract; **VAE=** Treated with 500 mg/kg *Vernonia amygdalina* extract; **TCE & VAE=** Treated with 500 mg/kg *Tetracarpidium conophorum* and 500 mg/kg *Vernonia amygdalina* extract. **METFORMIN=** Treated with 7.69 mg/kg metformin.

As shown in figure 2, it was observed that there was a significant (p<0.05) increase in the urea level of the diabetic group (untreated animals, when compared with that of other treated groups which showed a significant (p<0.05) decrease. On day 28, the group treated with combined extracts of *Tetracarpidium conophorum* and *Vernonia amygdalina* was in close range with the group treated with metformin.

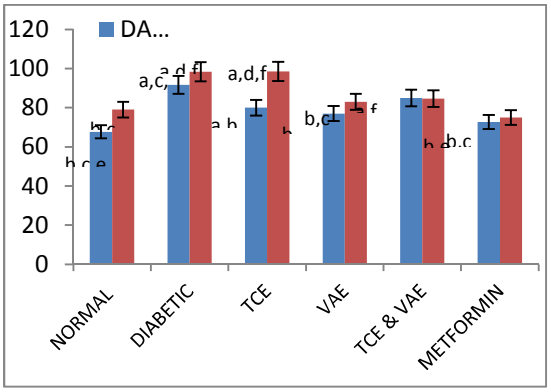

Fig 3: The effect of combined aqueous extracts of *Tetracarpidium conophorum* nuts and *Vernonia amygdalina* leaves on plasma creatinine level of Alloxan induced diabetic wistar rats. **KEY: NORMAL=** Non diabetic; **DIABETIC=** Diabetic & untreated; **TCE=** Treated with 500 mg/kg *Tetracarpidium conophorum* extract; **VAE=** Treated with 500 mg/kg *Vernonia amygdalina* extract; **TCE & VAE=** Treated with 500 mg/kg *Tetracarpidium conophorum* and 500 mg/kg *Vernonia amygdalina* extract. **METFORMIN=** Treated with 7.69 mg/kg metformin.

As shown in figure 3, it was observed that there was a significant increase ($p<0.05$) on the creatinine level of the diabetic untreated group on day 14 when compared with other treated groups which showed a significant ($p<0.05$) decrease. On day 28, the groups treated with *Vernonia amygdalina* extract and metformin showed a significant ($p<0.05$) decrease when compared to the group treated with *Tetracarpidiun conophorum* extract.

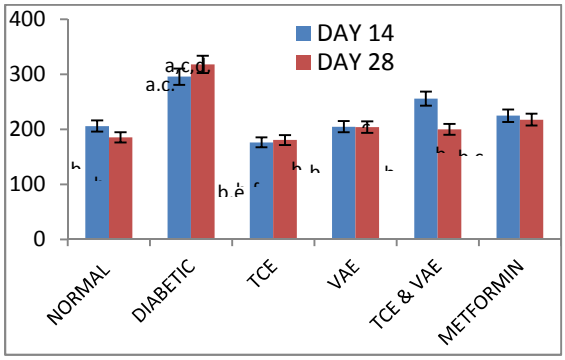

Fig 4: The effect of combined aqueous extracts of *Tetracarpidium conophorum* nuts and *Vernonia amygdalina* leaves on plasma amylase level of Alloxan induced diabetic wistar rats. KEY: NORMAL= Non diabetic; DIABETIC= Diabetic & untreated; TCE= Treated with 500 mg/kg *Tetracarpidium conophorum* extract; **VAE=** Treated with 500 mg/kg *Vernonia amygdalina* extract; **TCE & VAE=** Treated with 500 mg/kg *Tetracarpidium conophorum* and 500 mg/kg *Vernonia amygdalina* extract. **METFORMIN=** Treated with 7.69 mg/kg metformin.

As shown in figure 4, it was observed, that there was a significant increase ($p<0.05$) on the amylase level of the diabetic untreated group when compared with other treated groups which showed a significant ($p<0.05$) decrease.

On day 14, there was a significant decrease on the amylase level of the group treated with *Tetracarpidium conophorum* extract when compared to the group treated with combined extracts of *Tetracarpidium conophorum* and *Vernonia amygdalina*. Also on day 28, there was a significant decrease on the amylase level of the group treated with *Tetracarpidium conophorum* extract when compared to the group treated with metformin.

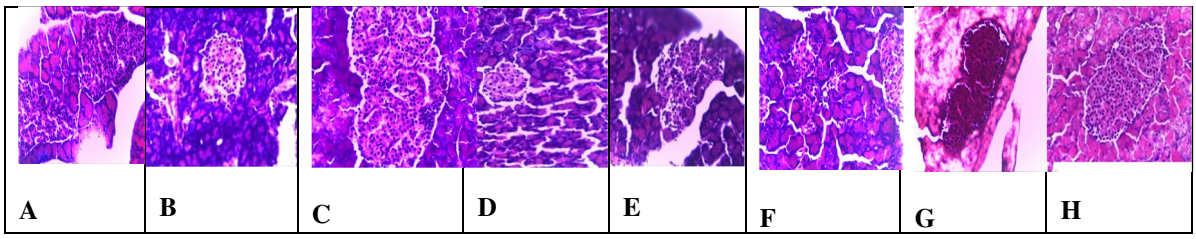

Plate A: Photomicrograph of the pancreas of the control rat after 14 days of treatment, stained with haematoxylin and eosin (x40) showing normal pancreas. **Plate B**: Photomicrograph of the pancreas of the diabetic control rat after 14 days of treatment stained with haematoxylin and eosin (x40) howing reduced endocrine pancreatic cell. mass with evidence of cytolysis **Plate C:** Photomicrograph of the pancreas of the rat treated with 500 mg/kg TCE and 500 mg/kg VAE after 14 days of treatment stained with haematoxylin and eosin (x40) showing increased endocrine pancreatic cell mass and normal cellular architecture. **Plate D:** Photomicrograph of the pancreas of the rat treated with 7.69mg/kg metformin after 14 days of treatment stained with haematoxylin and eosin (x40) showing increased endocrine pancreatic cell mass and normal cellular architecture **Plate E**: Photomicrograph of the pancreas of the control rat after 28 days of treatment stained with haematoxylin and eosin (x40) showing normal endocrine pancreas. **Plate F**: Photomicrograph of the pancreas of the diabetic control rat after 28 days of treatment stained with haematoxylin and eosin (x40) showing reduced endocrine pancreatic cell mass with cytolysis and mononuclear inflammatory cells infiltration. **Plate G**: Photomicrograph of the pancreas of the rat treated with 500 mg/kg TCE and 500 mg/kg VAE after 28 days of treatment stained haematoxylin and eosin (x40) showing increased endocrine pancreatic cell mass.

The consumption of *Tetracarpidium conophorum* and *Vernonia amygdalina* extract by diabetic rats can have a lot of effects on the glucose level, amylase level, urea and creatinine levels. There had been reports on the effect of *Tetracarpidium conophorum* and *Vernonia amygdalina* individually on these biochemical parameters on alloxan-induced diabetic rats.

This work was aimed at assessing the effects of the combined treatment or synergistic effect of aqueous extracts of *Tetracarpidium conophorum* nuts and *Vernonia amygdalina* leaves on the glucose level, amylase level, urea and creatinine levels on alloxan induced diabetic rats. The pancreas was assessed to know the extent of damage and effect of the combined treatment of these plant extracts in healing the damaged pancreas using diabetic Wistar rats as experimental model.

From the results of the glucose level, it was observed that there was a significant ($p<0.05$) increase in the glucose level of the diabetic untreated animals when compared to treated animals which have significant ($p<0.05$) decrease in glucose level as treatment progressed.

On Day 28, there was a significant ($p<0.05$) increase when the untreated animals were compared with the treated animals but most effective and efficient in the group treated with combined extracts of *Tetracarpidium conophorum* and *Vernonia amygdalina*, these combined extract competed favorably with the reference drug metformin. This result agreed with the advantages of polyherbal articulated by Tiwari and Rao (2002).

In addition to the antidiabetic properties of these plants, each of the plants have been reported of different activity geared towards alleviation of complication usually associated with diabetes. The findings of this present study also confirmed that *Tetracarpidium conophorum* extract alone and *Vernonia amygdalina* extract alone reduce blood glucose level significantly ($p<0.05$) on alloxan induced diabetic rats. This can be attributed to the bioactive molecules present in the indigenous plants. This report is in accordance with that of Donatus, *et al*. (2014) which reported the antihyperglycaemic effect of *Tetracarpidium conophorum* nuts on alloxan induced diabetic female albino rats. Ukpabi, *et al*. (2015) also reported the hypoglycaemic effect of *Vernonia amygdalina* on alloxan induced diabetic rats. Ayoola, *et al*. (2011) reported the presence of tannins, saponins, alkaloids, phenols and oxalate on *Tetracarpidium conophorum* nut. According to Ukpabi, *et al*. (2015), *Vernonia amygdalina* is rich in alkaloid, tannins, saponins, flavonoids and glycosides. Secondary metabolites of plants such as the ones listed above possess some alpha-glucosidase inhibitors and competively inhibit intestinal brush border enzymes with an eventual reduction in digestion and absorption of carbohydrates from the gut-postprandial hyperglycemia, hence resulting in an effective glucose control (Tiwari and Rao, 2002). A positive correlation has also been indicated between the presence in plants of flavonoids, glycosides and phytosterols with hypoglycaemic and anti-hyperglycaemic actions (Ekeocha, *et al*., 2012). The two plants have bitter taste which could be due to the presence of phytochemicals such as alkaloids, saponins, tannins and glycoside. The hypoglycaemic effect observed in the present study could be due to depression of key gluconeogenic or the increase in the levels of glucose transport and stimulation of uptake in peripheral tissues (Ji Su, *et al*., 2006). It could as well be that these plant extracts may have the potential of preserving the cells of islets of langerhans, which in turn result in an increase in insulin activity (Kamiya, *et al*., 2008; Yoshikawa, *et al*., 1995; Hossain, *et al*., 1992).

The inhibition of α-glucosidase and pancreatic α-amylase activity result in delaying carbohydrate digestion to absorbable monosaccharide, causing reduction of post prandial hyperglycemia. In diabetic condition, amylase is very high as it degrades long chain carbohydrate complexes into simpler glucose, maltose and other absorbable monosaccharides. From the result, it was observed that there was a significant increase in amylase level of the diabetic control when compared with other treated groups which showed a significant decrease. The group treated with *Tetracarpidium conophorum* extract showed the lowest value of amylase level and there was a significant decrease ($p<0.05$) when compared with the group treated with metformin a standard drug. This finding may be as result of phenolic compounds contained in *Tetracarpidium conophorum*. Published research suggests that there is a direct relationship between the phenolic compounds, flavonoids and condensed tannins and the ability to inhibit α-glucosidase and α-amylase activities (Adisakwattana, *et al*., 2009; Lee, *et al*., 2007; Mai, *et al*., 2007).

There was a significant increase ($p<0.05$) on the urea and cretinine levels of the diabetic group when compared with other groups which showed a significant ($p<0.05$) decrease. Plasma level of urea and creatinine are primarily used to determine the efficiency of the kidney. Urea and creatinine accumulate in the plasma when renal excretion is reduced. Causes of increased blood urea levels include high protein diet, intestinal haemorrhage, dehydration, severe haemorrhage, shock etc. Urea level could be decreased due to the following: liver failure, low protein diet, anabolic steroids, diabetes insipidus etc (Bush, 1991). In this present study, the combined extracts of *Tetracarpidium conophorum* and *Vernonia amygdalina* were able to reduce the plasma levels of urea and creatinine, this is an indication that the extracts possess the potentials of ameliorating renal failure.

The histological examination of the pancreas of the diabetic control rat on day 14 showed reduced endocrine pancreatic cell mass with evidence of cytolysis compared to that of the rat that was treated with 500 mg/kg TCE and 500 mg/kg VAE which showed increased endocrine cell mass and normal cellular architecture.

On day 28, the histology examination of the pancreas of the diabetic control rat showed reduced endocrine pancreatic cell mass with cytolysis and mononuclear inflammatory cells infiltration compared to that of the rat that received 500 mg/kg TCE and 500 mg/kg VAE which showed mild to moderate cytolysis of the endocrine pancreas with residual increase in cell

mass. From these changes observed in the histological examination of the pancreas, it can be inferred that the combined aqueous extracts of *Tetracarpidium conphorum* nuts and *Vernonia amygdalina* leaves were able to ameliorate the damage caused by alloxan.

Conclusion: The present work indicated that the combined aqueous extracts of *Tetracarpidium conophorum* nuts and *Vernonia amygdalina* leaves reduced the level of damage to the kidney and pancreas when administered to diabetic rats at the dosage used in this study.

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