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Effects of Aqueous Extracts of *Tetracarpidium Conophorum* (Nuts) and *Vernonia Amygdalina (Leaves)* on Lipid Parameters of Alloxan Induced Diabetic Wistar Rats

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Abstract:
This study investigated the effect of combined treatment of aqueous extracts of Tetracarpidium conophorum (TCE) walnuts and Vernonia amygdalina (VAE) bitter leaves on lipid parameters 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, cholesterol, triglyceride, HDL and LDL were measured. 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. There was a significant ($p<0.05$) reduction in the level of LDL, triglyceride, and cholesterol in all the treated groups compared to diabetic control. The plasma level of HDL showed no significant ($p>0.05$) difference. In conclusion, the combined aqueous extracts of Tetracarpidiumconophorum nuts and Vernonia amygdalina leaves reduced the levels of the lipid parameters when administered to diabetic rats at the dosage used in this study.

Keywords: *Cholesterol, Diabetes, Tetracarpidium conphorum, Triglyceride, Vernonia amygdalina*

1. Introduction

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 (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 such as 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).

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. Likewise, some medicinal plants have been associated with the management and control of diabetes (Edem *et al*., (2009;Ayodele, 2003).

Tetracarpidium conophorum and *Vernonia anygdalina* are widely distributed in Africa especially in Nigeria. The nut of *Tetracarpidium conophorum* are widely eaten in Nigeria and the leaves of *Vernonia amygdalina* are used to prepare delicacies.

Tetracarpidium conophorum is known as "Ukpa" in Igbo and "Awusa or "Asala" in Yoruba. Edem *et al*., (2009) reported on the proximate composition, ascorbic acid and heavy metals content of the nut. The nut contains some phytochemicals like oxalate, phytate and tannin (Ayodele, 2003). *T. conophorum* is a good source of calcium, copper, manganese, sodium, potassium and magnesium (Ayoola *et al*., 2011). Manganese is an essential mineral as it used in the management of diabetes. The sodium content of the nut makes it useful in the prevention and control of high blood pressure. Walnuts have been shown to reduce serum cholesterol. Furthermore, other medical studies have shown walnuts to ameliorate several physical illnesses, promote weight loss and enhance overall health (Ajaoyeoba and Fadare, 2006). These health benefits are as a result of the high content omega-3-polyunsaturated fats.

Omega-3-polyunsaturated fats are very vital in the body metabolism. They protect against cardiovascular diseases (Jeans *et al*., 2004). Anosike *et al*., (2015) reported that the nuts of *T. conophorum* exhibit antioxidant activity.The leafy juice of *T. conophorum* is used for the treatment of prolonged and constant hiccups. It is used to reduce asthma and constipation in elderly people (Okpero, 2001). The nut is used in formulating livestock feed. The bark of *T. conophorum* is used in tea as laxative and chewed for the treatment of toothache. Taking of 28g walnut daily could reduce serum cholesterol and LDL cholesterol by 4% and6% respectively (Ihemeje *et al*., 2012).

The plant *Vernonia amygdalina* has different names in different ethnic groups around the world. It is called 'Ewuro' in Yoruba, 'Etidot' in Ibibio, 'Onugbo' in Igbo (Ekeocha, *et al*., 2012). 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 (Ekeocha, *et al.,* 2012; Kamiya *et al*., 2008; Atangwho *et al*., 2007). Furthermore, the leaves of *V. amygdalina* have been shown to possess antimalaria activity. This antimalaria effect is as a result of its active compound sequisterpene lactones which include vernodalin, vernolin, vernolide, vernolin and hydroxy vernodalin (Tona *et al*., 2004). Madureira *et al*., (2002) reported that *in vitro* administration of different extract of the leaves of *V. amygdalina* possess antimalaria activity towards *Plasmodium falciparium*. Ayoola *et al*., (2008) reported that the ethanol extract of *V. amygdalina* showed antioxidant activity.The antioxidant activity is due to the flavonoid and phenolic content. 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 (Ukpabi, *et al*., 2015; Ayoola, *et al*., Atangwho *et al*., 2009).

Cholesterol is a sterol (or modified steroid), a lipid molecule and is biosynthesized by all animal cells because it is an essential structural component of animal cell membranes that is required to maintain both membrane structural integrity and fluidity. Triglyceride (TG, triacylglycerol, TAG, or triacylglyceride) is an ester derived from glycerol and three fatty acids. As a blood lipid, it helps enable the bidirectional transference of adipose fat and blood glucose from the liver. There are many triglycerides, depending on the oil source, some are highly unsaturated, some less so (Kizer *et al*., 2010).

The aim of this study was to investigate the effect of the combined aqueous extracts of *Tetracarpidium conophorum* nutsand *Vernonia amygdalina* leaveson blood glucose levels and also to investigate the effect on lipid profile using Wistar rats as experimental model.

2. Materials and Methods

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

2.1. 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.).

2.2. 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).

2.3. 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.

2.4. Preparation of Extract

2.4.1. 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.

2.4.2. 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.

2.5. 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.

2.6. 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:

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.

2.7. Method of Blood 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.

2.8. Estimation of Lipid Profile Parameters.

The plasma levels of all the Lipids were determined using Mindray test kits.

2.9. Plasma HDL Estimation

2.9.1. Method

The direct method of Lopes-Virella (Lopes-Virella *et al.,* 1977) was used to determine the level of high density lipoprotein – cholesterol in the samples.

Reaction Principle

(1) LDL,VLDL, Chylomicrons ↔ Cholestenone + H_2O_2

$2H_2O_2 \leftrightarrow 2H_2O + O_2$

(2) HDL ↔Cholestenone + H_2O_2

H_2O_2 + HDAOS + 4-aminoantipyrin ↔ Quinonimine

The System monitors the change in absorbance at 600 nm. This change in absorbance is directly proportional to the concentration of cholesterol in the sample and is used by the System to calculate and express the HDL-cholesterol concentration.

2.9.2. Procedure

Two test tubes labeled T1 (reagent blank) and T2 (test sample) were set up. T1 contained 900 µL of reagent (R1) and 12 µL of distilled water, while T2 contained 900 µL of reagent (R1) and 12 µL of test sample. The contents of each tube were mixed and incubated at 37℃ for 5 min. After incubating, 300 µL of the second reagent (R2) was added to both test tubes. The contents of each tube was incubated again for 5 minutes at 37^0C, the absorbance was read immediately.

2.9.3. Calculation

ΔA = [ΔA sample]- [ΔA blank].

Conc. of HDL = [change in absorbance of sample] – [change in absorbance of blank].

The result is expressed in mmol/L.

- Plasma Totalcholesterol Estimation.

Cholesterol oxidase- peroxidase (CHOD-POD) method according to Allain and Roeschlau (Roeschlau*et al.,* 1974) was used to determine the level of total cholesterol in the samples.

→ Reaction Principle

Cholesterol ester + H_2O ↔ Cholesterol + Fatty acid

Cholesterol + O_2 ↔ Δ4-Cholestenone + H_2O_2

$2H_2O_2$ + 4-Aminoantipyrine + Phenol ↔ Quinoneimine + $4H_2O$

By the catalysis of cholesterrol esterase and cholesterol oxidase, Cholesterol ester is catalyzed to yield H_2O_2, which oxidizes 4-aminoantipyrine with phenol to form a colored dye of quinoneimine. The absorbance increase is directly proportional to the concentration of cholesterol.

→ 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 thoroughly at 37℃. The absorbance was read 10 min. later.

→ Calculation

ΔA = [ΔA sample]- [ΔA blank]

Conc. of cholesterol = [change in absorbance of sample] – [change in absorbance of blank].

The result is expressed in mmol/L.

- Plasmatriglycerides (TG) Estimation.

Glycerokinase Peroxidase- Peroxidase method according to Tietz colorimetric method (Tietz, 1990) was used to determine the level of Triglyceride in the samples.

→ Reaction Principle

Triglycerides + $3H_2O$ ↔ Glycerol + fatty acid

Glycerol + ATP ↔ Glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O_2 ↔ Dihydroxyacetone Phosphate + H_2O_2

H_2O_2 + 4-Aminoantipyrine + 4-Chlorophenol ↔ Quinoneimine + HCl + H_2O

Through a sequence of enzymatic catalysis steps by lipase, glycerol kinase and Dihydroxyacetone phosphate dehydrogenase, triglycerides is catalyzed to yield H_2O_2, which oxidize 4-aminoantipyrinel to yield a colored dye of quinoneimine. The absorbance increase is directly proportional to the concentration of triglycerides.

→ 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 thoroughly at 37℃. The absorbance was read at a wavelength of 546 nm10 min. later.

→ Calculation

ΔA = [ΔA sample]- [ΔA blank]

Conc. of triglyceride = [change in absorbance of sample] – [change in absorbance of blank].

The result is expressed in mmol/L.

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 and lipid profile test (triglyceride, HDL, cholesterol, LDL) were analyzed to determine their concentration in the various samples. The results obtained are shown in Figures 1 to 5.

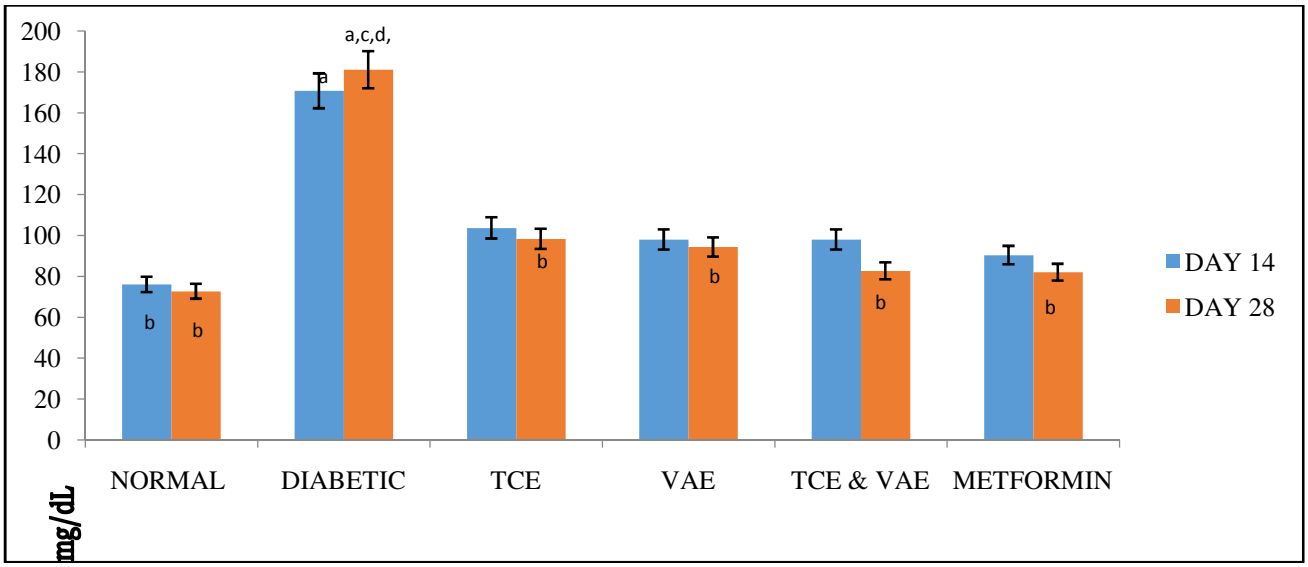

Figure 1: The effect of combined aqueous extracts of Tetracarpidium conophorumnuts and Vernonia amygdalina leaves on glucose level of alloxan – induced diabetic rats.
KEY: NORMAL= Non diabetic; DIABETIC= Diabetic & untreated; TCE= Treated with 500mg/kg Tetracarpidium conophorum extract; VAE= Treated 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 fig 4.1, 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.

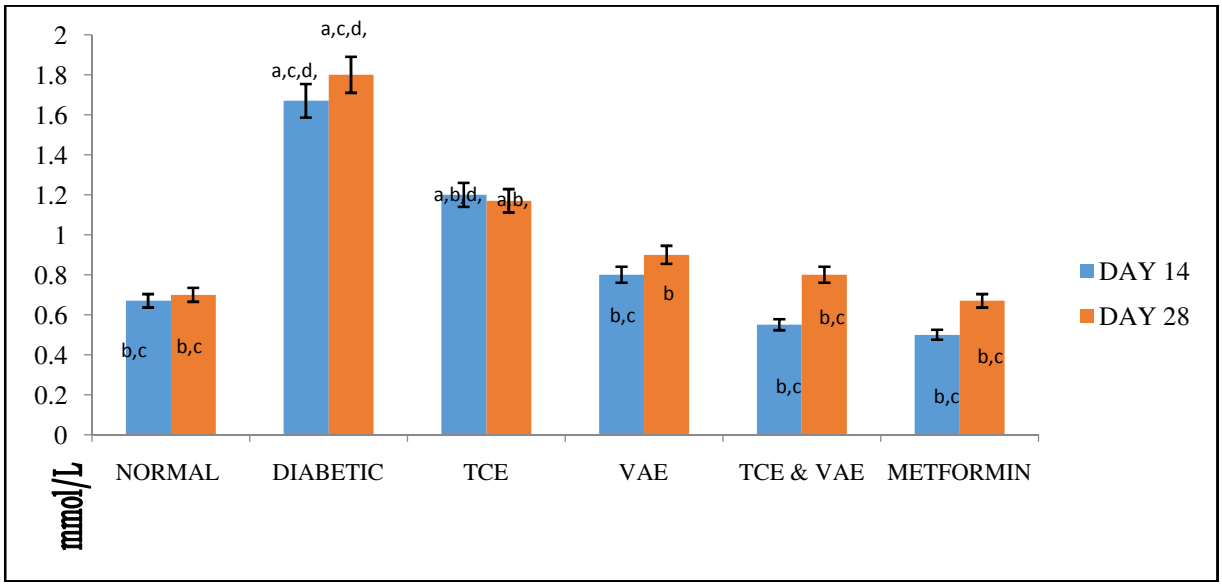

Figure 2 :The effect of the combined aqueous extracts of Tetracarpidium conophorum nuts and Vernonia amygdalina leaves on plasma triglyceride level of Alloxan induced diabetic wistar rats.
KEY: NORMAL= Non diabetic; DIABETIC= Diabetic & untreated; TCE= Treated with 500mg/kg Tetracarpidium conophorum extract; VAE= Treated with 500mg/kg Vernonia amygdalina extract; TCE & VAE= Treated with 500mg/kg Tetracarpidium conophorum 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 triglyceride level of the diabetic untreated group when compared with that of other treated groups which showed a significant ($p<0.05$) decrease. Also, there was a significant ($p<0.05$) increase in the plasma triglyceride level of the group treated with *Tetracarpidium conophorum*extract when compared to that of other treated groups.

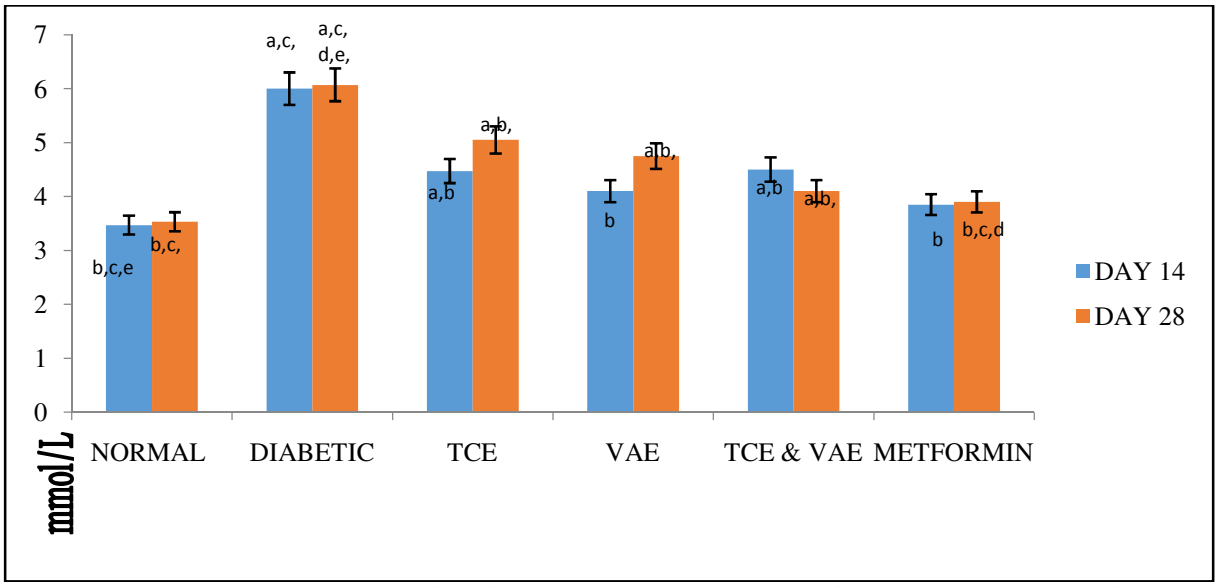

Figure 3: The effect of the combined aqueous extracts of Tetracarpidium conophorum nuts and Vernonia amygdalina leaves on plasma total cholesterol level of Alloxan induced diabetic wistar rats.
KEY: NORMAL= Non diabetic; DIABETIC= Diabetic & untreated; TCE= Treated with 500mg/kg Tetracarpidium conophorum extract; VAE= Treated with 500mg/kg Veronia amygdalina extract; TCE & VAE= Treated with 500mg/kg Tetracarpidium conophorum and 500mg/kg Veronia 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 total cholesterol level of the diabetic untreated group when compared with other treated groups. On Day 28, there was a significant decrease in the group treated with metformin when compared with group treated with *Tetracarpidium conophorum extract* and *Vernonia amygdalina* extracts. There was a significant($p<0.05$) decrease in the group treated with the combined extracts of *Tetracarpidium conophorum* and *Vernonia amygdalina* when compared with the groups treated with individual extract of *Tetracarpidium conophorum* and *Vernonia amygdalina*.

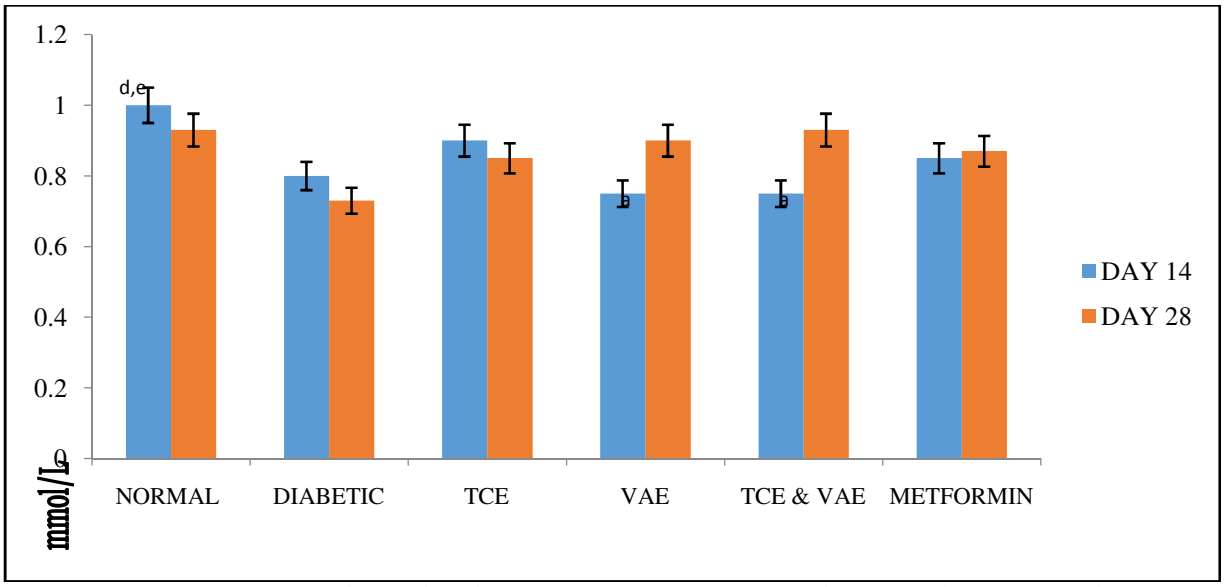

Figure 4:Effects of the combined aqueous extracts of Tetracarpidium conophorumnuts and Vernonia amygdalina leaves on plasma HDL (High density lipoprotein) level of alloxan induced diabetic wistar rats.
KEY: NORMAL= Non diabetic; DIABETIC= Diabetic & untreated; TCE= Treated with 500mg/kg Tetracarpidium conophorum extract; VAE= Treated with 500mg/kg Veronia amygdalina extract; TCE & VAE= Treated with 500mg/kg Tetracarpidium conophorum and 500mg/kg Veronia amygdalina extract. METFORMIN= Treated with 7.69mg/kg metformin.

As shown in Figure 4, it was observed that there was no significant difference in the HDL level of the diabetic untreated rats when compared with the treated animals. Also there was no significant difference among the treated groups both on Day 14 and Day 28 of treatment. There was an increase in the HDL levels of the group treated with *Tetracarpidium conophorum*extract though is not significant when compared with other groups.

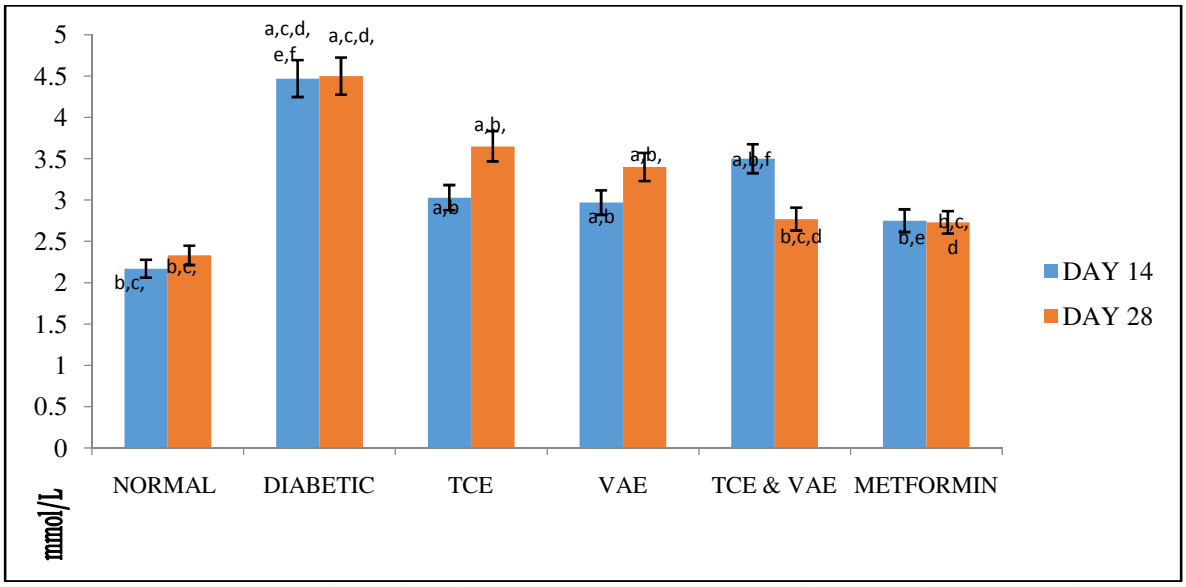

Figure 5: Effect of the combined aqueous extracts of Tetracarpidium conophorumnutsand Vernonia amygdalina leaves on plasma LDL (low density lipoprotein) of alloxan induced diabetic wistar rats.
KEY: NORMAL= Non diabetic; DIABETIC= Diabetic & untreated; TCE= Treated with 500mg/kg Tetracarpidium conophorum extract; VAE= Treated with 500mg/kg Veronia amygdalina extract; TCE & VAE= Treated with 500mg/kg Tetracarpidium conophorum and 500mg/kg Veronia amygdalina extract. METFORMIN= Treated with 7.69mg/kg metformin.

As shown in Figure 5, it was observed that there was a significant ($p<0.05$) increased in the LDL level of the diabetic untreated group when compared with other groups which showed a significant ($P<0.05$) decrease. On day 28, there was a significant decrease on the group treated with combined extracts of *Tetracarpidium conophorum* and *Vernonia amygdalina* when compared to group treated with *Tetracarpidium conophorum* extract and *Vernonia amygdalina* extract. Also, on day 28, there was a significant ($p<0.05$) decrease on the group treated with metformin when compared to groups treated with *Tetracarpidium conophorum* extract and *Vernonia amygdalina* extract.

5. Discussion

The consumption of *Tetracarpidium conophorum* and *Vernonia amygdalina* extract by diabetic rats can have a lot of effect on the glucose level, amylase level, liver enzymes and lipid profile and some other biochemical parameters. 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 lipid profile on alloxan induced diabetic rats. 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 was 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.

There was a significant ($p<0.05$) increase in the plasma level of triglyceride of the diabetic untreated animals when compared with that of the treated animals which showed a significant ($p<0.05$) decrease. The increase in plasma triglyceride in diabetic group is in agreement with previous reports documenting elevated serum triglyceride and lipid peroxide levels of diabetic subjects (Nwanjo, 2005). The combined aqueous extracts of *Tetracarpidium conophorum* and *Vernonia amygdalina* were able to significantly reduce the triglyceride level. This reduction could be by the inhibition of lipid absorption due to the presence of saponin and tannins (Ahmed *et al*., 2010).

There was a significant ($p<0.05$) increase in the total cholesterol level of the diabetic group when compared with the treated animals in other groups which showed a significant decrease. This shows that the extracts of *Tetracarpidium conophorum* and *Vernonia amygdalina* have high affinity for reduction of cholesterol level, therefore are very good in control of atherosclerosis and other metabolic diseases. This lowering effect of cholesterol may be due to the inhibition of cholesterol esterase, and production of

triglyceride precursors such as acetyl-coA and glycerol phosphate (Sharmila *et al*., 2007). This result is in line with what was reported by Vermeer *et al*. (2008) that phenolic compounds inhibit the formation of cholesterol micelles.

The result showed no significant difference in HDL-C level when the diabetic group was compared with other experimental groups. There was an increase in HDL-C of the animals treated with combined extracts of *Tetracarpidium conophorum* and *Vernonia amygdalina* though not significant. HDL-Cholesterol is essential in the transport of cholesterol from cells and artery to the liver where it is catabolised (Lacto *et al*., 2000). HDL-cholesterol constitutes a protective factor against cardiovascular disease (Lacto *et al*., 2000). An increase in the HDL-cholesterol level of 1mg/dl reduces the cardiovascular risk by 2-3 % (Garbon *et al*., 1989). Apolipoprotein A-1 (Apo A-1) the major protein in HDL activates the mobilization of cholesterol ester stored in the macrophages leading to reduction of cholesterol in the major cell type in atherosclerosis (Sacks, 2002). Since the combined extracts of *Tetracarpidium conophorum* and *Vernonia amygdalina* showed a slight increase in HDL-C, it s2hows that they have the possibility of reducing the incidence of atherosclerosis.

There was a significant ($p<0.05$) increase in the plasma LDL-cholesterol of the diabetic group compared with other groups which showed a significant decrease. The group that was treated with the combined extracts of *Tetracarpidium conophorum* and *Vernonia amygdalina* showed a reduction in the LDL-cholesterol level and it was competing favorably with the group treated with metformin, a standard drug. LDL-cholesterol is responsible for the accumulation of lipids in the arterial wall constituting a risk factor for coronary heart disease (Garbon *et al*., 1989). An increase in omega-3- fatty acid decreases the LDL-cholesterol and increases HDL-cholesterol. *T. conophorum* is rich in omega-3- fatty acid this could be responsible for the reduction of LDL-cholesterol observed in the present study. In conclusion, this study revealed that there was a significant reduction in the plasma levels of the lipids profile of all the treated groups compared to the diabetic control. Therefore, the combined aqueous extract of *Tetracarpidium conophorum* nuts and *Vernonia amygdalina* leaves possess the potential of ameliorating the complication of diabetes and it could be exploited in the formulation of antidiabetic agent.

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