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Lipid Profile Status of Streptozotocin-Induced Diabetic Rats Treated With Ethanol, N-Hexane and Aqueous Extracts of *Vitex Doniana* Leaves.

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ABSTRACT

The lipid profile of streptozotocin (STZ) induced diabetic rats treated with ethanol, aqueous and n-hexane extracts of *Vitex doniana* leaves was examined. 45 male wistar rats were divided into nine groups of five rats each. The animals were made diabetic with the intraperitoneal administration of streptozotocin (50mg/kg) and the extracts were administered orally once a day at a dose of 200mg/kg for a period of 4 weeks. In these groups, fasting blood glucose (FBS), change in body weight, thiobarbituric acid-reactive substances (TBARS), total cholesterol, LDL-cholesterol, HDL-cholesterol and triglyceride, were determined in the blood and serum respectively. The effect of the extracts was compared with an antidiabetic drug 'Glibenclamide'. The results revealed that the extracts treatment restored the elevated blood glucose level and lipid peroxidation to normal; produced 45.7%, 51.8% and 63.3% decrease in total cholesterol, triglycerides and LDL-C respectively in the treated rats when compared with the diabetic control. The extracts also prevented the diabetes-induced decrease in body weight of rats and HDL-C compared to the diabetic control. The results of the present study demonstrate that the extracts were able to alleviate elevations in lipid profile and oxidative stress induced by streptozotocin in albino rats.

Keywords: Streptozotocin, Diabetes Mellitus, *Vitex doniana*, Hypoglycaemic, Hypolipidemic.

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INTRODUCTION

Diabetes mellitus, often simply referred to as diabetes, is a metabolic disease in which a person has high blood sugar, either because the body does not produce enough insulin, or because the cells do not respond to the insulin that is produced [5]. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). Insulin itself is a hormone produced by the pancreas that helps to transport glucose (blood sugar) from the bloodstream to the cells where they are broken down and used as fuel. As a matter of fact, people cannot live without insulin [2].

The root causes of diabetes mellitus are complex. These among others include unhealthy lifestyle factors such as over-eating, physical inactivity and obesity. These can impair the body's ability to use insulin. Some other risk factors are genetic, family history and age.

Type-2 diabetes, also known as non- insulin dependent diabetes mellitus (NIDDM) is the commonest variety worldwide, accounting for about 70-90% of the diabetic cases [11] and usually affects people of over 40 years [10].

Globally as at 2010, an estimated 285 million people have type II diabetes, making up about 90% of all diabetes cases. Its incidence is increasing rapidly, and it is estimated that by the year 2030, this number will almost double [16]. The increase in the incidence of diabetes in the developing countries follows the trend of urbanization and life style changes, most importantly, western style ''diet'' [8].

Vitex doniana family (*Verbernaceae*) commonly known as black plum or "Ori-nla" is a widespread deciduous forest tree largely found in coastal woodlands and savannah. The fruits contain vitamins A and B and can be made into a jam or processed into wine. The leaves are often used as an herb for cooking. Earlier works have reported the use of the fruits and leaves for medicinal purposes [13]; [3].

Extracts of vitex doniana have been shown to have many pharmacological effects. These include anti-oxidant [1]; anti-bacterial, anti-hepatotoxic [9].

In the present study, the hypoglycaemic and hypolipidemic potentials of vitex doniana leaf extracts (aqueous, ethanol and n-hexane) were investigated in streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Preparation of plant extracts

Fresh leaves of *Vitex doniana* were collected from Ankpa, Ankpa local government area of Kogi State, Nigeria. The leaves were identified and autenticated at the department of biological sciences kogi state university (voucher specimen 735B). The leaves were then air- dried at room temperature and the dried leaves were grinded into powder using mortar and pestle, and then weighed. The aqueous extract was obtained by weighing about 100g of

the powdered leaves and dissolved in 1litre of distilled water. This was allowed to soak for 24 hours, after which it was fitered using whatman's filter paper, and the filtrate concentrated using a rotary evaporator. The ethanol and n-hexane extracts were obtained by weighing about 16g of the powdered leaves into a thimble and extracted with about 250 ml of n-hexane and ethanol at a temperature of 80 and 78ºc respectively using soxhlet extractor. The extracts were concentrated using rotary evaporator and stored in the refridgerator for further use.

Animals and treatments:

Forty-five male albino rats (Wister strain), weighing between 120-170g were used. The animals were housed in standard metallic cages, kept under room temperature and fed with broiler starter/ broiler finisher (mixed) purchased from Vital feeds Jos, Plateau state Nigeria. The animals were allowed access to food and water *ad libitum*. They were distributed randomly into nine groups of five animals each and an acclamitization period of seven days was allowed for the animals before the commencement of treatments.

Induction of diabetes:

The animals in the test groups were induced with diabetes after an overnight fasting with a single dose intraperitoneal injection of streptozotocin (STZ) at a dose of 50mg/kg body weight. STZ was freshly prepared by dissolving in 0.1M cold citrate buffer, PH 4.5[12]. 10% glucose solution was provided to the induced animals for 24 hours to prevent severe hypoglycaemia. Three days were allowed for the development of diabetes and only animals with blood glucose concentration of 250mg/dl were selected as the test groups [4].

Group A serve as the diabetic control rats no treatment. Animals in groups B, C, and D were diabetic but treated orally with 200mg/kg preparation of *Vitex doniana* extracts; aqueous, ethanol and n-hexane respectively. Groups E, F and G were non diabetic animals (normal animals) but however treated with 200mg/kg of *Vitex doniana* extracts (aqueous, ethanol and n-hexane) respectively. Group H are the normal control animals, non-diabetic rats, no treatment; while group I were diabetic rats treated orally with gibenclamide, a standard antidiabetic drug at a therapeutic dose of 2.5mg/kg. Water served as the vehicle for both the extracts and gibenclamide. These treatments (extracts and drug) were administered orally to the animals daily for a period of 28days.

Blood glucose determination:

The fasting blood glucose levels were determined by collecting blood samples from the tails of the animals on day; 0,7,14, 21 and 28. Blood glucose estimation was done by one touch electronic glucometer (Accu-Check Active 444, Roche Diagnostic GmbH, Germany) using glucose test strips according to the method of Giordano *et al*., 1989.

Chemicals:

Thiobarbituric acid (TBA) was purchased from Sigma Chemicals (St Louis, MO, USA). Gibenclamide (BLISS GVS PHARMA LTD INDIA) was purchased from a local chemist in lokoja,

Kogi State, Nigeria. Diagnostic kits for cholesterol, triglyceride and high density lipoprotein-cholesterol (HDL-C) precipitants were purchased from AGAPPE DIAGNOSTICS SWITZERLAND GmbH. All other chemicals used were of analytical grade and of the purest quality available.

Collection of samples:

At the end of the 4th week, the animals were fasted overnight prior to sacrifice. They were anaesthesized using mild chloroform and sacrificed by humane decapitation. Blood was collected in non heparinised tubes, and allowed to clot. These were then centrifuged at 3000 rpm for 10 mins using MSE bench centrifuge (Beckman and Hirsch, Burlington, IO, USA); and the sera collected in fresh sample tubes and stored at -4ºc for subsequent use.

Preparation of Tissue Homogenate

The whole kidneys, liver and heart from each animal was removed after sacrifice, rinsed in normal saline, blotted dry and weighed. The samples were homogenized in four volumes of ice-cold isotonic phosphate buffer, pH, 7.4; centrifuged at 3000 rpm for 15mins and the supernatant collected and stored at -4ºc untill use.

METHODS

Serum triglyceride and cholesterol were assayed using commercial kits (Agappe diagnostics). The lipoproteins were measured using the enzymatic colorimetric method; very-low density lipoprotein (VLDL) and low –density lipoprotein (LDL) were precipitated by the addition of phosphotungstic acid and magnesium chloride. After centrifugation at 3000g for 10mins, at 25ºc, the clear supernatant contained HDL fraction, which was assayed for cholesterol with the Agappe diagnostic kit. The LDL- cholesterol (LDL-C) was calculated using the formula of [7]. Lipid peroxidation (LPO) was assesed by measuring the thiobarbituric acid – reactive substances (TBARS) formation as described by [14].

STATISTICAL ANALYSIS

Results are expressed as the mean ± SD (n = 5). One way analysis of variance (ANOVA) was used for data analysis and post hoc Duncan's multiple range-test using SPSS Version 18. Differences between groups were considered significant at $p<0.05$ levels.

RESULTS

Fig1.0 shows the level of blood glucose in the experimental animals. All the extract treated groups and gibenclamide treated group (B, C, D, and I) have reduced level of FBS when compared to the diabetic control group (A). The levels of FBS in non-diabetic treated groups (E, F and G) were higher than that of normal control group.

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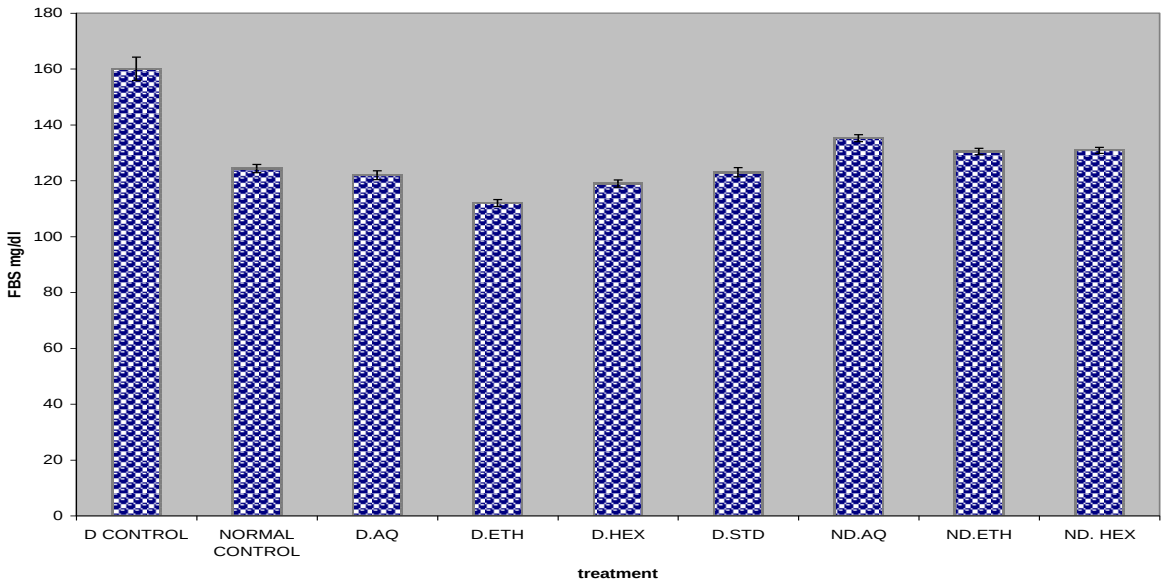

Fig. 1.0 Effect of *Vitex doniana* extracts and standard drug gibenclamide on the fasting blood sugar level of streptozotocin induced diabetic rats. Data are mean ± SD P<0.05 compared to the diabetic control. D control = diabetic control, D.AQ = dibetic rats +aqueous extract, D.ETH. = diabetic rats + Ethanol extract, D.HEX = dibetic rats + n-hexane extract, D.STD = diabetic rats + standard drug, ND.AQ. = non diabetic rats + aqueous extracts, ND.ETH. =on diabetic rats + ethanol extract, ND.HEX. = non diabetic rats + n-hexane extract.

Figures. 2.0, 3.0, 4.0 and 5.0 show the levels of Total cholesterol, HDL -C, LDL-C and Triglyceride in the serum. There was a significant increase ($p< 0.05$) in Total cholesterol, LDL-C and triglyceride levels in diabetic control rats when compare to normal control group. There was also a decrease in HDL-C levels of the diabetic control rats compared with the normal controls. Treatment of the rats with *Vitex doniana* extracts (i.e. aqueous, ethanolic and n-hexane extracts) and standard drug gibenclamide significantly ($p<0.05$) reduced the levels of LDL-C and total cholesterol in the treated groups (B, C, D, and I) compared to the diabetic control. Also the groups treated with aqueous and ethanolic extracts (B and C) have significantly ($p<0.05$) reduced levels of triglyceride when compared with the diabetic control group. On the other hand, both the extracts and gibenclamide groups were able to sigificantly ($p < 0.05$) raise the level of HDL-C higher than the normal and diabetic control groups. The non-diabetic treated groups also have higher levels of HDL-C when compared to the normal control group.

Fig. 6.0 shows the level of malondialdehyde (MDA) in kidneys, heart and serum. The highest level of MDA was found in the kidneys, heart and serum of the diabetic control group A. However, treatment with Vitex doniana extracts and standard drug gibenclamide was able to significantly ($p< 0.05$) lower the MDA levels in the treated groups (B, C, and I) when compared to diabetic control group. Likewise, the normal treated groups (E, F, and G) have relatively lower levels of malondialdehyde when compared to the normal group (H).

Fig. 7.0 shos the effect of vitex doniana extracts on the body weight of the treated rats. Dabetic control rats showed significant weight loss as the experiment progressed; whereas the diabetic rats treated with the xtracts showed slight increase or stable body weight.

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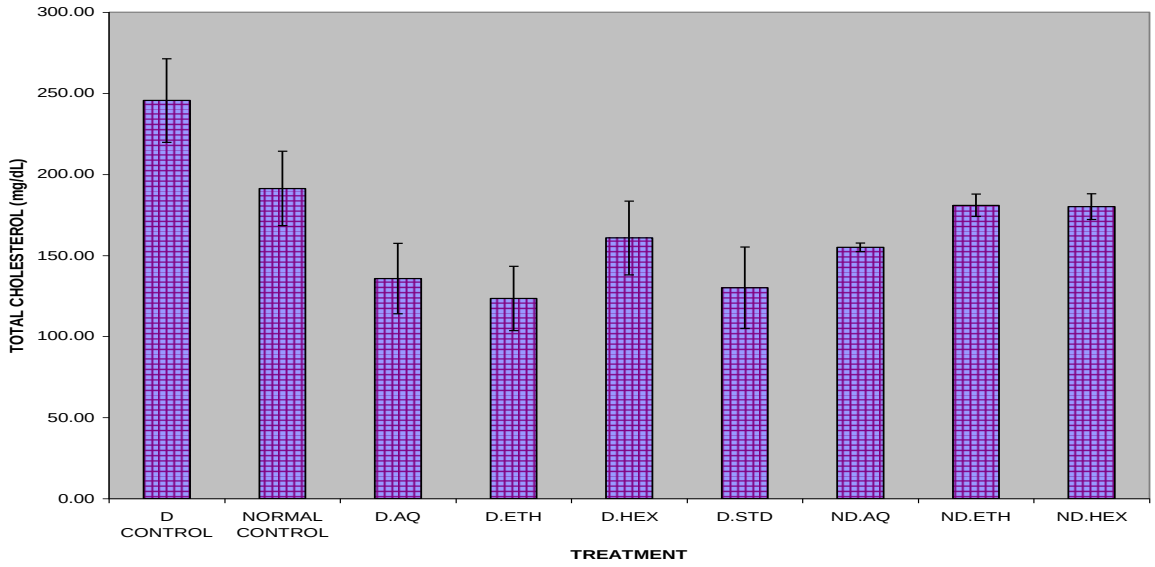

Fig. 2.0 Effect of *Vitex doniana* extracts and standard drug gibenclamide on the cholesterol level of streptozotocin induced diabetic rats. Data are mean ± SD P<0.05 compared to the diabetic control. D control = diabetic control, DAQ = dibetic rats +aqueous extract, D.ETH. = diabetic rats + Ethanol extract, DHEX = dibetic rats + n-hexane extract, D.STD = diabetic rats +s tandard drug, ND.AQ. = non diabetic rats + aqueous extracts, ND.ETH. =on diabetic rats + ethanol extract, ND.HEX. = non diabetic rats + n-hexane extract.

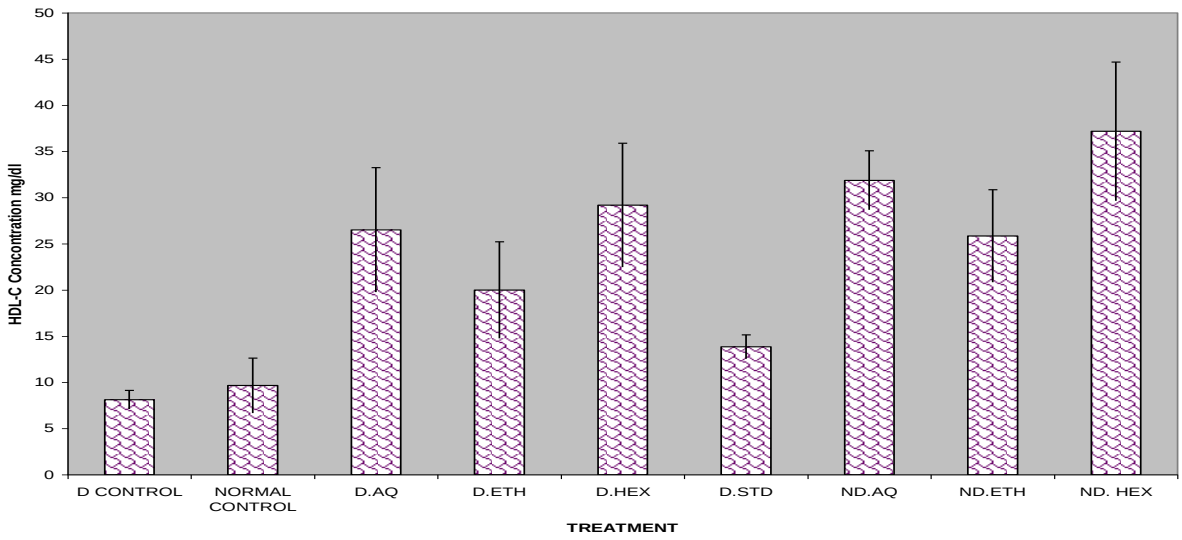

Fig.3.0 Effect of *Vitex doniana* extracts and standard drug gibenclamide on the HDL-C level of streptozotocin induced diabetic rats. Data are mean ± SD P<0.05 compared to the diabetic control. D control = diabetic control, DAQ = dibetic rats +aqueous extract, D.ETH. = diabetic rats + Ethanol extract, DHEX = dibetic rats + n-hexane extract, D.STD = diabetic rats +s tandard drug, ND.AQ. = non diabetic rats + aqueous extracts, ND.ETH. =on diabetic rats + ethanol extract, ND.HEX. = non diabetic rats + n-hexane extract.

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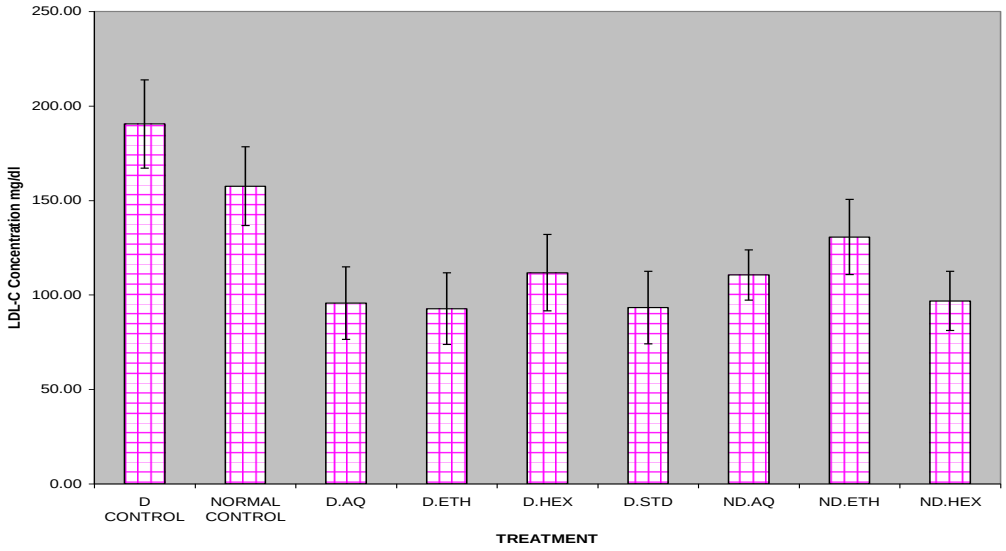

Fig. 4.0 Effect of *Vitex doniana* extracts and standard drug gibenclamide on the LDL-C level of streptozotocin induced diabetic rats. Data are mean ± SD P<0.05 compared to the diabetic control. D control = diabetic control, DAQ = dibetic rats +aqueous extract, D.ETH. = diabetic rats + Ethanol extract, DHEX = dibetic rats + n-hexane extract, D.STD = diabetic rats +s tandard drug, ND.AQ. = non diabetic rats + aqueous extracts, ND.ETH. =on diabetic rats + ethanol extract, ND.HEX. = non diabetic rats + n-hexane extract.

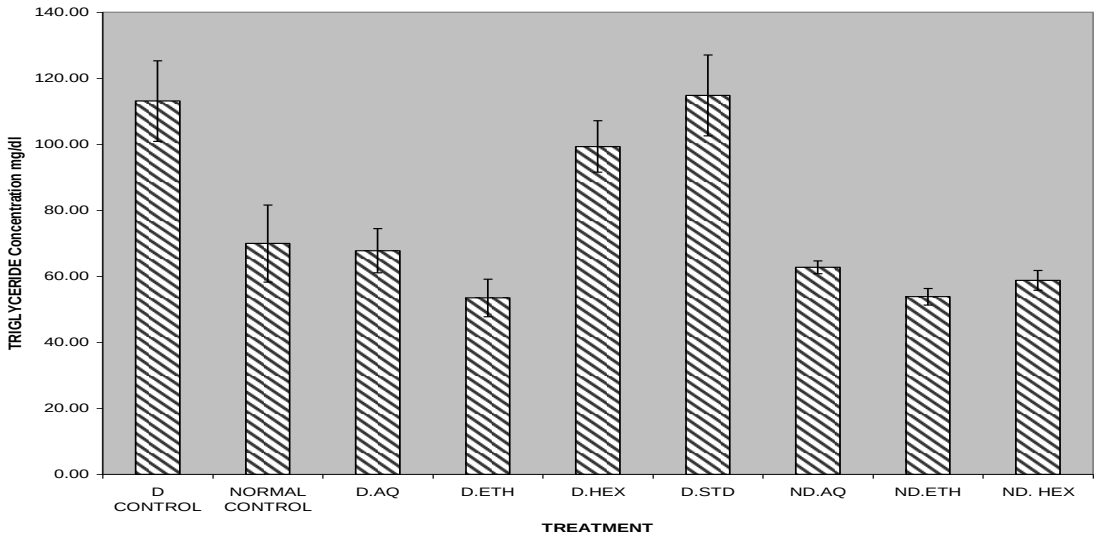

Fig. 5.0 Effect of *Vitex doniana* extracts and standard drug gibenclamide on the triglyceride level of streptozotocin induced diabetic rats. Data are mean ± SD P<0.05 compared to the diabetic control. D control = diabetic control, DAQ = dibetic rats +aqueous extract, D.ETH. = diabetic rats + Ethanol extract, DHEX = dibetic rats + n-hexane extract, D.STD = diabetic rats +s tandard drug, ND.AQ. = non diabetic rats + aqueous extracts, ND.ETH. =on diabetic rats + ethanol extract, ND.HEX. = non diabetic rats + n-hexane extract.

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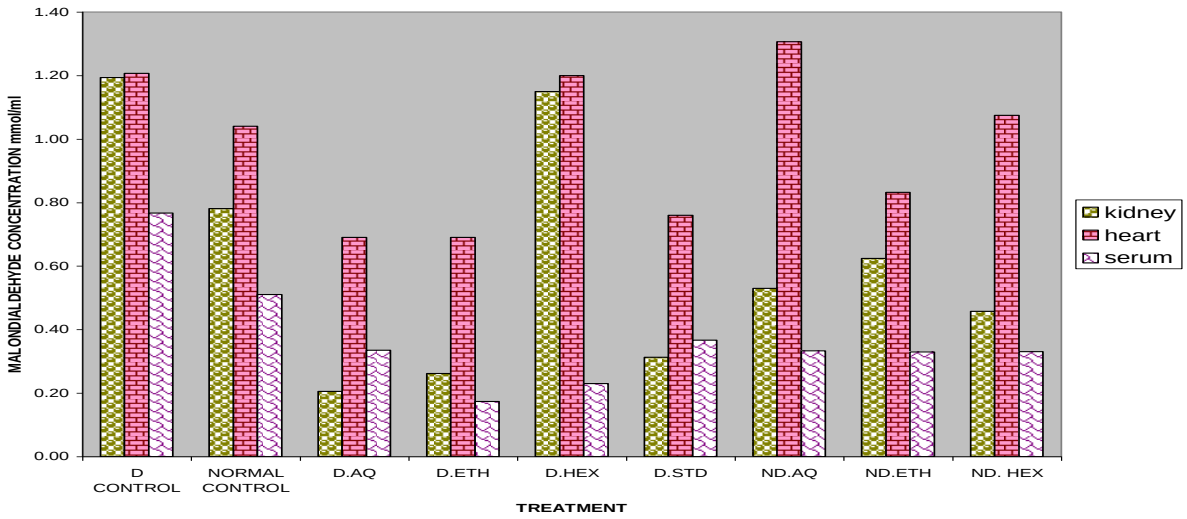

Fig. 6.0 Effect of *Vitex doniana* extracts and standard drug gibenclamide on lipid peroxidation of streptozotocin induced diabetic rats. Data are mean ± SD P<0.05 compared to the diabetic control. D control = diabetic control, DAQ = dibetic rats +aqueous extract, D.ETH. = diabetic rats + Ethanol extract, DHEX = dibetic rats + n-hexane extract, D.STD = diabetic rats +s tandard drug, ND.AQ. = non diabetic rats + aqueous extracts, ND.ETH. =on diabetic rats + ethanol extract, ND.HEX. = non diabetic rats + n-hexane extract.

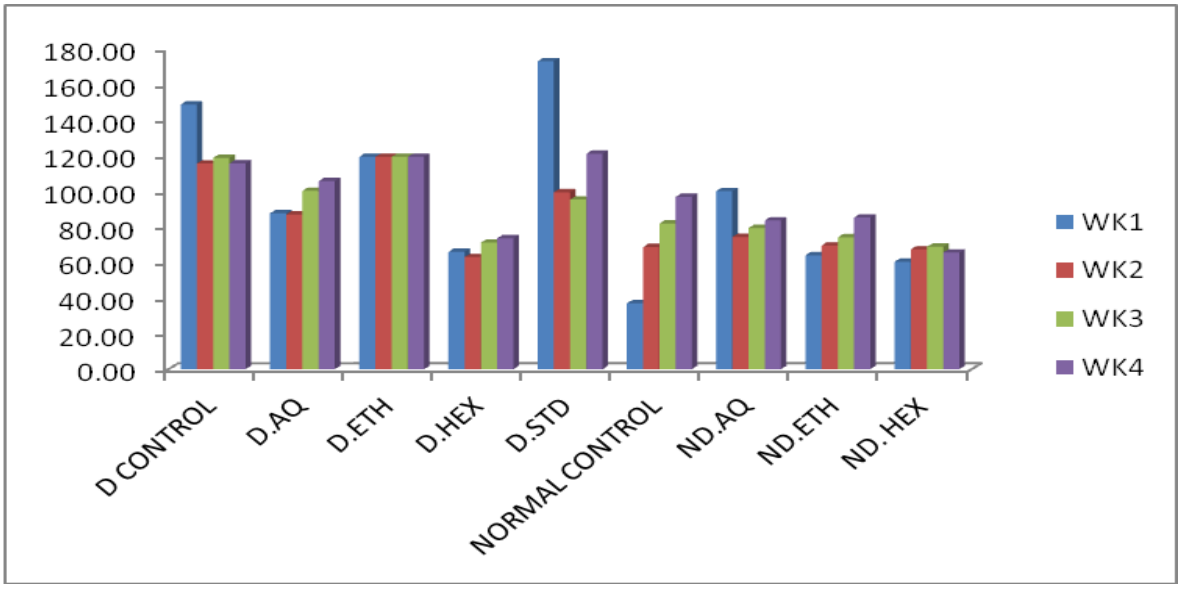

Fig. 7.0 Effect of *Vitex doniana* extracts and standard drug gibenclamide on body weight of streptozotocin induced diabetic rats. Data are mean ± SD P<0.05 compared to the diabetic control. D control = diabetic control, DAQ = dibetic rats +aqueous extract, D.ETH. = diabetic rats + Ethanol extract, DHEX = dibetic rats + n-hexane extract, D.STD = diabetic rats +s tandard drug, ND.AQ. = non diabetic rats + aqueous extracts, ND.ETH. =on diabetic rats + ethanol extract, ND.HEX. = non diabetic rats + n-hexane extract.

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DISCUSSION

Diabetes is now recognized as one of the major killer diseases and a leading cause of death, claiming many lives worldwide. Oral hypoglycemic agents especially the sulphonyureas and biguanides have been commonly used in the disease management especially type II diabetes but are not without serious side effects. Consequently, attention has been focused on the use of plants and herbal remedies believed to be safer and devoid of serious side effects as alternatives in the treatment of diabetes.

The blood glucose level of the diabetic non treated animals as observed in this experiment was significantly high ($p<0.05$). However, the administration of 200mg/kg of both the aqueous, ethanol and n-hexane extracts of *v.doniana* reduced the blood glucose to normal in the treated groups; indicating a hypoglycemic effect of the extracts. This result conforms to the work of [6] which demonstrated the hypoglycemic effect of aqueous extract of *Ocimum gratissimum* in streptozotocin induced diabetic rats. The effective lowering of blood glucose level demonstrated by the leave extracts of *V. doniana* supports its local use as a hypoglycemic agent.

Blood cholesterol, triglyceride and LDL-C were significantly increased ($p<0.05$) in the diabetic control group. This increase might be due to the increased action of hormone sensitive lipase, which promotes lipolysis, and hence increase in the level of free fatty acids in the plasma which is subsequently catabolised to acetyl CoA. The acetyl CoA can be channeled to cholesterol synthesis, thus increasing blood cholesterol level.

The lowering of plasma Total cholesterol, Triglyceride and LDL- cholesterol levels and significant increase in HDL-cholesterol level in the treated animals clearly demonstrated the presence of hypolipidaemic agents in the extracts of *V. doniana* and standard drug gibenclamide (figure 2.0, 3.0, 4.0 and 5.0). The ability of the extracts to manage hyperlipidaemia is a potential beneficial effect on cardiovascular risk factors which is a major cause of death in diabetes mellitus [15].

Lipid Peroxidation is considered to be a primary mechanism of cell membrane destruction by free radicals. The extent of lipid peroxidation is measured by the formation of malondialdehyde (MDA) which conjugate with amino group of proteins to form intra and extra molecular cross-links. These cross-links inactivate the membrane bound enzymes and receptors. The decrease in MDA levels of the *Vitex doniana* extracts treated rats suggest the ability of the leaf constituents (phytochemicals) to prevent oxidative damage by interfering with the processes leading to intermolecular cross-link formation and hence reductions in the rate of progression of diabetic complications.

The observed weight loss in the diabetic control rats could be as a result of fluid depletion and accelerated breakdown of fats and muscles. The relative weight gain observed in the diabetic rats treated with extracts could be due to the presence of phytochemicals such as alkaloids and flavonoids in the extracts which are believed to effect facilitated glucose utilization in peripheral tissues [6].

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CONCLUSION

The present study demonstrated that the extracts exhibited promising hypolipidemic and antidiabetic effects on the treated animals. The anti diabetic and anti lipidemic effects induced by the extracts at a dose of 200mg/kg were comparable to that of standard drug, glibenclimide (2.5mg/kg). *Vitex doniana* leaves therefore could be a potent dietary chemopreventive agent for diabetes and its related diseases.

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