Original Research Article

PROPHYLACTIC AND ANTIDIABETIC EFFECT OF *Andrographis paniculata* AND ITS COMBINATION WITH *Allium sativum* ON BLOOD GLUCOSE LEVEL AND SOME BIOCHEMICAL INDICES OF WISTER RATS

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ABSTRACT

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Background: Diabetes Mellitus is a chronic health problem with devastating effect that is characterized by high blood glucose levels resulting from faults or flaws in insulin production or action. The unaffordability and adverse effects of conventional drugs in the treatment and management of many diseases has spurred the interest of scientists to the use of herbal medicine to treat and manage many ailments. **Aim:** The purpose of this study was to investigate the prophylactic and anti-diabetic effects of *Andrographis paniculata* on the blood glucose level and on some biochemical indices of wistar rats. The bodyweight, fasting blood glucose level, lipid profile and lipid peroxidation were determined using standard biochemical methods. **Result:** All the treatment groups indicated gradual increase in their body weights which was not above normal control and significant reduction in fasting blood glucose level was also observed in all groups but with far greater reduction seen in the pretreated groups with a percentage reduction of 61.25%. The result of the lipid profile showed that varying doses of *A. paniculata* and *A. sativum* significantly reduced the LDL, TCHOL, TRIG and VLDL levels of diabetic rats. However, the pretreatment of *A. paniculata* gave a higher reduction in the LDL,TCHOL,TRIG and VLDL levels. The MDA level of all the test groups decreased when compared to the diabetic untreated group and the standard drug group with significant ($p< 0.05$) reduction seen in the groups treated with 100mg/kg and 200mg/kg of *A. paniculata.* **Conclusion;** The findings from this research showed that *Andrographis paniculata* has the potential to be used as a remedy for the treatment and management of diabetes mellitus and its complications, however pretreatment with *A. paniculata* is highly recommended as it gives better results in reducing the complications associated with diabetes mellitus.

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Keywords: *Andrographis paniculata*, Bodyweight, Diabetes mellitus, Fasting blood glucose, Lipid peroxidation Lipid profile, Pretreatment.

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INTRODUCTION

Diabetes Mellitus is a chronic health problem with devastating, yet preventable consequences. It is a global epidemic and a non-communicable disease that affects both affluent and non-affluent society [1]. Diabetes is described by high blood glucose levels resulting from faults or flaws in insulin production or action [2]. This chronic health problem consists of several types; Type 1diabetes, Type 2 diabetes, pre-diabetes and

gestational diabetes. The occurrence of Diabetes mellitus comes with many complications like diabetic peripheral neuropathy (nerve damage), diabetic retinopathy (most common blindness among working-age individuals, diabetic nephropathy (kidney disease) [3]. Complications associated with diabetes is said to be the seventh leading cause of death worldwide [3] ; In 2012, 1.5 million deaths were recorded worldwide as a result of the micro and macro vascular complications of diabetes and of which about 50 % of these death were due to stroke which is a major and common complications of diabetes [4]. The World Health Organization (WHO) noted in 2016 that the occurrence of diabetes rose faster in low and middle income countries over the past decade with Nigeria currently having the highest incidence of diabetes in sub-Saharan Africa. It was estimated that diabetes killed more than 40,000 Nigerians in 2015 due to lack of efficient and effective healthcare delivery as there are millions of Nigerians who are diabetic but are yet to be diagnosed and treated [5]. The prevalence of this chronic disease called diabetes mellitus is however dependent on several factors such as ethnic group, age, urbanization, diet and increased westernization of life style [6,7].

The global increase of diabetes mellitus and its adverse effect on the individual, economy and the world at large attracts the need for more research on treatment and prevention options for the disease and its complications, especially as the cost of diabetes drugs like Metformin, Loperamide and other diabetes treatment continues to rise [8] hence medicinal Plants are vastly used in the treatment of various diseases as they contain essential phytochemicals that are therapeutic with lesser or no side effect and are cost-effective [9].

Garlic (*Allium sativum*) is the second most wildly used *Allium* and could be used as a spice, an additive or a medicinal plant [10]. Garlic is well-known to be used in food preparation, especially dried foods for storage and some types of soup and it can be utilized in both fresh and dehydrated states [11]. Conventionally, *Allium sativum* and its related compounds have been stated to have several biological activities including anti-carcinogenic, antioxidant [12], anti-diabetic, renoprotective, anti-atherosclerotic, antibacterial, antifungal [13], and antihypertensive activities [14]. Allium sativum is wildly used for treatment of various disease because it contains a very high concentration of sulfur compounds that have been reported to be responsible for its pungent odor and therapeutic properties [15,16]. This commonly used spice could be consumed either raw (fresh leaves or dried cloves) or processed (garlic oil, garlic extracts, and garlic powder). It has also been reported that some of the biological active compound of garlic does not exist until it is in a particular form. For example, *Allicin* (diallyl thiosulfinate or diallyl disulfide) does not exist in

cooked garlic but exist in crushed or cut raw garlic bulbs. The crushing of the garlic bulb activates the enzyme allinase, and within minutes at room temperature, allinase metabolizes alliin to allicin. Allicin which is very unstable is further metabolized to other compounds like vinyldithiines, diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), ajoene, and dithiins [11], hence it is conventionally advised to consume raw garlic in order to get maximum overall health benefits [17,18].

Andrographis paniculata belongs to the family *Acanthaceae* with seeds from India origin and is widely cultivated and grown in Southeast Asia including Malaysia and Nigeria [19]. It grows best in moist and shady places, forests, and wastelands [20]. The leaves and stem of *Andrographis paniculata* are also reported to be dark green with extreme bitter taste [21].

Phytochemical studies have also revealed that *Andrographis paniculata* contains diverse compounds that are usually used in extracting the active component of this plant which may show some variability due to geographical region, harvest time and processing method [22]. However phytochemical studies show that among all the plant metabolites found in *Andrographis paniculata*, terpenoids (entalabdane diterpene lactones) account for a large proportion of its components and therapeutic activities such as antibacterial, anti-inflammatory, antidiabetic, antimalarial and antioxidant activities [23,24]. According to [25], Andrographolide stimulates innate immune response in mice. The immunomodulatory property of diterpene lactone andrographolide was reported to be associated with the enhancement of the proliferation of human peripheral blood lymphocytes, as well as the production of key cytokines and the expression of Y Xu 21 immune activation markers in whole blood cells in culture in vitro [25]. This study however investigates the prophylactic and antidiabetic effect of *Andrographis paniculata* on blood glucose level and some biochemical indices of wistar rats.

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METHODS

Sample Collection and Identification

The leaves of *Andrographis paniculata* and the bulbs of *Allium sativum* were purchased from Afor market in Ogwashi uku Delta State. Both samples were identified by a taxonomist in the Department of Botany, Nnamdi Azikiwe University Awka, Anambra State. The voucher number for *Andrographis paniculata* as deposited in the herbarium of Nnamdi Azikiwe University Awka is NAUH-198^{A} while that of *Allium sativum* is NAUH-27.

ANIMAL STUDIES AND GROUPINGS

A total of 63 Male Wistar Albino rats were procured from Chris Animal Farm and Research Laboratory Mgbakwu Awka, Anambra State and used for the experiment. They were maintained and housed in cages at the Chris Experimental Animal Farm and Research Laboratory Mgbakwu Awka, according to the Institutional Animal Care and Use Committee (IACUC) guidelines on the care and handling of experimental animals.

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The animals were allowed to acclimatize for 7 days, and fed *ad libitum* (ie the diet was available at all times) with vital grower's mash pellets purchased from Vital Feed Distributor at Awka, Anambra state. At the end of the 7 days acclimatization period, the animals were weighed and then randomized into 9 groups of 7 rats each. Group A was the normal control, group B was the Diabetic untreated, group C was treated with standard drug (100 mg/kg bw. gluformin), groups D and E were pre-treated with 100 mg/kg and 200 mg/kg body weight of the ethanol leaf extract of *Andrographis paniculata* for a period of 14 days before the induction of diabetes to check the ability of the extracts in preventing or delaying the onset of diabetes. Diabetes was induced intraperitoneally using 140 mg/kg bodyweight of Alloxan. Bodyweights and Fasting blood glucose levels of the rats were determined before initiating the daily treatment with 100mg/kg and 200mg/kg of the ethanol extracts of *Andrographis paniculata* for a period of 14 days. Groups F and G were treated with 100mg/kg and 200mg/kg of *Andrographis paniculata* respectively. Groups H and I were treated with 100mg/kg and 200mg/kg of a combination of *Andrographis paniculata* and *Allium sativum* respectively. After 28 day of treatment, the animals were sacrificed for biochemical analysis.

ACUTE TOXICITY (LD_{50}) EVALUATION

The median lethal dose (LD_{50}) for *Andrographis paniculata* and for a combination of *Andrographis paniculata* and *Allium sativum* was determined using [26].

INDUCTION OF DIABETES.

Induction of diabetes was carried out using [27]. After 16 hours of fast, diabetes was induced intraperitonally in the rats using 140 mg/kg bodyweight of Alloxan before the commencement of treatment. Condition of Hyperglycemia was determined in rats following 72 hours of alloxan treatment using fasting blood sugar (FBS) cut off point set at 200mg/dL. Rats exhibiting FBS (≥ 200mg/dL) were collected and accepted as been diabetic [28].

ANIMAL SACRIFICE AND SAMPLE COLLECTION

After 14 days of treatment, the animals were anaesthetized with chloroform and blood samples collected via cardiac puncture. The samples were collected into the universal bottles and allowed to clot, after which they were centrifuged for 10 minutes at 4000 rpm. The serum obtained was transferred into another set of test tubes. The serum were used for the biochemical analysis

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DETERMINATION OF FASTING GLUCOSE LEVELS

The Fasting blood glucose levels of the rats were checked before the induction of diabetes, and at an interval of four (4) days using One Touch Glucometer (Life Scan, USA) and test strips based on the method of [29].

LIPID PEROXIDATION

Lipid peroxidation was determined by the thiobarbituric acid-reacting substances (TBARS) assay method of [30], and used by [31]. Exactly 0.4ml of serum was collected into the test tube; 1.6ml of 0.25N HCl was added together with 0.5ml of 15% trichloroacetic acid and 0.5ml of 0.375% of thiobarbituric acid and then mixed thoroughly. The reaction mixture was then placed in 100^{o}C boiling water for 15 minutes, allowed to cool and centrifuged at 3000 rpm for 10 minutes. The supernatant was collected and the optical density recorded at 532nm against reagent blank containing distilled water. The lipid peroxidation activity was calculated using the formula:

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$$\frac{\text{Optical density}}{\text{Time}} \times \frac{\text{Extinction co-efficient}}{\text{Amount of sample}}$$

Where the extinction coefficient value is 1.56 x $10^{-5}M^{-1}CM^{-1}$.The unit was expressed as umol/MDA/mg of protein.

LIPID PROFILE TEST

The lipid profile (Total Cholesterol, Triglycerides, High-Density Lipoprotein-cholesterol, Low-Density Lipoprotein-cholesterol and Very Low-Density Lipoprotein-cholesterol) was determined using Randox test kits [32,33]. Low-density Lipoprotein-cholesterol (LDL-c) was calculated using a standard formula [34]. The procedure used was according to the manufacturer's instructions provided in the manual.

DATA ANALYSIS

Data obtained from the experiments was analyzed using the Statistical Package for Social Sciences software for windows version 23 (SPSS Inc., Chicago, Illinois, USA). All the data collected were expressed as Mean ± SEM. Statistical analysis of the results obtained was performed by using ANOVA Tests to determine if significant difference exists between the mean of the test and control groups. The level of significance was set at $p<0.05$.

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RESULTS

RESULTS OF BODYWEIGHT

The test animals showed gradual increase in their bodyweight before diabetes was induced. After the induction of diabetes, a decrease in body weight of the rats was observed but during the treatment period, the test animals showed increase in body weight which was within the range of their weights before induction. Significant increase ($p<0.05$) when compared to the week initial was observed on week 2 in all groups with exception to the normal control group, groups treated with 200mg/kg of *A. paniculata* and *A. sativum* and the group pretreated and treated with 200mg/kg of *A. paniculata* (figure1). The group pretreated and treated with 100mg/kg and 200mg/kg indicated significant increase ($p<0.05$) in bodyweight on week 4 with respect to the initial week and the week of induction (week 0).

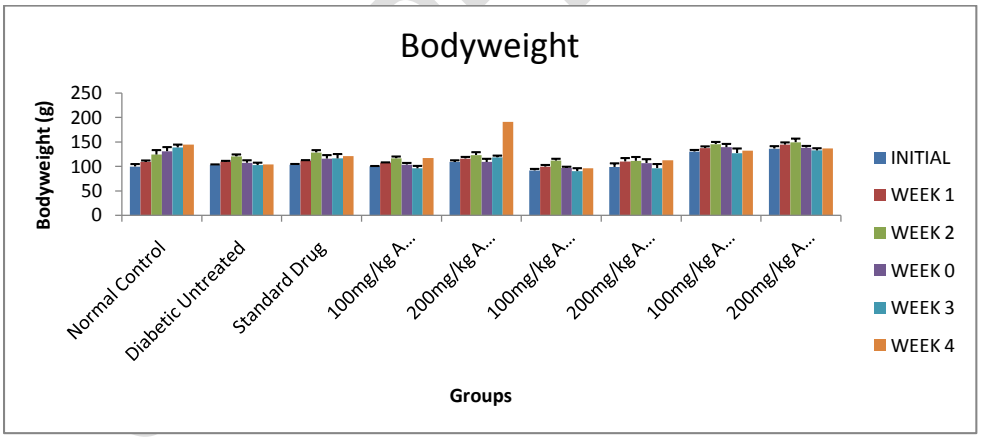

Figure 1: Effect of ethanol extract of *A. paniculata* and *A. sativum* on bodyweight of Alloxan-induced diabetic rats during pretreatment and treatment period.

RESULTS OF FASTING BLOOD GLUCOSE LEVEL

The fasting blood glucose (FBG) concentration of the normal control group remained normal throughout the experiment (figure 2). The induction of diabetes after two weeks of pretreatment caused a significant ($p<0.05$) increase in the fasting blood glucose concentration of all the test groups including the diabetic untreated and standard drug groups (Figure 8). The percentage increase in the fasting blood glucose concentration 48 hours after the induction of diabetes was between 83% and 87% . The FBG level of the diabetic untreated group remained high after induction but showed significant decrease ($p<0.05$) on day 28 with respect to day 0. The group treated with the standard drug showed significant reduction ($p<0.05$) on day 20 and 28 when compared to the day of induction which is 25% and 51% respectively. The groups that were pretreated with 100mg/kg and 200mg/kg of *A. paniculata* before treatment showed significant reduction in FBG levels from day 20 to day28 with highest reduction on day 28 which is 34.92% and 61.25% reduction respectively. The highest percentage reduction in fasting blood glucose concentration for the groups treated with 100 and 200mg/kg bw of *A. paniculata* on day 28 are 53.21% and 57.83%. However, the groups that were treated with 100mg/kg and 200mg/kg bw of a combination of *A. paniculata* and *A. sativum* only showed highest significant reduction in the fasting blood glucose concentration on day 20 and day 28 which is 50.58% and 52.62% respectively. Comparing the reductions in the fasting blood glucose levels for the groups pretreated and treated with ethanol extract of *A. paniculata* and the groups only treated with ethanol extract of *A. paniculata*, significant reduction was observed in both groups with a far better reduction seen in the pretreated groups especially in the group pretreated with 200mg/kg bw which had a percentage reduction of 61.25%.

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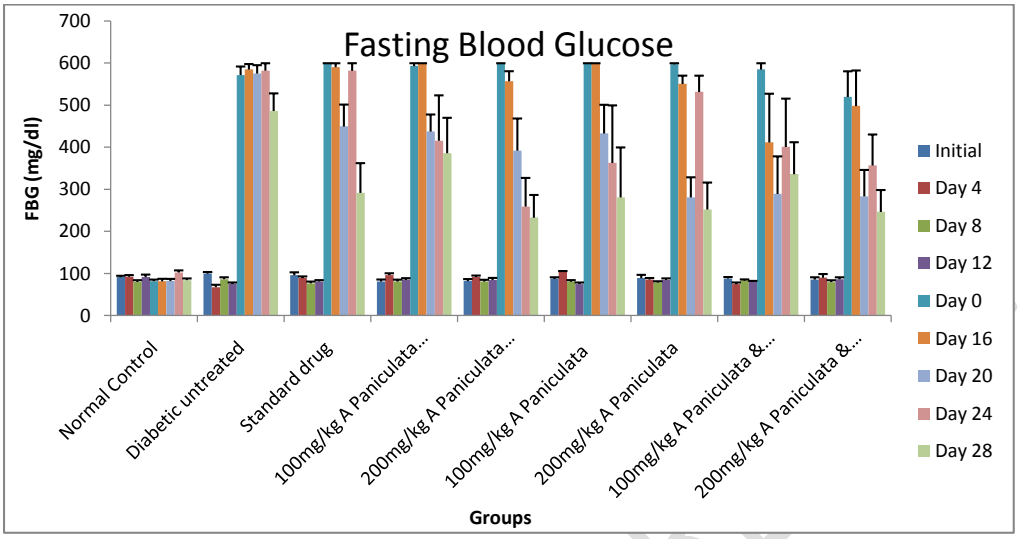

Figure 2: Effect of ethanol extract of *A. paniculata* and *A. sativum* on Fasting Blood Glucose of Alloxan-induced diabetic rats during pretreatment and treatment period.

RESULTS OF LIPID PEROXIDATION ANALYSIS

The result for the lipid peroxidation test showed that all the test group decreased in their Malondialdehyde concentration when compared with the normal control group, the diabetic untreated group and the group treated with the standard drugs but significant decrease ($p<0.05$) was only observed in the groups treated with 100mg/kg and 200mg/kg bw of *A. paniculata* and the group treated with 100mg/kg bw of a combination of *A. paniculata* and *A. sativum* (figure 3).

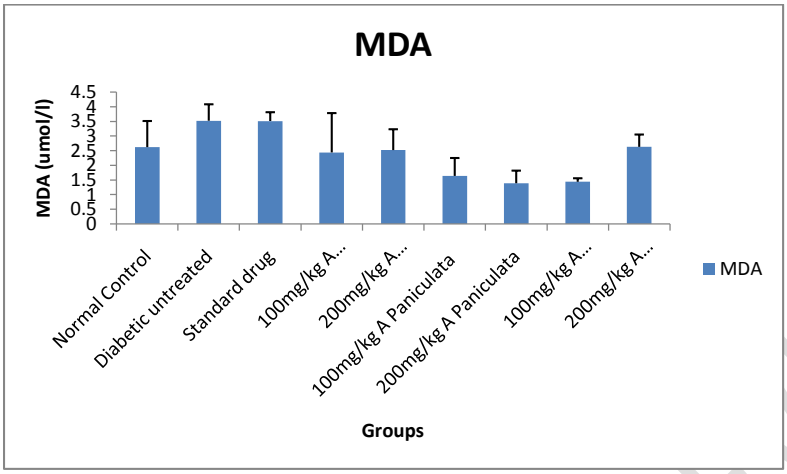

Figure 3: Effect of ethanol extract of *A. paniculata* and *A. sativum* on Malondialdehyde concentration of Alloxan-induced diabetic rats.

RESULTS OF LIPID PROFILE ANALYSIS

Result of High density Lipoprotein Concentration

The result of the high-density lipoprotein concentration seen in figure 4 reduced throughout the period of the experiment with no significant decrease statistically observed ($p>0.05$).

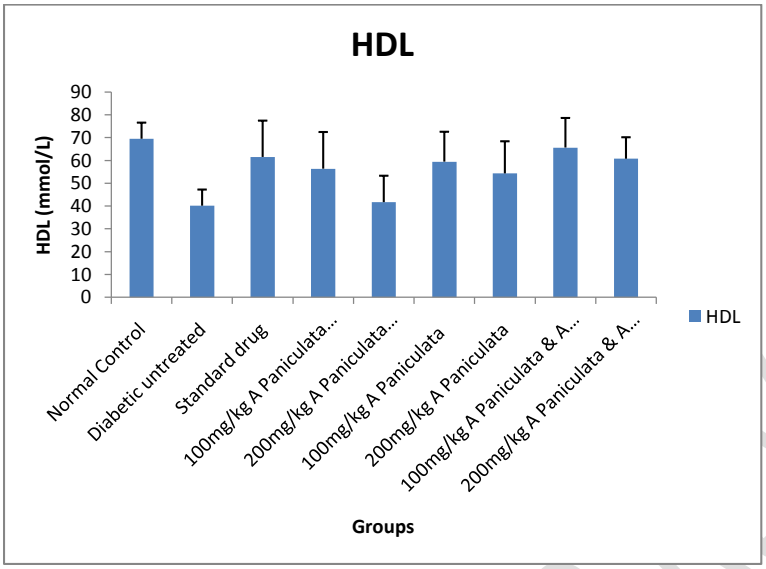

Figure 4: Effect of ethanol extract of *A. paniculata* and *A. sativum* on High density lipoprotein concentration of Alloxan-induced diabetic rats.

Result of Low density Lipoprotein Concentration

The group treated with standard drug and all test groups showed significant reduction ($p<0.05$) in their Low density lipoprotein concentration when compared with the diabetic untreated group, however higher reduction in LDL concentration was observed in the group pretreated with 200mg/kg bw of *A. paniculata* and the group treated with 100mg/kg bw of a combination of *A. paniculata* & *A. sativum* (figure 5).

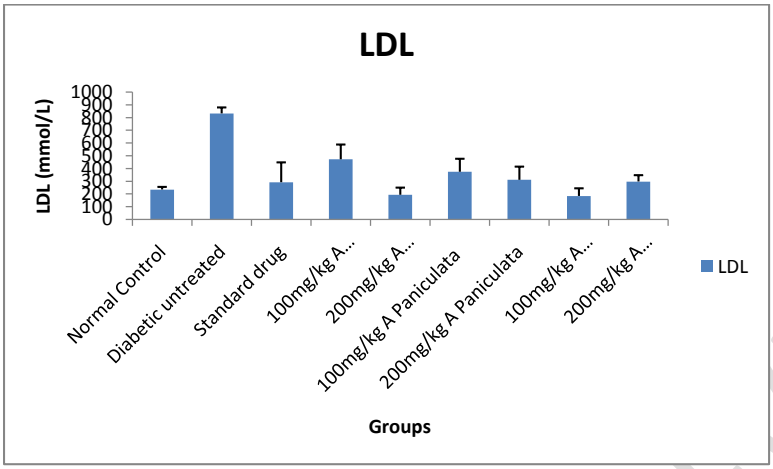

Figure 5: Effect of ethanol extract of *A. paniculata* and *A. sativum* on Low density lipoprotein concentration of Alloxan-induced diabetic rats.

Result of Total Cholesterol Concentration

The group treated with standard drug and all test groups showed significant reduction ($p<0.05$) in their Total Cholesterol concentration when compared with the diabetic untreated group, however, higher reduction in TCHOL concentration was observed in the group pretreated with 200mg/kg bw of *A. paniculata* and the group treated with 100mg/kg bw of a combination of *A. paniculata* and *A. sativum* (figure 6).

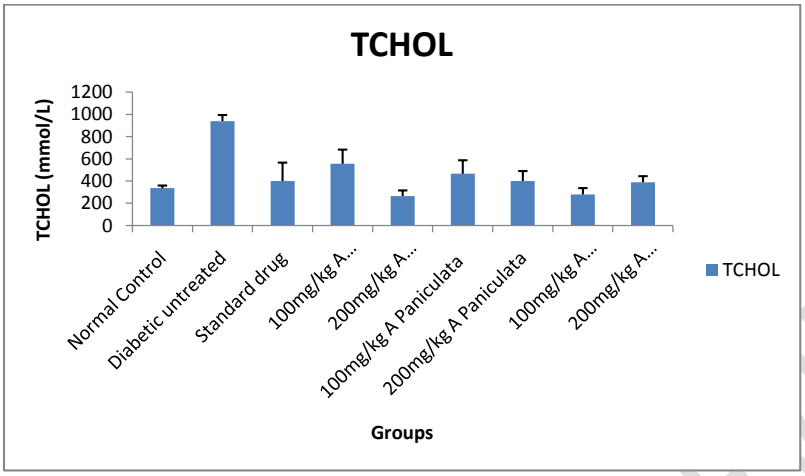

Figure 6: Effect of ethanol extract of *A. paniculata* and *A. sativum* on Total Cholesterol concentration of Alloxan-induced diabetic rats.

Result of Triglyceride Concentration

The group treated with standard drug and all test groups showed significant reduction ($p<0.05$) in their Triglyceride concentration when compared with the diabetic untreated group, however highest reduction in TRIG concentration was observed in the group pretreated with 100mg/kg bw of *A. paniculata* (figure 7)

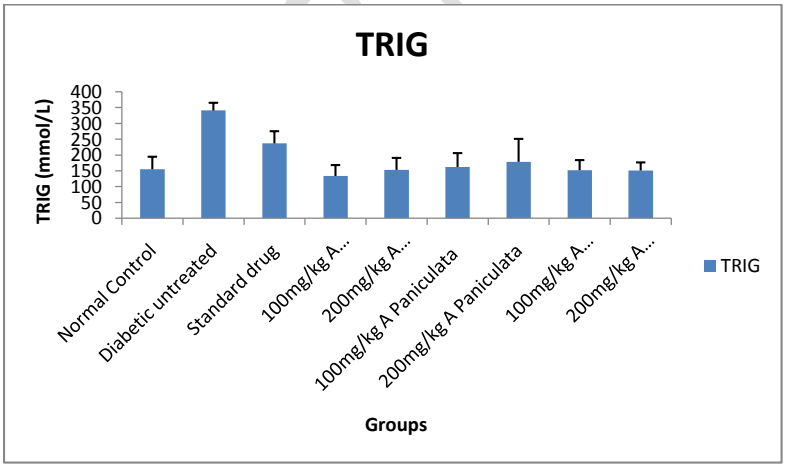

Figure 7: Effect of ethanol extract of *A. paniculata* and *A. sativum* on Triglyceride concentration of Alloxan-induced diabetic rats.

Result of Very low density Lipoprotein Concentration

The group treated with standard drug and all test groups showed significant reduction (p<0.05) in their Very low density lipoprotein concentration when compared with the diabetic untreated group, however highest reduction in VLDL concentration was observed in the groups pretreated with 100mg/kg and 200mg/kg bw of *A. paniculata* and groups treated with 100mg/kg and 200mg/kg bw of a combination of *A. paniculata* and *A. sativum* (figure 8).

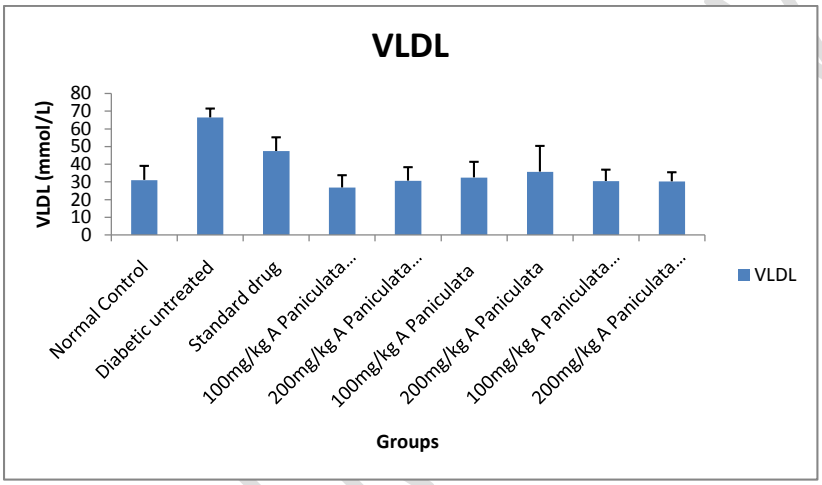

Figure 8: Effect of ethanol extract of *A. paniculata* and *A. sativum* on Very low density lipoprotein concentration of Alloxan-induced diabetic rats.

DISCUSSION

The results of the LD_{50} of the ethanol extract of *Andrographis paniculata* leaf and for a combination of *Andrographis paniculata* and *Allium sativum* bulbs obtained showed no death record thus the extracts could be said to be largely safe for consumption.

The test animals showed gradual increase in their bodyweight before diabetes was induced. However, after the induction of diabetes, a decrease in body weight of the rats was observed but during the treatment period, the test animals showed increase in body weight which was within the range of their weights before induction. The result from figure 7 showed that when compared with week zero, all the treatment groups indicated gradual increase in their body weights especially in the groups pretreated and treated with 100mg/kg and 200mg/kg of *A. paniculata* which showed significant increase on week 4. However this increase observed after induction did not exceed the body weight of the normal control group. This result can however infer that the ethanol extracts of *A. paniculata* and *A. sativum* restores close to normal the increase or decrease in body weight that may occur in Alloxan induced diabetic rats. The results of the animal study suggest that the ethanol extract of both *A. paniculata* and *A. sativum* has some prophylactic and antidiabetogenic effects as the pretreatment of groups D and E helped boost the immunity of groups D and E by reducing the severity of the symptoms of diabetic condition after the induction of diabetes. The boost in the immunity of the pretreatment groups could be as a result of the compound andrographolide that is found in *A. paniculata*. According to [25] andrographolide stimulates innate immune response in mice. The immunomodulatory property of diterpene lactone andrographolide was reported to be associated with the enhancement of the proliferation of human peripheral blood lymphocytes, as well as the production of key cytokines and the expression of Y Xu 21 immune activation markers in whole blood cells in culture in vitro [25]. The result of the Fasting blood glucose level showed that the groups that were pretreated with 100mg/kg and 200mg/kg of *A. paniculata* before treatment indicated significant reduction in FBG levels from day 20 to day28 with highest reduction on day 28 which is 34.92% and 61.25% reduction respectively. The highest percentage reduction in fasting blood glucose concentration for the groups treated with 100 and 200mg/kg bw of *A. paniculata* on day 28 are 53.21% and 57.83%. However, the groups that were treated with 100mg/kg and 200mg/kg bw of a combination of *A. paniculata* and *A. sativum* only showed highest significant reduction in the fasting blood glucose concentration on day 20 and day 28 which is 50.58% and

52.62% respectively. Comparing the reductions in the fasting blood glucose levels for the groups pretreated and treated with ethanol extract of *A. paniculata*, the groups only treated with ethanol extract of *A. paniculata* and the groups treated with a combination of *A. paniculata* and *A. sativum*, significant reduction was observed in all groups but with a far better reduction seen in the pretreated groups especially in the group pretreated with 200mg/kg bw which had a percentage reduction of 61.25%. This reduction in fasting blood glucose could also be as a result of the presence of Saponin present in the plant extract as reports have shown that the presence of Saponin helps to mop up glucose by lysing the red blood cells [35]. This result is consistent with the study by [36] who reported that oral administration of *A. paniculata* considerably reduces streptozotocin induced diabetes mellitus and was further confirmed by [25] who also reported that orally administered glucose-induced hyperglycemia in nondiabetic rabbits could be prevented by the extract of *A. paniculata* and that a dose of 400mg/kg was found to lower the blood glucose levels of streptozotocin-induced animals.

The result for the lipid peroxidation test agrees with the findings of [37] and indicates that all the test groups inhibited lipid peroxidation by lowering the levels of thiobarbituric-acid-reactive substances in the liver and kidney of diabetic rats (as compared to normal rats) but the groups treated with 100mg/kg and 200mg/kg bw of *A. paniculata* and the group treated with 100mg/kg bw of a combination of *A. paniculata* & *A. sativum* showed highest significant decrease ($p<0.05$) in their Malondialdehyde concentration and this could help improve diabetic condition since it reduces the severity of the symptoms after the induction of diabetes.

Increase in lipids can cause plaque to grow over time and lead to obstructions in blood flow that could result in heart attack or Stroke. Hence, it is critical to actively decrease blood lipid counts to prevent and cure cardiovascular and cerebrovascular diseases [38]. The result of the lipid profile showed that varying doses of *A. paniculata* and *A. sativum* will significantly reduce the LDL, TCHOL, TRIG and VLDL levels of diabetic rats. However, the pretreatment of *A. paniculata* and the combination of both *A. paniculata* and *A. sativum* will give a higher reduction in the LDL,TCHOL,TRIG and VLDL levels. The result also showed that the extract had no significant effect on the HDL levels as the HDL levels remained low throughout the experiment. Although the reduction observed was not statistically significant. These findings align with the research of [39], where it was reported that garlic extract reduced greatly the serum glucose, total cholesterol, triglycerides, urea, uric acid, aspartate aminotransferase, and alanine aminotransferase of diabetic mice. A recent study by [40] further demonstrated that *A. paniculata* has active hypolipidemic effects and protects the cardiovascular system by lowering TC, TG, HDL-TC, and LDL-TC in mice and

rats. [41] also reported that the purified extract of andrographolide (an active compound in *A. paniculata*) significantly ($P < 0.05$) decreased the levels of blood glucose, triglycerides, and LDL.

CONCLUSION

King of bitters (*A. paniculata*) and Garlic (*A. sativum*) are natural plants that are easily available and show their effect in treatment of various diseases. This study has shown that both king of bitters and Garlic possess Y. Vasudeva Rao Visva-Bharati (A Central University)

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West Bengal, India Ms_AJRCD_58146 yvrao31@gmail.com Prophylactic Efficacy of Bitter Kola (Garcinia kola) against Egg-Yolk induced Hypertension in Wistar Rats as they both reduce blood glucose level and tries to correct the changes in some biochemical parameters that is caused by diabetes. The combination of both king of bitters and garlic showed high efficacy in significantly reducing blood glucose level and the adverse effect that could arise from diabetes mellitus while the pretreatment with king of bitters provided prophylactic effect against diabetes as it showed higher efficacy in significantly reducing blood glucose level and the adverse effects associated with diabetes mellitus when compared with other treatment groups. Hence these plant extracts can be used as a remedy for the treatment and management of diabetes mellitus and its complications.

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CONSENT

It was not applicable in this research

ETHICAL APPROVAL

All experiments carried out in this research have been examined and approved by the ethics committee of Nnamdi Azikiwe University, Awka, Nigeria in accordance with the Institutional Animal Care and Use policy in Research, Education and Testing.

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