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Ameliorating Effect of Combinedaqueous Extracts of *Tetracarpidium Conophorum* (Nuts) and *Vernonia Amygdalina (Leaves)* on Liver Enzymes of Alloxan Induced Diabetic Wistar Rats

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Abstract:
The present study evaluated the effect of combined treatment of aqueous extracts of Tetracarpidium conophorum (TCE) walnuts and Vernonia amygdalina (VAE) bitter leaves on some liver enzymes 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 14^{th} and 28^{th} day of treatment. The plasma levels of glucose, alkaline phosphatase (ALP), aspartate amino transferase (AST), alanine amino transferase (ALT). The result showed that there was a significant reduction ($p<0.05$) in the blood glucose level of all the treated groups compared to the diabetic control. Also, the plasma levels of AST, ALT ALP ($p<0.05$) decreased in all treated groups compared to the untreated diabetic group. The histology of the liver of the treated groups showed that the plant extracts ameliorated the effect of alloxan on the organ. In conclusion, the combined aqueous extracts of Tetracarpidiumconophorum nuts and Vernonia amygdalina leaves reduced the level of damage to the liver when administered to diabetic rats at the dosage used in this study.

Keywords: *Alanine amino transferase, Alkaline phosphatase, Aspartate amino transferase, Diabetes, Enzymes, Liver, Tetracarpidium conphorum, Vernonia amygdalina*

1. Introduction

The African walnut, *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).

T. conophorum is rich in linoleic and linolenic acids and other compounds such as arginine, vitamins, folate and polyphenols. This arginine is an amino acid that is required by the body to produce nitric oxide that is necessary for keeping the blood vessels flexible (Nus *et al*., 2004). It is used to boost male fertility and also inhibits microbial activity (Okon and Atai, 2014). Ajaiyeoba and Fadare, 2006).

Vernonia amygdalina is commonly called bitter leaf because of the characteristics odour 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). Arhoghro *et al*. (2009) reported that oral administration of the aqueous extract of the plant could ameliorate liver damage through reduction of liver marker enzymes.

The chronic condition of diabetes mellitus 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).

It is therefore very important for a biomedical research on development of hypoglycaemic agent for the control of the disease from natural sources to be instituted. There are more than 800 plant species with hypoglycaemic activity (Donatus *et al*., 2014).
Aspartate aminotransferase (AST)is an important enzyme in amino acid metabolism. AST is found in the liver, heart, skeletal muscle, kidneys, brain, and red blood cells. Alanine aminotransferase (ALT) is found in plasma and in various body tissues, but is most common in the liver. SerumAST/ALT ratio are commonly measured clinically as biomarkers for liver health. These enzymes have become increasingly important in clarifying the etiology, pathonogenesis as well as diagnosis of a number of diseases associated with the different organs in which they are present or concentrated (Watkins and Kaplowitz, 2006, Okoye *et al*, 2012a).Phosphatases are enzymes capable of hydrolyzing organic esters of phosphoric acid to organic compounds and free inorganic phosphate. Alkaline phosphatase (ALP) has optimum pH of about of about 9.8. It is present in the blood plasma, bone, kidney, intestine, lungs (Okoye *et al*, 2012b).
Reports on the combined hypoglycaemic effects of these plants are scanty and limited. Therefore, in this present study, the effects of combined aqueous extracts of *Tetraaridium conophurm* nuts and *Vernonia amygdalina* leaves on some liver enzymes of alloxan induced diabetic rats were investigated. The liver enzymes analysed were ALT, AST and ALP. Also, the histology of the liver was investigated.

2. Materials and Methods

The reagents and materials used during this research work were of analytical grade.
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.).

2.1. 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 (120mg/kg).

2.2. 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.

2.3. Preparation of Extract

2.3.1. 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 50ml 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.

2.3.2. 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 5g was soaked in 50ml 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.

2.4. Administration of Alloxan

One gram (1g)of Alloxan was dissolved in 20ml of distilled water from which a single dose of 120mg/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.

2.5. Experimental Design

The acclimatized animals were sortedinto 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 1:

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 500mg/kg *T.conophorum* extract only.
4	Diabetic rats	The animals in this group were treated with 500mg/kg *V. amygdalina*extract only.
5	Diabetic rats	The animals in this group were treated with 500mg/kg T. conophorum and 500mg/kg *V. amygdalina* Extract.
6	Diabetic rats	The animals in this group were treated with 7. 69 mg/kg Metformin only.

Table 1: Grouping of the Experimental Animals

The dosage of metformin was obtained by using the standard dose of an average adult which is 500mg/65kg body weight. So if 500mg is for 65kg, therefore 7.69mg will be for per kg body weight. Osinubi *et al.* (2007) reported the use of 500mg/kg body weight of *V. amygdalina* leaves and Okon and Atai (2014) reportedtheuseof500 mg/kg of *T.conophorum* nuts.

2.6. 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} dayof 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 was cut off with surgical blade and placed in a sample bottle containing 10% Formal- saline solution for histological examination.

2.7. Assay of Liver Enzyme Activities

The plasma activities of all the liver enzymes were assayed using Mindray test kits.

ASSAY OF ALANINE AMINOTRANSFERASE (ALT).

The plasma activity of alanine transaminase was assayed using Reitman and Frankel method (Reitman and Frankel, 1957).

Reaction Principle

α-oxoglutarate + L-alanine ↔ L-glutamate + pyruvate

pyruvate + NADH + H^+ ↔ L-lactate + NAD^+

Alanine aminotransferase catalyzes the reversible transamination of L-alanine and α-oxoglutarate to pyruvate and L-glutamate.

The pyruvate is then reduced to lactate in the presence of lactate dehydrogenase (LDH) with the concurrent oxidation of reduced β-nicotinamide adenine dinucleotide (NADH) to β-nicotinamide adenine dinucleotide (NAD^+). There is change in absorbance which is directly proportional to the activity of ALT in the sample.

Procedure

Two test tubes labeled T1 (reagent blank) and T2 (test sample) were set up. T1 contained 1000 µL of reagent (R1) and 100 µL of distilled water, while T2 contained 1000 µL of reagent (R1) and 100 µL of test sample. The contents of each tube were mixed for 5 min. After incubating at 37°C, 250 µL of the second reagent (R2) was added to both test tubes (T1 and T2). After mixing thoroughly, the absorbance was read after 1 min and time was monitored. The absorbance was read again after additional 3 minutes at 546nm.

Calculation

ΔA/min = [ΔA/min sample] - [ΔA/min blank]

Plasma activity of ALT= [change in A/min of Sample]-[change in A/min of blank].

The result is expressed in IU/L.

2.8. Assay of Aspartate Aminotransferase (AST) Activity

The plasma activity of aspartate transaminase was assayed using Reitman and Frankel method (Reitman and Frankel, 1957).

2.8.1. Reaction Principle

L-aspartate + α-oxoglutarate ↔ oxaloacetate + L-glutamate

oxaloacetate + NADH + H^+ ↔ L-malate + NAD^+

In the assay reaction, the AST catalyzes the reversible transamination of L-aspartate and α-oxoglutarate to oxaloacetate and L-glutamate. The oxaloacetate is then reduced to malate in the presence of malate dehydrogenase (MDH) with NADH being oxidized to NAD^+. The rate of the photometrically assayed NADH decrease is directly proportional to the rate of formation of oxaloacetate and thus the AST activity.

2.8.2. Procedure
Two test tubes labeled T1 (reagent blank) and T2 (test sample) were set up. T1 contained 1000µL of reagent (R1) and 100µL of distilled water, while T2 contained 1000 µL of reagent (R1) and 100 µL of test sample. The contents of each tube were mixed for 5 min. After incubating at 37°C, 250 µL of the second reagent (R2) was added to both test tubes (T1 and T2). After mixing thoroughly, the absorbance was read after 1 min and time was monitored. The absorbance was read again after additional 3 minutes at 546nm.

2.8.3. Calculation
ΔA/min = [ΔA/min sample] - [ΔA/min blank]
Plasma activity of AST= [change in A /min of Sample] -[change in A /min of blank].
The result is expressed in IU/L.

2.9. Assay of Plasma Alkaline Phosphatate (ALP) Activity
The plasma activity of alkaline phosphatase (ALP) was assayed using colorimetric method of Deuptsche Gesellschaft für Klinische Chemie (GSCC, 1972).

2.9.1. Reaction Principle
p-Nitrophenyl phosphate + H_2O ↔ p-nitrophenol + Phosphate
By the action of ALP and magnesium ions, p-nitrophenyl phosphate is catalysed to p-Nitrophenol, and the absorbance increase is directly proportional to the activity of ALP.
Procedure
Two test tubes labeled T1(reagent blank) and T2 (test sample) were set up. T1contained 1000 µL of reagent (R1) and 20 µL of distilled water, while T2 contained 1000 µL of reagent (R1) and 20 µL of test sample. The contents of each tube were mixed and incubated for 2 min at 37℃. After incubating, 250 µL of the second reagent (R2) was added to both test tubes (T1 and T2). After mixing thoroughly, it was incubated at 37℃ for 1 min. and the absorbance was read after 1 min and time monitored. The absorbance was read again after additional 3 minutes at 546nm.

2.9.2. Calculation
ΔA/min = [ΔA/min sample] - [ΔA/min blank]
Plasma activity of ALP= [change in A /min of Sample]-[change in A /min of blank].
The result is expressed in IU/L.

2.10. Histopathological Analysis
The livers 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.

3. 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 ($p<0.05$).

4. Results
Biochemical parameters such as glucose level, liver function tests (ALT, AST and ALP) were analyzed to determine their concentration in the various samples. The results obtained are shown in Figures 1 to 4.

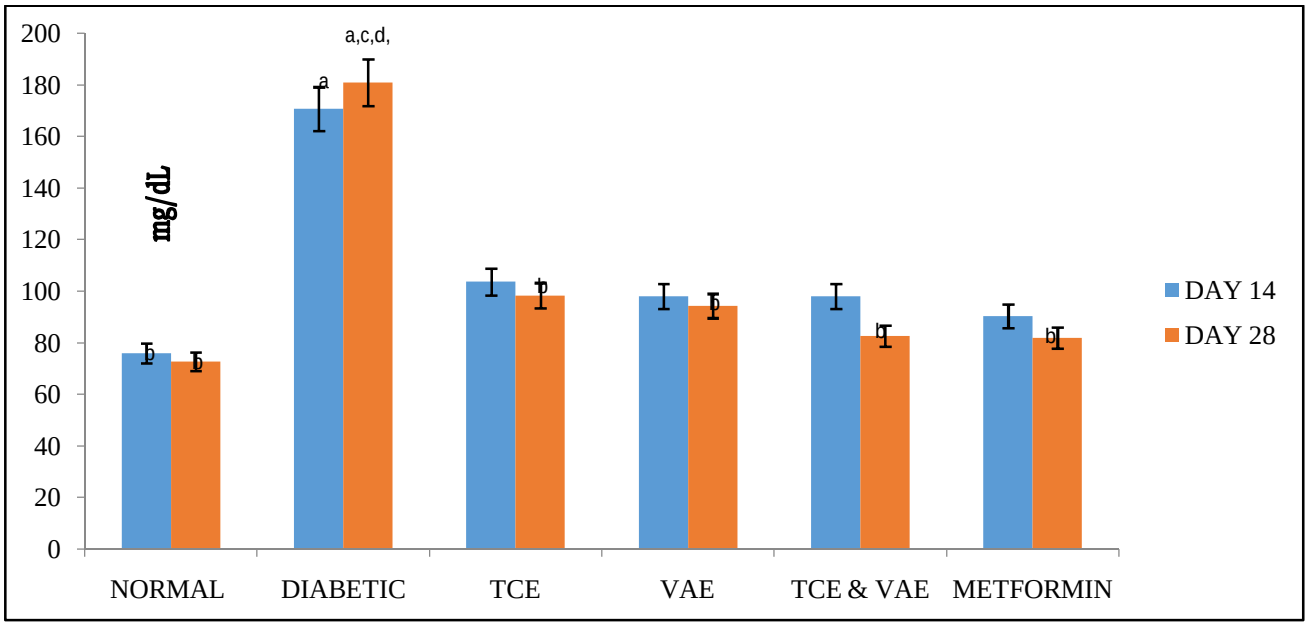

Figure 1: The effect of combined aqueous extracts of Tetracarpidium conophorumnuts and Vernonia amygdalina leaves on glucose level of alloxan – induced diabetic rats.

KEY: NORMAL= Non diabetic; DIABETIC= Diabetic & untreated; TCE= Treated with 500mg/kg Tetracarpidium conophorum extract; VAE= Treated with 500mg/kg Vernonia amygdalina extract; TCE & VAE= Treated with 500mg/kg Tetracarpidium conophorum and 500mg/kg Vernonia amygdalina extract. METFORMIN= Treated with 7.69mg/kg metformin.

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.

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 *Vernoniaamygdalina* were compared with the group treated with metformin a standard drug both on day 14 and day 28 of treatments.

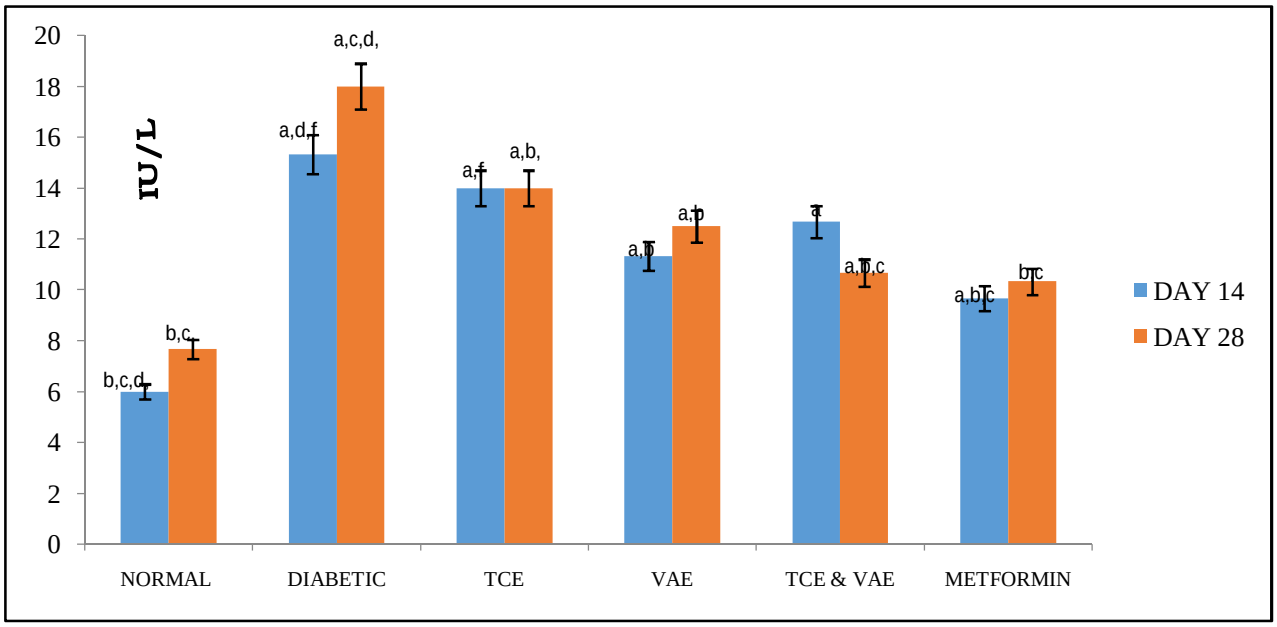

Figure 2: The effect of combined aqueous extracts of Tetracarpidium conophorum nuts and Vernonia amygdalina leaves on plasma AST level of Alloxan induced diabetic rats

KEY: NORMAL= Non diabetic; DIABETIC= Diabetic & untreated; TCE= Treated with 500mg/kg Tetracarpidium conophorum extract; VAE= Treated with 500mg/kg Vernonia amygdalina extract; TCE & VAE= Treated with 500mg/kg Tetracarpidium conophorum and 500mg/kg Vernonia amygdalina extract. METFORMIN= Treated with 7.69mg/kg metformin.

As shown in Figure 2, it was observed that there was a significant ($p<0.05$) increase in the plasma AST level of the diabetic group (untreated animals) when compared with that of the treated groups which showed a significant ($p<0.05$) decrease both on day 14 and day 28 of treatment. There was a significant increase ($p<0.05$) when the group treated with *Tetracarpidium conophorum* were compared to group treated with combined extracts of *Tetracarpidium conophorum* and *Vernonia amygdalina*. Also there was a significant ($p<0.05$) increase when the group treated with *Tetracarpidium conophorum* extract were compared to the group treated with metformin both on day 14 and day 28 of treatments.

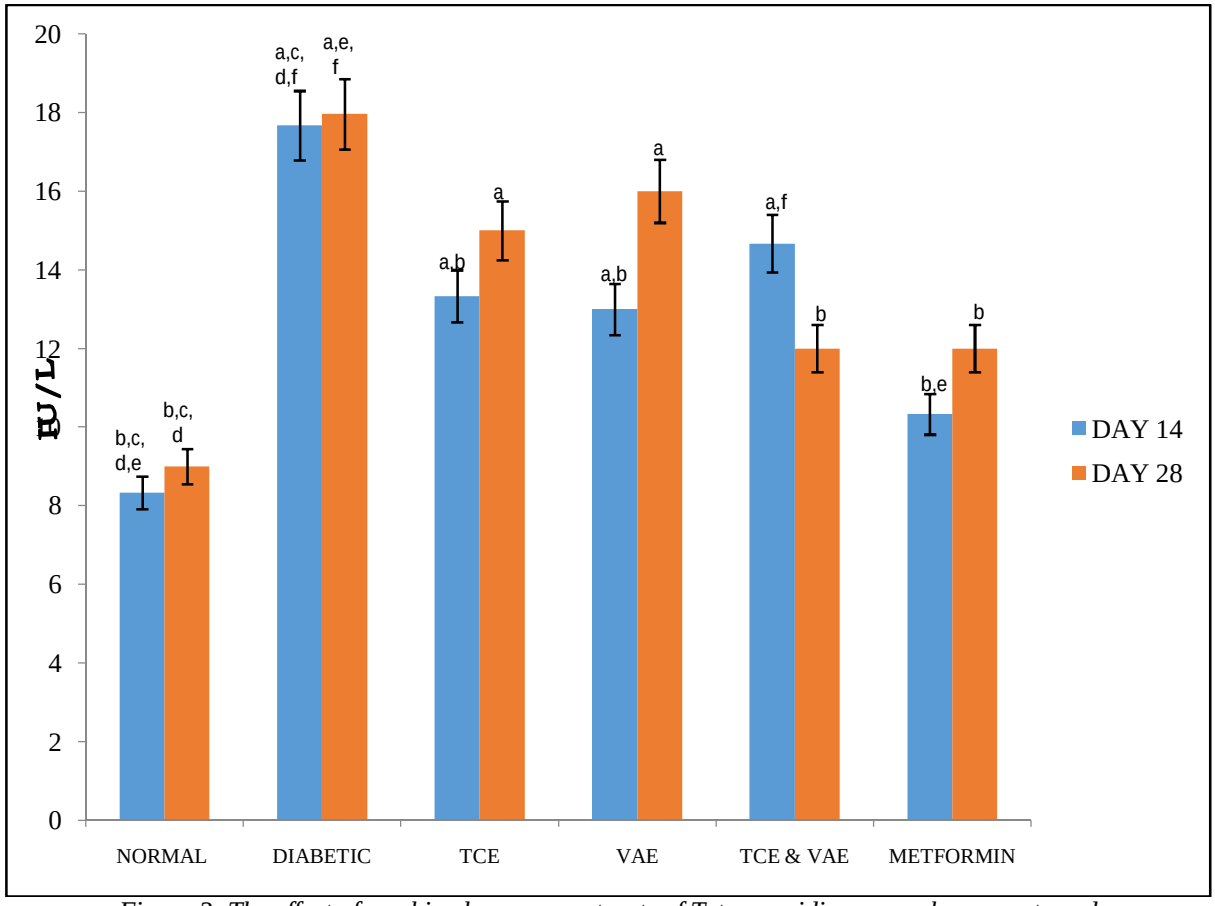

Figure 3: The effect of combined aqueous extracts of Tetracarpidium conophorum nuts and Vernonia amygdalina leaves on plasma ALT level of alloxan induced diabetic rats

Superscript "a" shows significant difference, ($p<0.05$) when normal group is compared with other groups.
Superscript "b" shows significant difference, ($p<0.05$) when diabetic group is compared with other groups.
Superscript "c" shows significant difference, ($p<0.05$) when TCE group is compared with other groups.
Superscript "d" shows significant difference, ($p<0.05$) when VAE group is compared with other groups.
Superscript "e" shows significant difference, ($p<0.05$) when TCE & VAE group is compared with other groups.
Superscript "f" shows significant difference, ($p<0.05$) when metformin group is compared with other groups.

KEY: NORMAL= Non diabetic; DIABETIC= Diabetic & untreated; TCE= Treated with 500mg/kg *Tetracarpidium conophorum* extract; VAE= Treated with 500mg/kg *Vernonia amygdalina* extract; TCE & VAE= Treated with 500mg/kg *Tetracarpidium conophorum* and 500mg/kg *Vernonia amygdalina* extract. METFORMIN= Treated with 7.69mg/kg metformin.
As shown in Figure 3, it was observed that there was a significant ($p<0.05$) increase in the plasma ALT level of the diabetic group (untreated animals, when compared with that of the treated groups which showed a significant ($p<0.05$) decrease. On day 28 of treatment, the group treated with the combined extracts of *Tetracarpidium conophorum* and *Vernonia amygdalina* competed favorably with the group treated metformin as they were in close range.

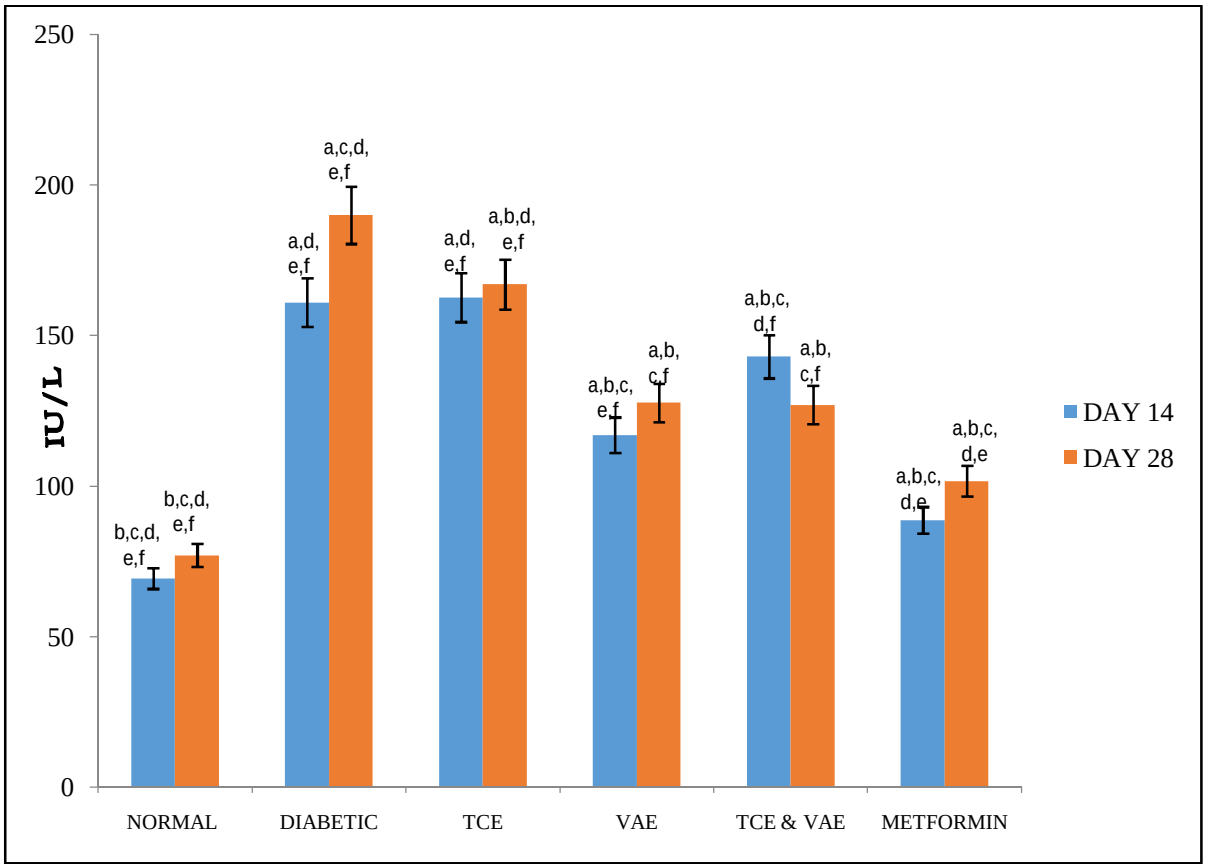

Figure 4: The effect of combined aqueous extracts of Tetracarpidium conophorumnuts and Vernonia amygdalina leaves on plasma ALP level of alloxan induced diabetic wistar rats

KEY: NORMAL= Non diabetic; DIABETIC= Diabetic & untreated; TCE= Treated with 500mg/kg *Tetracarpidium conophorum* extract; VAE= Treated with 500mg/kg *Vernonia amygdalina* extract; TCE & VAE= Treated with 500mg/kg *Tetracarpidium conophorum* and500mg/kg *Vernonia amygdalina* extract. METFORMIN= Treated with 7.69mg/kg metformin.

As shown in Figure 4, it was observed that there was a significant increase ($p<0.05$) in the plasma ALP level of the diabetic untreated group, when compared to that of the treated groups which showed a significant ($p<0.05$) decrease both on day 14 and 28 of treatment. There was a significant difference ($p<0.05$) among the treated groups, the group treated with *Tetracarpidium conophorum*extract showed a significant ($p<0.05$) increase when compared to groups treated with *Vernonia amygdalina*extract, combined extracts of *Tetracarpidium conophorum*and *Vernonia amygdalina* and metformin. There was a significant decrease ($p<0.05$) when metformin group is compared with other treated groups.

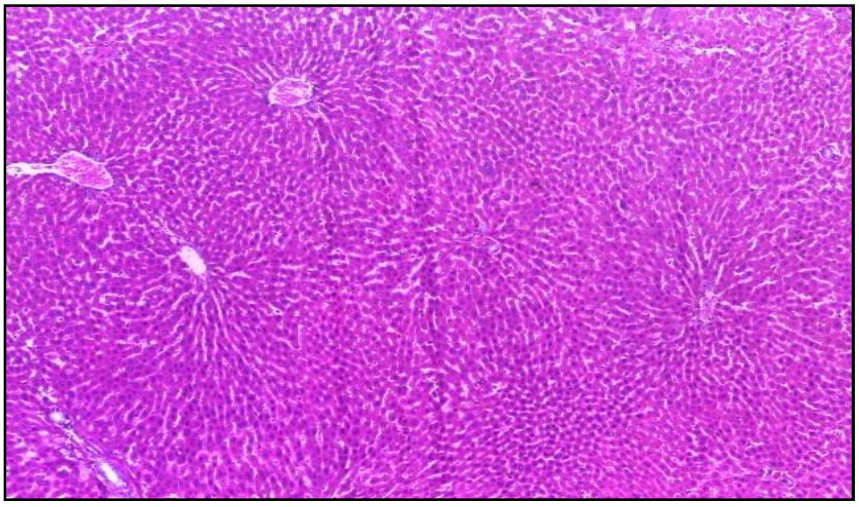

Figure 5: Photomicrograph of the liver of control rat after 14 days of treatment, stained with haematoxylin and eosin (x20) showing normal liver

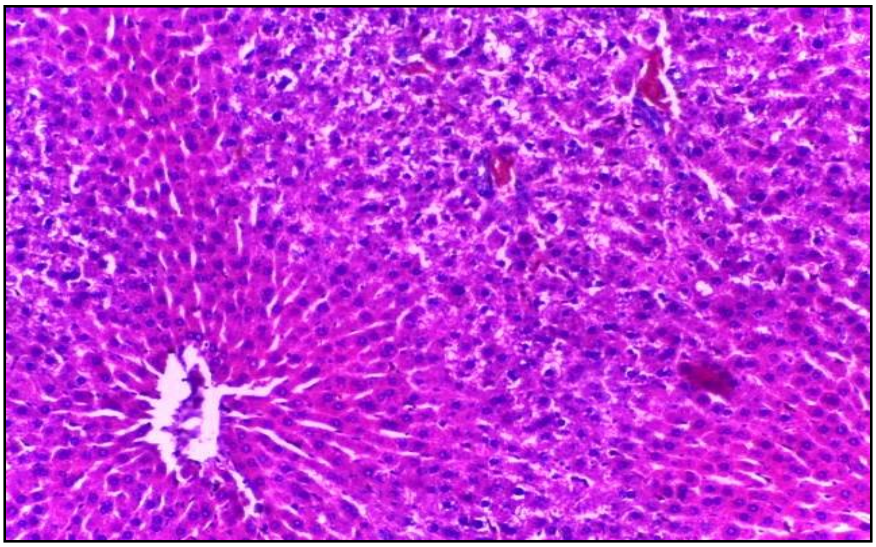

Figure 6: Photomicrograph of the liver of diabetic control rat after 14 days of treatment, stained with haematoxylin and eosin (x20) showing early fatty change in the liver

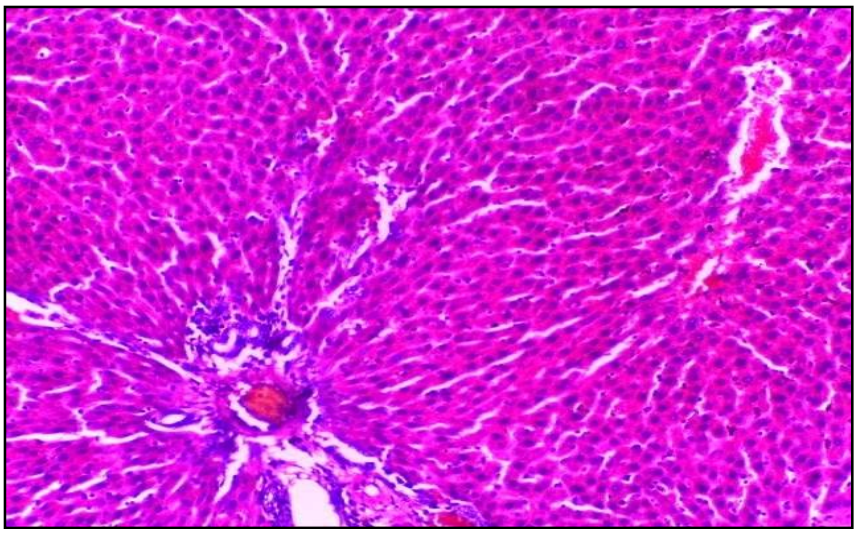

Figure 7: Photomicrograph of the liver of the rat treated with 500mg/kg TCE and 500mg/kg VAE after 14 days of treatment stained haematoxylin and eosin (x20) showing restored lobular architecture and minimal diffuse mononuclear inflammatory cells infiltration

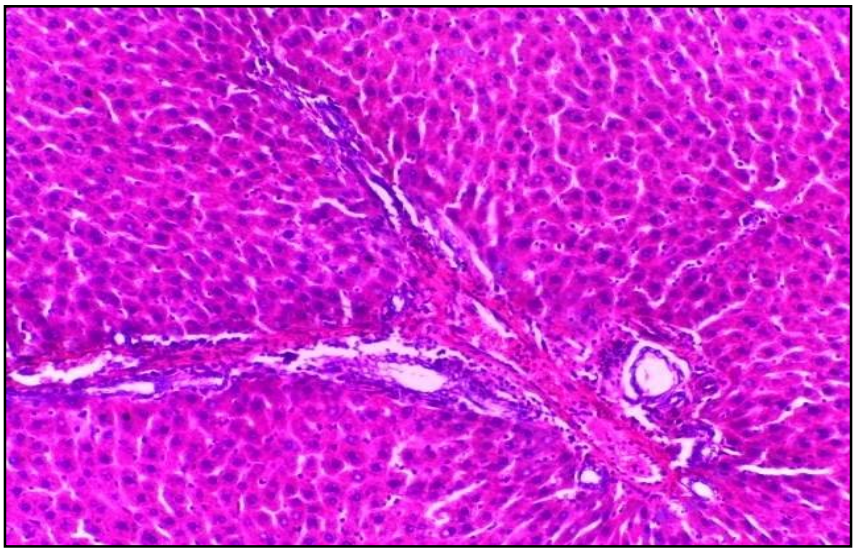

Figure 8: Photomicrograph of the liver of the rat treated with 7.69mg/kg metformin after 14 days of treatment stained with heamatoxylin and eosin (x20)) showing normal liver architecture with residual mononuclear inflammatory cells infiltrate

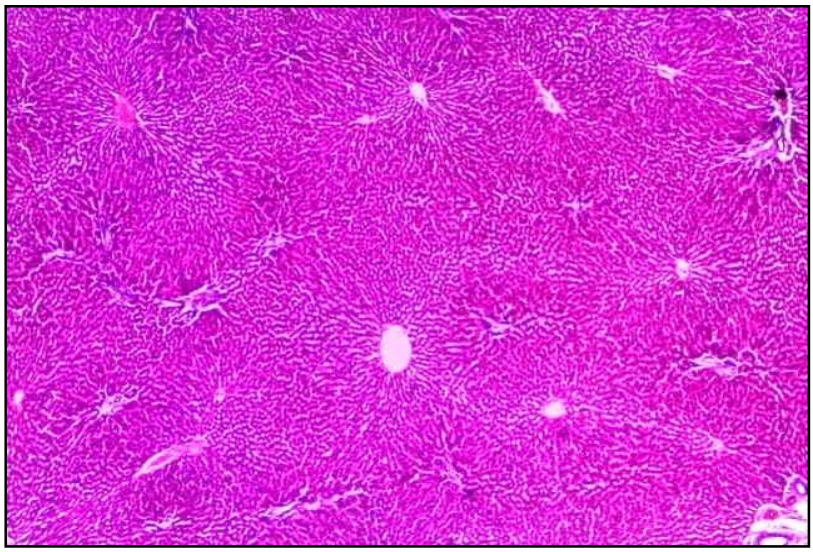

Figure 9: Photomicrograph of the liver of the control rat after 28 days of treatment, stained with haematoxylin and eosin (x10) showing normal liver

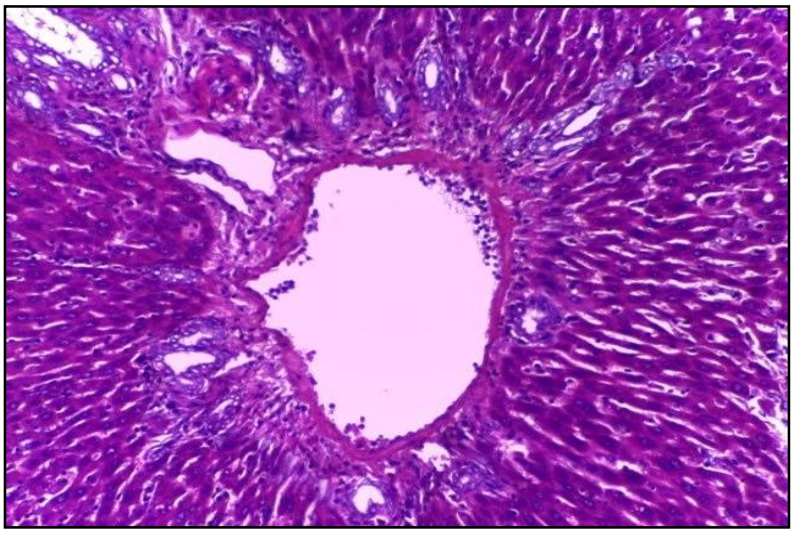

Figure 10: Photomicrograph of the liver of the diabetic control rat after 28 days of treatment stained with haematoxylin and eosin (x40) showing early fatty liver change with portal tract fibrosis and infiltration by mononuclear inflammatory cells infiltration

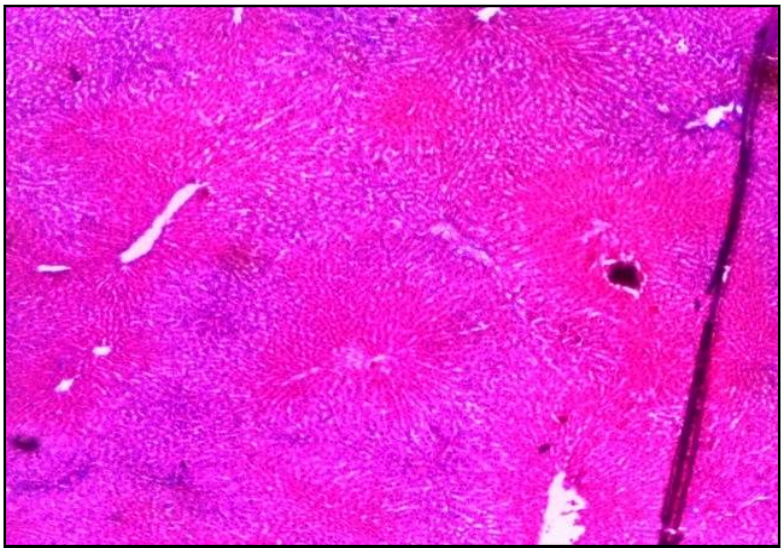

Figure 11: Photomicrograph of the liver of the rat treated with 500mg/kg TCE and 500mg/kg VAE after 28 days of treatment stained with heamatoxylin and eosin (x10) showing normal lobular architecture but residual inflammatory cells infiltration

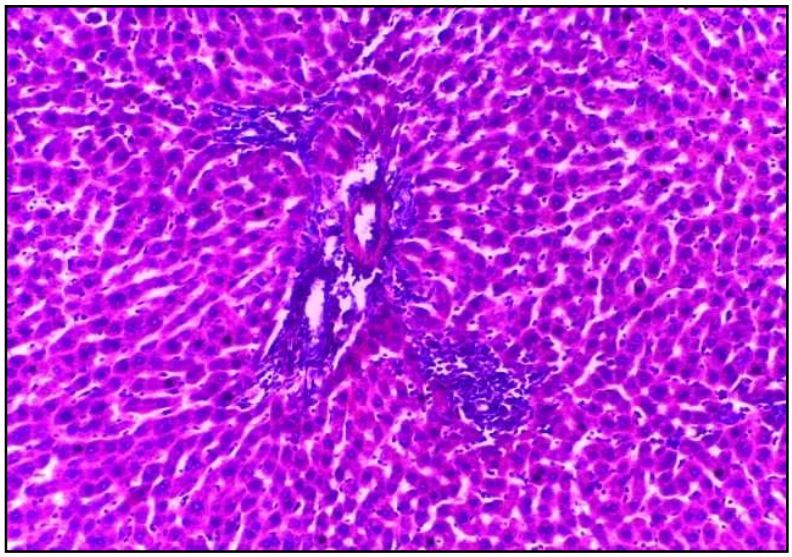

Figure 12: Photomicrograph of the liver of the rat treated with 7.69 mg/kg metformin after 28 days of treatment stained with heamatoxylin and eosin (x40) showing patchy collection of mononuclear inflammatory cells and fibrosis of the limiting plate

5. Discussion

The consumption of *Tetracarpidium conophorum* and *Vernonia amygdalina* extract by diabetic rats can have a lot of effect on the glucose level and liver enzymes. 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 and liver enzymes on alloxan induced diabetic rats. The liver was assessed to know the extent of damage and effect of the combined treatment of these plant extracts in healing the damaged liver, using diabetic Wistar rats as experimental model.

From the result, 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 competitively 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*., 2013). 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 (Hossain *et al*., 1992, Yoshikawa *et al*., 1995, Kamiya *et al*., 2008).

There was a significant ($p<0.05$) increase in the activities of all liver enzymes (ALT, AST and ALP) of the diabetic group (untreated animals) when compared with that of treated groups which showed a significant ($p<0.05$) decrease in the activities of liver enzymes.

Diabetic patients have higher tendency of developing liver function abnormalities (Ikebukuro*et al*., 2002). The plasma levels of specific enzymes activity show the extent of liver damage, thus the degree of elevation of a particular enzyme activity in plasma is often used as basis of the state of health of thepatient. Increase in the plasma levels of liver enzymes is an indicator of liver injury, which may be inflammation or damage to the hepatic cells (Harris, 2005). When these cells are inflamed or injured, they tend to leak higher than normal amount of liver enzymes into the blood stream, which eventually result in elevated liver enzymes in liver function test.

The present research work observed increase in the plasma levels ALP, AST and ALT levels of the diabetic group (untreated animals). This could be that the prolonged hyperglycaemia resulted in complications of metabolism and release of reactive free radicals that interfere with the integrity of liver cells. This interference in turn disrupts the liver cell membrane which leads to the resultant damage and leakage of liver enzymes into the plasma. The overall effect is the elevated plasma levels of AST, ALT and ALP observed in the diabetic rats in the present study.

Oral administration of the combined extracts of *Tetracarpidium conophorum* nuts and *Vernonia amygdalina* leaves significantly lowered the plasma levels of liver enzymes. These extracts may have stopped the process of hepatocellular damage due to their antioxidant and antidiabetogenic properties vested in the flavonoids, alkaloids and tannins content (Ogbonna *and* Abraham 2013); Udia *et al*., 2013). This is in line with the proposition based on the fact that flavonoids and flavonoid containing herbals possess antidiabetogenic and cytoprotective properties (Adaramoye and Adeyemi, 2006).

Histology of the liver was done to ascertain the effect of the plant extracts on the organs. The histological analysis of the liver of the diabetic control rat on day 14 showed early fatty liver change compared to that of the rat that received 500mg/kg TCE and 500mg/kg VAE which showed restored lobular architecture and minimal diffuse mononuclear inflammatory cells infiltration.

On day 28, the histological examination of the liver of the diabetic control rat showed early fatty liver change with portal tract fibrosis and infiltration by mononuclear inflammatory cells compared to that of the rat that received 500mg/kg TCE and 500mg/kg VAE which showed normal lobular architecture but residual inflammatory cells infiltration.

6. Conclusion

The present work indicated that the combined aqueous extract of Tetracarpidium conophorum nuts and Vernonia amygdalina leaves possess the potential of ameliorating the complication of the liver that might suffice as a result of diabetes. Therefore these plants could be exploited in the formulation of antidiabetic agent.

7. References

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