Hypoglycemic potentials and Effects of Nigerian Leech *(Aliolimnatis michaelseni)* Saliva Extract on biochemical parameters in Alloxan-induced Diabetesin Rats

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Abstract

Diabetes is a chronic metabolic disorder that results in increased blood glucose levels.Glucose tolerant test (OGTT), α-Amylase inhibition and acute toxicity tests of the Leech Saliva Extract (LSE) were conducted, while blood glucose levels were monitored. Also biochemical parameters including Aspartate amino transaminase (AST), Alanine amino transaminase (ALT), Alkaline Phosphatase (ALP) and lipid profile were also analyzed. A total of 25 rats (120.0 ± 5.0 g) were divided into five groups (A-E) of 5 rats each. All treatments were administered intraperitoneally. LSE had LD_{50}greater than 5ml/kg bodyweight and exert dose dependent α- Amylase Inhibitory with the highest Inhibitory effects of 13.46% at 3.0ml. A significant ($p<0.05$) reduction in fasting blood glucose levels of 170.00 ± 5.64 mg/dl and 148.04 ± 4.56 mg/dl coupled with improvements in body weight of 129.05 ± 3.65g and 132.05 ± 3.00g were observed in alloxan-induced diabetic rats treated with the LSE when compared with the untreated rats with 375.57 ± 12.56mg/dl and 108.34 ± 6.76g body weight respectively. The administration of 1ml and 2ml/kg body weight of LSE to diabetic rats significantly ($p<0.05$) decrease the total cholesterol (TC) (94±5.52 and 88±4.55 mg/dl), Triglycerides (TAG) (68±9.31 and 61±1.19 mg/dl), LDL-C (57 ±8.62 and 56±5.55 mg/dl) and AST (38±2.64 and 69±3.00 U/L) HDL-C (105±6.18 and 101±3.39 mg/dl) while a significant ($p<0.05$) increase in the level of AST (92±6.80U/l), TAG (135±19.50 mg/dl), TC (138±19.87mg/dl), LDL (69 ±0.59 mg/dl) and decrease HDL (74 ±7.4mg/dl) were observed in the diabetic untreated rats.

Keywords; Diabetics, Glucose levels, Hypoglycemia, Leech saliva extract, Biochemical parameters

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by elevated blood glucose levels (hyperglycemia) resulting from defects in insulin secretion(Mohammed *et al.*, 2009).Insulin is a hormone manufactured by the beta cells of the pancreas, which is required to utilize glucose from digested food as an energy source (ADA, 2010). It has been considered as a global pandemic due to the progressive increasing rates of people suffering from diabetes, recent estimates indicate there were 171 million people in the world with diabetes in the year 2000 and this is projected to increase to366 million by 2030 (Wild*et al.*, 2004). Recurrent or persistent hyperglycemia during diabetes causes glycation of body proteins, which in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries (ADA, 2010). One of the main complications associated with diabetes is hyperlipidaemia. In diabetes, high serum total triglyceride level, high level of transaminase; creatinine kinase and urea has been implicated (Nagappa *et al.*, 2003). Pharmacological treatment of DM is based on oral hypoglycemic agents and insulin which are best with manyundesirable effects in human system (Andreoli *et al.*, 1990). The evaluation of natural products used traditionally in treating diabetes is of growing interest.

Since Africa is blessed with diversity of natural products with healing practices (Lawal *et al.*, 2015; Mohammed *et al.*, 2014; Bashir *et al.*, 2015), World Health Organization has recommended and encouraged the use of these natural products against diabetes especially in countries where access to conventional treatment of diabetes is inadequate. It however emphasized their scientific evaluations of the efficacy/safety.

Leeches are blood worms with segmented body commonly used traditionally for healing practice (Fig. 1). Leeches have been used for medicinal purpose since the era of Ayurvedic medicine dating back to 2000 BC (Borda and Siddall, 2004).Leeches secrete Hirudin (haematin) in its saliva which has anticoagulant property that prevents blood clotting. Due to this property they improve macro and microcirculation and clearing blockage. Leeches inject powerful anaesthetic and anti-inflammatory enzymes while sucking the blood and patient feels no pain (Baskova, 2008). Leech extracts have also been used for treating a solid tumour, liquid tumour, diabetes, virial diseases, parasitic diseases, bacterial diseases (Whitaker *et al.*, 2004) and has been reported for its anti-oxidant properties (Omalu *et al.*, 2015). Leech application has been used traditionally for the treatment of *Diabetes mellitus* complications. In order to scientifically validate the traditional uses of leech saliva in managements of diabetics, the present study was set out to evaluate the hypoglycemic activities and effects of Nigerian Leech *Aliolimnatis michaelseni* saliva extract on biochemical parameters in alloxan-induced diabetic rats.

Figure 1.Medicinal Leech (*Aliolimnatis michaelseni*) (Field photograph)

MATERIALS AND METHODS

Leech Sampling

Leech (*Aliolimnatis michaelseni)*, were collected from the natural lake and ponds in Minna, Niger State, Nigeria. Taxonomic authentication was conducted at the Department of Biological Sciences, Federal University of Technology, Minna. They were maintained in well-aerated plastic aquaria with non-chlorinated water. Water was regularly changed every three days. The collected leeches were kept in a room under 12h:12h light and dark cycle at the room temperature (25°C).

Experimental Animal

A total of twenty five (25) white albino rats (*Rattusnovergicus*) of both sexes weighing between 120 and 200g were obtained from the Small Animal Holding Unit of the Department of Biochemistry, Federal University of Technology, Minna. The rats were kept in clean plastic cages and maintained under standard laboratory conditions; temperature: 22±3°C; photoperiod: 12 hours natural light and 12 hours dark; humidity: 40-45%. The animals were maintained on standard animal feedsand tap water *ad libitum*. The principles governing the use of laboratory animals as laid out by the Federal university of Technology, Minna Committee on Ethics for Medical and Scientific Research and also existing internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol (RILARCLS, 1997), were duly observed.

Extraction of leech saliva

Leech saliva extract (LSE) was collected without sacrificing the animal as described previously (Abdualkader *et al.*, 2011). Two hundred (200) starved leeches at different periods (weeks) vomited colourless salivary fluids. Leeches were starved for two weeks after feeding before the commencement of saliva extraction and prior to another extraction; the leeches were fed with sugar solution and at a particular stage of the experiment animal blood or sodium chloride were used. Saliva was extracted by the method outlined by Rigbi (1987). Leeches were then immobilized by putting them into test tubes and inserting the test tubes into plastic container surrounded by ice for 5-10 min. This technique forces the leeches to vomit whatever they have sucked. To complete the saliva collection, leeches were squeezed smoothly from the posterior toward anterior (mouth) sucker which is a modified method of extraction without animal scarification.

Acute Toxicity Test

The acute toxicity test was conducted in accordance with OECD guidelines (OECD, 2008). The limit test was adopted by given oral dose of 5ml/kg.bw to five Wister rats of body weight between 140-180g after 16 hours of fasting. Animals were observed continuously up to 4hrs, for detailed autonomic behavioural and neurological changes. Signs of delayed toxicity or mortality were monitored up to a period of fourteen days.

α-Amylase Inhibition test

The α-amylase inhibition assay was performed using the chromogenic method as described by Thalapaneni *et al.* (2008). Percentage inhibition by the LSE was calculated as:

$$\%\ \text{inhibition} = \frac{Acontrol - Aexperiment}{Aconctrol} \times 100$$

Oral glucose tolerance test (OGTT)

The OGTT was conducted as described by Anyakudo (2015). A total of eighteen Wister ratswere grouped into 6 (A- F) blocks of 3 rats each. Groups A-C were administered 0.5, 1, 2ml leech *Aliolimnatis michaelseni* salivary extract and500µg/kg.bw standard drug glibenclamide respectively, while groups E and F serve as the normal and diabetics control respectively. After which an oral D-glucose load of 2 gm kg-1 (dissolved in distilled water) were administered by means of cannula. Blood samples were withdrawn from the tail vein of each animal (tail snipping) to determine the fasting blood sugar concentration at time 0 minute (before ingestion of glucose) and subsequently at intervals of 30, 60, 90 and 120 minutes respectively after oral glucose administration.

Antidiabetic Studies

Induction of Diabetes

The Animals were allowed to fast for 24 hours and were injected with freshly prepared solution of alloxan monohydrate (Sigma) (120mg/kg) intra-peritoneally. After 3 days, blood was collected in vials from the tail vein of

overnight starving rats as selected under guidance of a vet using the aseptic conditions and disposable kits. Fasting Blood Glucose (FBG) level of blood was checked regularly up to the stable hyperglycemia stage, usually one week after alloxan monohydrate injection. Animal with marked hyperglycemia were selected for the study Etuk *et al.* (2010).

Animals Grouping and Extract Administration

A total of twenty five (25)rats were divided into five groups (A-E) of five rats each.

Group A: normal control rats,

Group B: Diabetic control rats, given 2ml/kg b.w of normal saline after diabetes induction

Group C: Diabetic rats given 1ml/kgb.w leech salivary extract

Group D: Diabetic rats given 2ml/kgb.w leech salivary extract

Group E: Diabetic rats given Glibenclamide (2.5mg/kgb.w.)

All the treatment were administered interperitoneally once daily for 2 weeks. After the 14th day dose the rats were sacrificed. Blood sample were collected in a centrifuge tubes for biochemical analysis.

Determination of Blood Glucose Level

Fasting blood glucose levels were determined with ACHU-CHECK® Glucometer (LIFESCAN, Inc 2001 Milpitas, CA 95035, USA). The fasting Blood Glucose Levels (FBGL) was monitored at3, 7, 14 days respectively by collecting the blood through orbital puncture of the tail vain of rats (Aladodo *et al.*, 2013).

Collection of Blood, serum and organs

The collection of sample for biochemical analyses has been described (Yakubu *et al.*, 2003). At the end of the fourteen days treatment, the animals were denied their feeds overnight but still had water *ad libitum* for 24 hours before they were sacrificed under ether anaesthesia. The blood was collected in a clean, dry centrifuge tubes. The blood sample was allowed to stand for 10minutes at room temperature and then centrifuged at 1000rpm for 15minutes to get the serum. The animals were thereafter quickly dissected and the liver, kidneys and spleen were removed, cleaned and weighed.

Determination of Biochemical Parameters

The levels of serum alkaline phosphatase (ALP), Aspartate transaminase (AST) and alanine transaminase (ALT) were determined using standard procedures (Tietz, 1995; Reitman and Frankel, 1957). The levels of cholesterol, triacyglycerol, HDL-C and LDL-C were determined in the serum of the animals using standard procedures (Fredrickson *et al.*, 2009; Agrawal *et al.*, 2010).

Data Analysis

Data were analyzed using statistical package for social science (SPSS) version 16 and presented as means ± SEM. Comparisons between different groups was done using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Values of $P<0.05$ were considered as statistically significant.

RESULTS

Acute Toxicity Test

The behavioural profile (alertness, restlessness, irritability and fearfulness), neurological (spontaneous activities, reactivity, touch and pain responses) and autonomic profile (defecation and urination) were all normal during the side cage observation and beyond. Although, they were less active after the first four hours but their activities became normal afterwards. No mortality was recorded throughout the fourteen days of observation (Table 1). Therefore, LD_{50} of the Leech Saliva Extractwas found to be greater than 5ml/kg bodyweight (LD_{50}>5ml/kg.bw).

α–Amylase Inhibition Test

Leech saliva Extract exert dose dependent α-Amylase Inhibitory activities with percentage Inhibitory effects of 6.42% (at 1.0ml LSE) - 13.46 (at 3.0 ml LSE) (Table 2).

Oral glucose tolerance test (OGTT)

The glycemic responses of Leech (*Aliolimnatis michaelseni*) salivary extract during OGTT are presented in Figure 1. There was a significant ($p<0.05$) increase in glucose level at 30 minutes between all the experimental groups when compared with the control group. At 60 – 120 minutes a progressive decrease in blood glucose level was observed for all the experimental groups. The glucose levels were however, significantly ($p>0.05$) lower in rats

administered LSE at 0.5, 1.0 and 2.0ml/kg when compared with the group that received D-glucose only (Negative control).

Blood Glucose Level

Effect of the leech *Aliolimnatis michaelseni* salivary extract on fasting blood glucose level of alloxan induced diabetes in rat was presented in Figure 2. A significant ($P<0.05$) and progressive increase in blood glucose level was observed in diabetic untreated rats throughout the experimental periods. The group of rats treated with leech (*Aliolimnatis michaelseni)* salivary extract at 1 and 2ml/kg and rats treated with 5mg/kg glibenclimide also showed initial increase in blood glucose level on the first day of treatments, after which a significant ($p<0.05$) dose dependent and progressive decrease in blood glucose level compared to the untreated rats were observed. However, rat treated with glibenclimide showed more significant decrease in blood glucose than those treated with leech (*Aliolimnatis michaelseni)* salivary extract at 1 and 2ml/kg.

Body Weight Changes

A significant ($P<0.05$) and progressive decrease in body weight changes was observed in diabetic untreated rats throughout the experimental periods. The group of rats treated with Leech (*Aliolimnatis michaelseni)* salivary extract at dose of 1 and 2ml/kg and rats treated with 5mg/kg glibenclimide showed an initial decrease in body weight on day 7th after which a significant improvements in body weight gains was observed on the 14th day (Fig. 3).

Serum lipid profile

A significant ($P<0.05$) increase in triglycerides (TAG), total cholesterol and reduction in high density-lipoprotein (HDL-C) levels was observed in diabetic untreated rat when compared with the normal glycemic rats and other experimental groups. However there was no significant difference ($p>0.05$) in the level of low density lipoprotein (LDL- C) in diabetes untreated rats when compared with normal control rats. Administration of leech (*Aliolimnatis michaelseni)* salivary extract at dose of 1 and 2 ml/kg for 2 weeks caused a significant ($P<0.05$) decrease in the elevated triglycerides, total cholesterol, low density lipoprotein and increase high density-lipoprotein compared to the diabetic untreated rats. The total cholesterol and LDL- C were significantly ($P<0.05$) lowered in rats treated with leach salivary extract (1 and 2ml/kg) than the normal control rats (Fig. 4). However, level of triglyceride and HDL- C was compared well ($P>0.05$) with the normal control rats.

Serum Enzymes Activities

Figure 5 showed the effect of leech (*Aliolimnatis michaelseni)* salivary extract on serum activities of some liver based enzymes in alloxan induced diabetic rat. The serum AST and ALP activities were significantly ($P<0.05$) raised in diabetic untreated rat when compared with the normoglycaemic rats and other experimental groups. However there was no significant difference ($p>0.05$) in serum ALT in diabetics untreated rats when compared with control rats and other experimental groups. Administration of leech (*Aliolimnatis michaelseni)* salivary extract at dose of 1 and 2 ml/kg for 2 weeks causes a significant ($P<0.05$) decrease in the elevated serum AST when compared to the untreated rats. However, serum ALP in diabetics rat treated with leeches salivary extract (1 and 2 ml/kg) were not significantly ($P>0.05$) different from the untreated rats.

Relative organ weight

Effect of leech (*Aliolimnatis michaelseni)* salivary extract on relative organ weight ratios in alloxan induced diabetic rat was presented in Table 3. The computed relative organ weight ratios indicated that the liver and spleen and kidney/body weight ratios of the diabetic untreated rats and those treated with leeches' salivary extract and Glibenclamide were not significantly ($P>0.05$) different from those of the control rats. However, the leech salivary extracts caused a significant ($P<0.05$) increased in kidney/body weight ratio when compared with the diabetics untreated rats and the control rats.

Table 1. Acute Toxicity Profile of Leech saliva Extract (LSE)

Group Dose (5ml/kgbw)	Extract toxicity		
Behavioural profile	Autonomic Profile	Neurological profile	Mortality
Leech saliva (5ml/kgbw) Normal	NormalNormal	Nil	

Table 2. α- Amylase Inhibitory effects of Leech saliva Extract (LSE)

Volume	Absorbance	Enzyme Activity	% Inhibition
1.0	0.598	93.58	6.42
1.5	0.596	93.27	6.73
2.0	0.567	88.73	11.27
2.5	0.553	86.54	13.30
3.0	0.554	86.70	13.46

Table 3. Effect of leech (*Aliolimnatis michaelseni)* salivary extract on relative organs weight ratios in alloxan induced diabetic rat.

	LIVER	KIDNEY	SPLEEN
NORMAL	3.43±0.06^{a}	0.60±0.18^{a}	0.38±0.02^{a}
Negative Control	3.50±0.29^{a}	0.74±0.08^{a}	0.57±0.02^{a}
Glibenclamide(5mg/kg)	3.53±0.37^{a}	0.65±0.18^{a}	0.58±0.05^{a}
1 ml/kg leech salivary extract	3.96±0.83^{a}	0.81±0.10^{ab}	0.45±0.16^{a}
2 ml/kg leech salivary extract	3.80±0.64^{a}	1.23±0.06^{b}	0.52±0.47^{a}

Values are mean ± SEM of 5 determinations. The values along the same row with different superscripts are significantly different ($p < 0.05$).

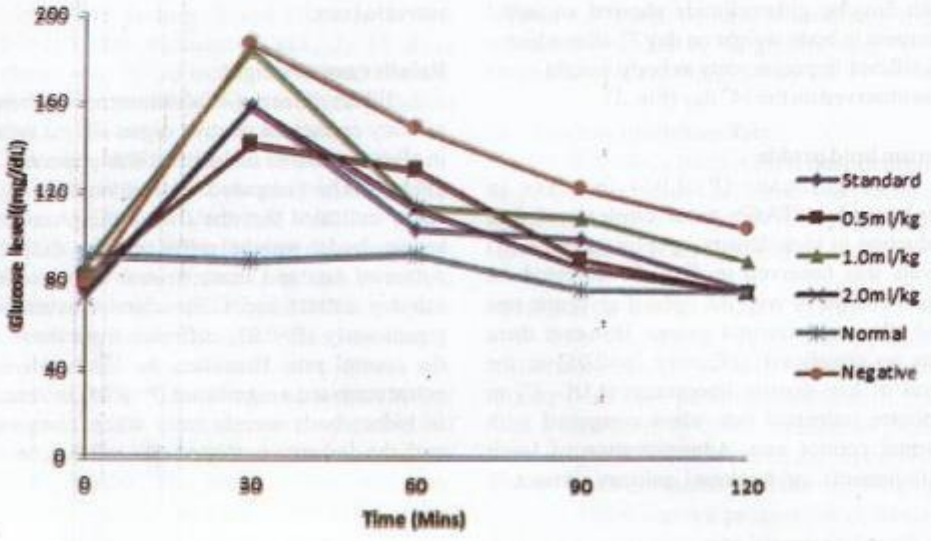

Figure 1 Glycemic responses of Leech *Aliolimnatis michaelseni* salivary extract during OGTT

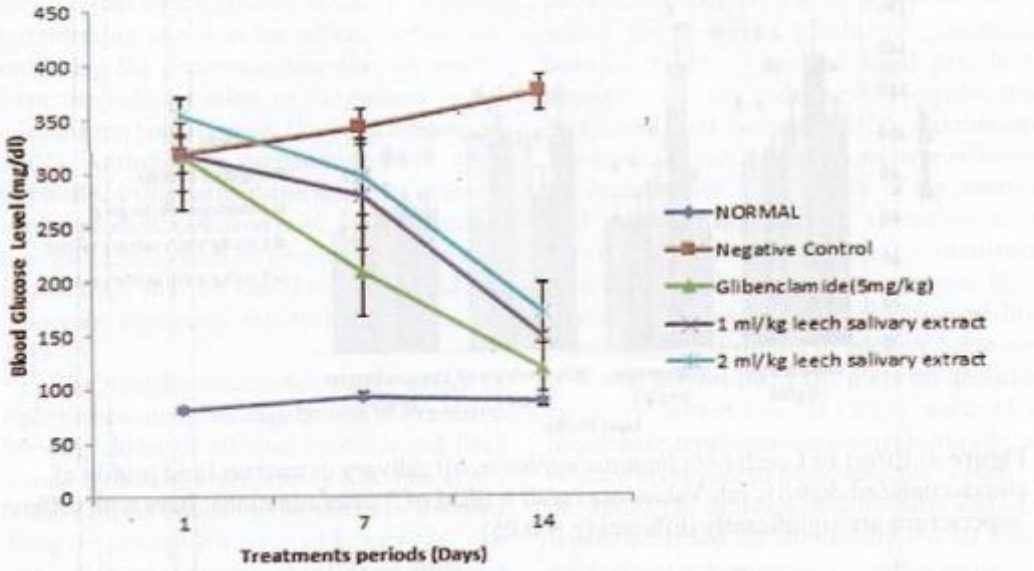

Figure 2. Effect of leech (*Aliolimnatis michaelseni)* salivary extract on fasting blood glucose level of alloxan induced diabetic rat.

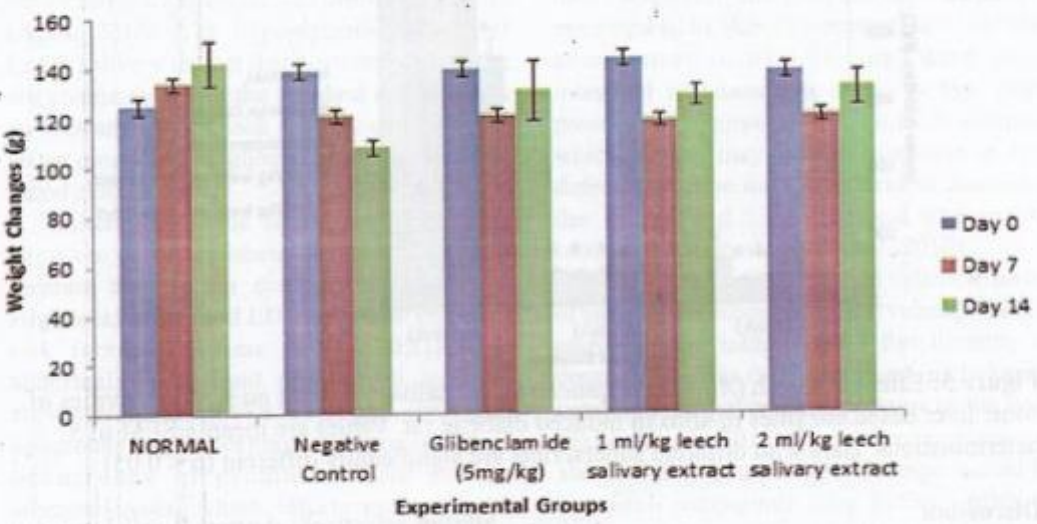

Figure 3. Effect of Leech (*Aliolimnatismichaelseni)* salivary extract on body weight changes in alloxan induced diabetic rat.

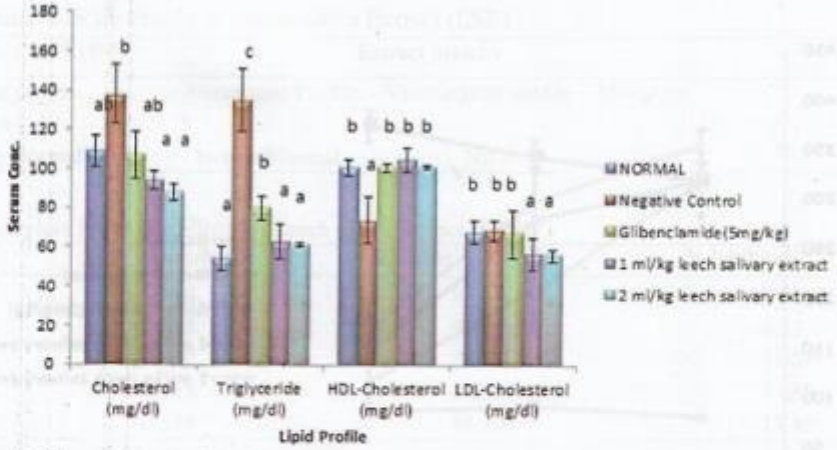

Figure 4: Effect of Leech (*Aliolimnatis michaelseni)* salivary extract on lipid profile of alloxan induced diabetic rat. Values are mean ± SEM of 5 determinations. Bars with different superscripts are significantly different ($p < 0.05$).

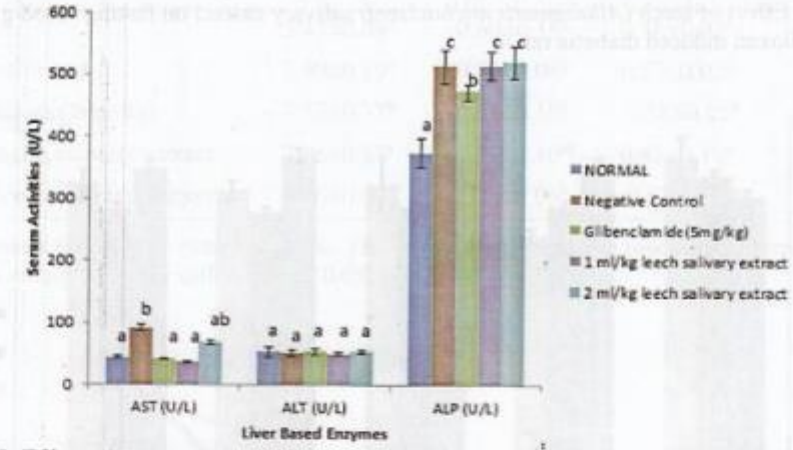

Figure 5: Effect of Leech (*Aliolimnatismichaelseni)* salivary extract on serum activities of some liver based enzymes in alloxan induced diabetic rat. Values are mean ± SEM of 5 determinations. Bars with different superscripts are significantly different ($p < 0.05$)

Discussion

Medicinal uses of natural product are gaining popularity in developing countries.Over the last decades toxins and secretions from poisonous and venomous animals have been used as drugs and drug leads for treatment of numerous untreatable human ailments (Sudhanshu, 2013). Several pharmacological, biologicalandclinical applications of Leech salivary secretionabounds in literature. However, few studies have been reported on its hypoglycemic effects.

Blood glucose is a key marker for diagnosis and prognosis of *Diabetes mellitus*. Since alloxan selectively destroy B-cell leaving the less active cell and resulting in diabetic state, the evaluation of hypoglycemic activity of antidiabetic agent using alloxan-induce hyperglycemia model has been widely accepted (Szkudelski, 2001).

In the present study, alloxan administration causes significant and progressive increase in blood glucose level accompanied with decrease body weight. However, treatment with Leech salivary extract at 1 and 2 ml/kg b.w produced a dose dependent reduction in BGL and improvement in body weight when compared to

diabetic untreated rats. The hypoglycemic action of the Leech salivary extract may be by potentiating the insulin effect, either by increasing the pancreatic secretion of insulin from the cells of islets of langerhans or its release from bound insulin (Pari and Amarnath, 2004). Antioxidants compounds have been implicated in the antidiabetic activities of many natural products (Okokon *et al.*, 2006). Thus the hypoglycaemic effect of Leech salivary extract in this study may be linked to its antioxidants properties previously reported (Omalu *et al.*, 2015).

The significant decrease in body weight of diabetic untreated rats may be due to excessive breaking down of all tissue protein and lipid caused by insulin insufficiency (Okokon *et al.*, 2006). Glibenclimide can be used as a standard drug to compare the efficacy of the hypoglycemic agents in alloxan-induced diabetes. It acts by increasing fatty acid oxidation, decreasing hepatic glucose production and intestinal absorption, increase peripheral glucose uptake and insulin sensitivity (ADA, 2010). The hypoglycemic effects of Leech salivary extract demonstrated at 2 ml/kg are comparable with the standard drug. This is an indication that Leech salivary extract could be as promising as glibenclimide in lowering blood glucose level.

Alteration in the serum lipid profile is known to occur in diabetes and this is likely to increase the risk for coronary heart disease. High level of TC and LDL are major coronary risk factors (Temme *et al.*, 2002). The abnormalities in lipid metabolism leads to elevation in the levels of serum lipid and lipoprotein that in turn plays an important role in occurrence of premature and severe atherosclerosis, which affects patients with diabetes (El-harzmi and Warsy, 2001). Therefore ideal treatment of diabetes, in addition to glycemic control, should have a favourable effect on lipid profiles.

In the present study, administration of alloxan leads to significant increase in cholesterol, triglycerideand HDLC which are responsible for several cardiovascular disorders. The higher lipid levels seen in diabetic rats was due to increased mobilization of free fatty acids from peripheral depots and also due to lipolysis caused by hormones (Dineshkumar *et al.*, 2010). Moreover, administration of leech (*Aliolimnatis michaelseni)* salivary extract at dose of 1 and 2 ml/kg for 2 weeks produced significant beneficial effects on the lipid profile in hyperglycemic rats, reducing triglycerides, total cholesterol, and increasing HDL significantly. The leech salivary extract might have enhanced the regeneration of the β-cells of the pancreas and potentiating insulin secretion from surviving β-cells; the increase in insulin secretion and the consequent decrease in blood glucose level may lead to inhibition of lipid peroxidation and control of lipolytic hormones. It is well known that LDL plays an important role in arteriosclerosis and that hypercholesterolemia is associated with a defect relating to the lack of LDL receptors (Temme *et al.*, 2002).The decrease of cholesterol and LDL levels achieved by administration of leech salivary extract suggests a possible protection against hypercholesterolemia and associated complication. However, complete hypolipidemia (decreased levels of LDL-C, total cholesterol, and TAG and increase LDL-C as compared to diabetic untreated rat) following administration of LSE suggested small intestinal malabsorpion of lipids that could prevent the absorption of fat soluble vitamins, which in turn may lead to blindness or eyes defects that arise from degenerative changes in the retina, and also, physical and mental retardations (Dineshkumar *et al.*, 2010).

Evaluation of biochemical indices in serum of animals has become the most valuable tool for assessing the integrity and functionality of organs as well as risk assessment, pathological condition and general health status of the body (Shittu *et al.*, 2015).Biomarker enzymes can also indicate tissue cellular damage caused by chemical compounds long before structural damage that can be picked by conventional histological techniques (Akanji *et al.*, 1993). Alkaline phosphatase has been widely used as biomarker enzyme for assessing the integrity of endoplasmic reticulum and plasma membrane. The increase in ALP activities observed in diabetic untreated rats suggested that the integrity and functionality of endoplasmic reticulum and plasma membrane has been comprised (Shittu *et al.*, 2015). Such increase in ALP activities could constitute threat to the life of cells that are dependent on a variety of phosphate esters for their vital process since the

cells might be deprived of the much needed energy as a result of indiscriminate hydrolysis of the phosphate ester (Oyewo *et al.*, 2012).The transaminase (ALT and AST) are 'markers' of liver damage and can thus be used to assess liver cytolysis with ALT being a more sensitive biomarker of hepatotoxicity than AST (Yakubu *et al.*, 2003). Consequently, in the present work marked increase in AST observed in diabetic untreated rats is an indication that there is a leakage of this enzymes from the liver into serum Such increase AST will negatively influence the metabolism of macromolecules, thus affecting adenosine triphosphate generation (Oyewo *et al.*, 2012). Although, leech salivary extract was not able to reduce the elevated level of serum ALP, it has ameliorated the diabetic induced liver impairment by lowering the level of serum AST levels towards their normal values.

Organ body weight ratios are normally investigated to determine whether the size of the organ has changed in relation to the weight of the whole animal. The absence of an effect on the computed organs/body weight ratios of the diabetic untreated rats and those treated with leech (*Aliolimnatis michaelseni)* salivary extract when compared with the control rats suggest that the diabetic condition as well as administration of leech salivary extract did not cause any form of swelling, atrophy and hypertrophy on the organs (Shittu *et al.*, 2015). Although, there was mild degeneration of the hepatocytes as revealed by elevated AST and ALP, it is possible that the degenerative changes was not sufficient enough to produce atrophy or organ constriction. In addition, changes in the levels of biochemical indices during diabetes could be an earlier event preceding gross morphological changes in the organ(s). It is not in all cases that the alterations in biochemical parameters of an organ are supported by histo-architectural alterations (Aboyade *et al.*, 2009). However, the significant increase in kidney/body weight ratio of rats following leech salivary extract administration may be attributed to tissue necrosis since the organ is concerned with the excretion of foreign substances (Yakubu *et al.*, 2003)

Conclusion

The results from this study showed that leech (*Aliolimnatis michaelseni)* salivary extract has a beneficial effect in reducing the elevated blood glucose level although not as effective as the standard drug and significant ameliorative effects on diabetes induced dyslipidemia and liver impairments.

Acknowledgements

This study was sponsored by TETFUND/ FUTMINN/2014/06, from the Federal University of Technology Minna.

The Nigeria leech was identified to species level through molecular methods by Mark Siddall, Curator and Professor, Sackler Institute of Comparative Genomics & Invertebrate Zoology, American Institute of Natural History, Central Park West NY., USA.

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