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LIPID PROFILE AND BLOOD GLUCOSE LEVELS OF ALLOXAN-INDUCED DIABETIC RATS TREATED WITH *AJỤ MBAISE* POLYHERBAL FORMULATION

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ABSTRACT: *Ajụ mbaise* polyherbal extract, widely consumed for medicinal and nutritional purposes is composed of stems, leaves, roots and barks of different plant species wrapped together in various proportions. The current study was carried out to determine the effects of orally administered ethanolic *Ajụ mbaise* polyherbal extract on lipid profile and blood glucose levels of alloxan-induced diabetic rats. Twenty male rats of Wistar strain were randomly assigned into four groups of five rats each, following a 7-day acclimatization. Group A served as normal control, Group B as diabetic control, while Groups C and D were diabetic and orally administered with 200 and 400 mg/kg body weight of *Ajụ mbaise* polyherbal extract respectively. Extract administration lasted for fourteen days. After which, blood glucose level was checked on overnight fasted rats. Thereafter, animals were anesthetized and blood samples obtained via cardiac puncture were used for biochemical estimation of the lipid profile. Two weeks post extract administration, the blood glucose level was significantly increased ($P < 0.05$) in the diabetic non-treated group, while no significance changes were observed in the treated-groups when compared to normal control. Also, there were no significant differences ($P < 0.05$) observed in the lipid parameters (HDL-c, LDL-c, TC, TG and VLDL-c) of the treated groups when compared to the normal and diabetic control groups. In conclusion, results obtained in the present study suggest that *Aju mbaise* polyherbal ethanolic extract administered for a 2-week period had no effect on blood glucose level and lipid parameters of the alloxan-induced hyperglycemic rats.

INTRODUCTION: Glucose, the main fuel in the body tissues which has the function to generate energy is an important carbohydrate which is absorbed more into the bloodstream and other sugars are also converted to glucose in the liver. Blood glucose level is closely related to diabetes mellitus [1].

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Diabetes mellitus (DM) is one of the most prevalent metabolic and common endocrine disorders in both developed and developing countries [2, 3]. It is a pathological state which leads to long term complications causing damage of different tissue and organs as heart and blood vessels [3].

One such complication is hyperlipidemia, which is characterized by elevated levels of cholesterol, triglycerides and changes in lipoproteins [4], and an increased risk of complications from vascular diseases which are attributed to an insufficient supply of insulin. DM is classified into two main groups viz- type 1 (T1DM) and type 2 (T2DM).

T1DM requires insulin treatment. T2DM is complex, heterogeneous, polygenic disease defined primarily by insulin resistance, ongoing hyperglycemia, and β cells' dysfunction. Metabolic abnormalities such as dyslipidemia, hyperinsulinemia, or insulin resistance and obesity play key roles in the induction and progression of type 2 diabetes mellitus (T2DM) [5].

According to Capcarova and Kalafova, [6] the rising incidence of diabetes mellitus (DM) worldwide presents a global public health problem. The use of medicinal plants and their derived bioactive compounds to cure various ailments, including metabolic disorders, has been a preference since the earliest of times. There is still a continuous rising interest in the use of medicinal plants either in their crude or pure form to fight disorders such as diabetes mellitus [7]. It has been reported that traditional medicines are particularly useful in developing countries as most individuals have limited resources and access to orthodox medicines. Thus, the use of natural extracts in the treatment of diabetes mellitus has grown recently [8].

Ajụ mbaise is a polyherbal formulation, composed of combination of different leaves, roots and trunk of different medicinal plants wrapped together in various proportions and taken in the form of concoctions [9, 10]. This formulation is native to the Mbaise Community in Mbaise Local Government area of Imo State, Nigeria, from where it derived its name.

Reports had it that it is used for various purposes, including- enhancing labour, facilitating the expulsion of retained placenta, relieving menstrual and post-delivery pains and promoting involution of the uterus, detoxification and cleansing of the womb, reduction of stomach size following administration to women after childbirth [9–12]. More so, Ijioma *et al.* [13] reported that the formulation had potential anti-diarrhoeal agent and anti-motility effect on the gastrointestinal tract smooth muscles. Its effectiveness in weight reduction has also been reported by [14]. *Ajụ mbaise* polyherbal formulation is composed of parts of six different plants including *Barteria fistulosa, Napoleona vogelli, Euphorbia convolvuloids*, *Spondias mombine, Uvaria chamae* and *Ceiba petandra* [12]. Phytochemical screening of *Ajụ mbaise* has also shown that it contained appreciable amount of alkaloids, tannins, flavonoids, cyanogenic glycoside, phenols, steroids, terpenes and saponin [10, 12, 15]. It has also been reported by Ogueke *et al.* [9] that this polyherbal formulation has good amounts of quality proteins, minerals and vitamins.

Following the upsurge in the indiscriminate use of this herbal formulation, notwithstanding the traditional claims justifying its use, there is need for a thorough scientific evaluation to validate the supposedly therapeutic effects of this herbal concoction. There are insufficient and dearth of data on the lipidemic and glycemic indices of this formulation. On this note, the present study was designed to test the influence of orally administered ethanolic *Ajụ mbaise* polyherbal extract on lipid profile and blood glucose levels of experimentally-induced diabetic rats.

MATERIALS AND METHODS:

Plant Collection and Identification: Fresh samples of *Ajụ mbaise* polyherbal heads, (comprising all plants that make up *Ajụ mbaise*) used in this study were purchased at AfoOgbe market in Mbaise, Imo State, Nigeria. The plants were identified and authenticated at Biological Sciences Department of Alex Ekwueme Federal University, Ndufu-Alike. The fresh plants after collection were cut into small pieces, air dried and blended to a powdered form using multipurpose mechanical grinder before the extraction process. The extraction was done with ethanol as the solvent.

Extraction of Plant Materials: A powdered sample weighing 1000 g was soaked in 3000 mL of 95% ethanol for 48 hours after which it was sieved using a muslin cloth and afterwards filtered through a Whatmann filter paper No. 1. The filtrate was concentrated using a rotary evaporator at 45^{o}C and thereafter placed on a thermostatic water bath for further drying. The concentrate (paste) was collected, weighed, kept in sterile bottles and stored in the refrigerator.

Experimental Animals: A total number of 20 male rats of the Wistar strain weighing between 120-200g used for the study were purchased from Animal house, Physiology Department of Alex Ekwueme Federal University Ndufu-Alike. The

rats were acclimatized for two weeks prior to the study and fed with standard chow (Vital feed®, Nigeria). The animals were kept in cages, and maintained under standard conditions with light-dark cycle. They were maintained in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals [16].

Induction of Diabetes: Following acclimatization, 160mg/kg body weight of freshly prepared solution of alloxan was injected intraperitoneally to the overnight fasted experimental rats. Three days after the alloxan injection, blood samples were collected from the tail vein to determine the blood glucose concentration using glucose meter (Accu-Answer ®, India) prior to the commencement of the administration of the extract. The alloxan-treated rats with fasting blood glucose level >200mg/dL were considered diabetic and included in the study.

Grouping of Animals: The animals were randomly selected into four groups of five rats per group (n=5) as follows:

Group 1: Normal control (received distilled water vehicle for alloxan).

Group 2: Diabetic rats.

Group 3: Diabetic rats treated with 200 mg/kg of *Ajụ mbaise* extract.

Group 4: Diabetic rats treated with 400mg/kg of *Ajụ mbaise* extract.

All the animals were allowed free access to feed and water *ad libitum.*

Sample Collection and Analysis: 14 days post extract administration, rats were fasted overnight and blood glucose levels were measured using glucose meter. Thereafter, the animals were anesthetized and whole blood sample of about 5 mL was obtained via cardiac puncture into plain tubes and was allowed to clot. Sera were obtained from the clotted sample after centrifuging at 3000 rpm for 10minutes.

Biochemical Assay: Total cholesterol and HDL –c were measured colorimetrically by automatic analyzer using Randox commercial diagnostic kit, while the TG was by Teco diagnostic kits in accordance with the procedures described in the manufacturers operation manual.

VLDL and LDL were estimated using Friedewald's equation as reported in our previous work [17]:

VLDL= TG/5(mg/dl); LDL= TC- (HDL+VLDL).

All the laboratory analysis was performed at Clinical Chemistry Laboratory Department of Alex Ekwueme Federal University Teaching Hospital Abakaliki (AE-FUTHA) Ebonyi State, Nigeria.

Statistical Analysis: Data were expressed as mean ± SEM and were analyzed using graphpad prism 7.0 software. Differences in mean were compared using Analysis of variance (ANOVA) and student's t-test. P values < 0.05 were taken as statistically significant.

RESULTS: The results of all the experiments carried out are as shown in **Table 1** and **Fig. 1** to **6**. All the values given are expressed as mean ± SEM and asterisks in the table and bar charts indicates the values that are significantly different from the control values or treatment group of all the variables measured.

Effect of *Ajụ mbaise* Polyherbal Extract on Glucose Level and Lipid Parameters of Alloxan-Induced Diabetic Rats: The effect of *Ajụ mbaise* polyherbal extract (APE) on glucose level, Triglycerides (TG), Total Cholesterol (TC), High Density Lipoprotein cholesterol (HDL-c), Very Low Density Lipoprotein cholesterol (VLDL-c) and Low Density Lipoprotein cholesterol (LDL-c) are shown in **Table 1.**

Fig. 1 shows the effect of *Ajụ mbaise* polyherbal extracton blood glucose level. There was a significant increase in blood glucose level of the alloxan-induced diabetic control group when compared to the normal control and other treated groups. However, *Ajụ mbaise* polyherbal extract had no effect on the treatment groups. **Fig. 2-6**, show the effect of *Ajụ mbaise* polyherbal extracton the lipid profile levels. The extract showed no significant effect on all the lipid profile parameters (TG, TC, HDL-c, VLDL-c and LDL-c) measured between the treatment groups and the normal control.

TABLE 1: EFFECT OF *AJụ MBAISE* POLYHERBAL EXTRACT ON GLUCOSE LEVEL AND LIPID PARAMETERS OF ALLOXAN-INDUCED DIABETIC RATS. VALUES ARE EXPRESSED AS MEAN ± SEM (n=5)

Parameter	Group A	Group B	Group C	Group D
Glucose (mg/dl)	95.7±2.5	310.4±55.2*	151.3±19.9	127.0±1.3
TG (mg/dl)	42.1±4.2	46.3±6.5	31.6±3.1	43.4±4.2
TC (mg/dl)	87.0±2.8	67.3±8.0	78.8±11.4	68.3±3.2
HDL-c (mg/dl)	52.2±0.8	56.3±3.5	48.6±4.4	53.3±0.3
VLDL-c (mg/dl)	8.4±0.8	9.3±1.3	6.3±0.6	8.7±0.8
LDL-c (mg/dl)	26.4±4.4	1.7±11.0	23.9±16.2	6.3±2.7

Key: n = 5, *=p<0.05, TG- Triglyceride, TC- Total cholesterol, HDL-c-High density lipoprotein cholesterol, VLDL-c- Very low density lipoprotein cholesterol, LDL-c- Low density lipoprotein cholesterol.

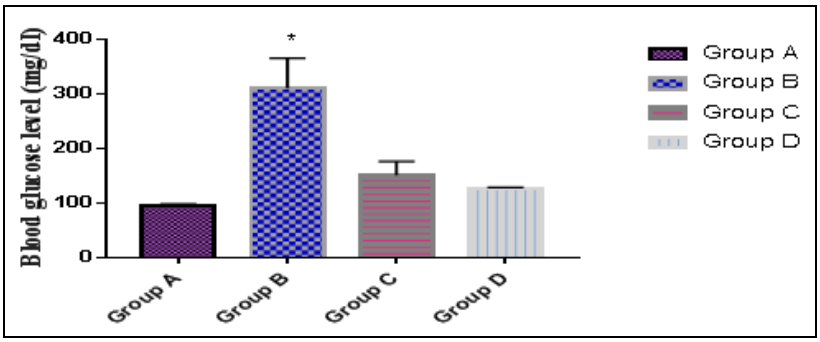

FIG. 1: EFFECT OF *AJỤ MBAISE* POLYHERBAL EXTRACTON BLOOD GLUCOSE LEVEL (n=5; *=P<0.05)

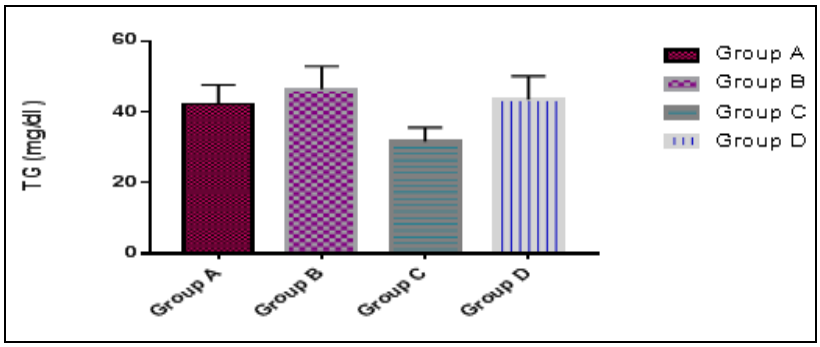

FIG. 2: EFFECT OF *AJU MBAISE* POLYHERBAL EXTRACT ON TRIGLYCERIDE (TG). (n=5)

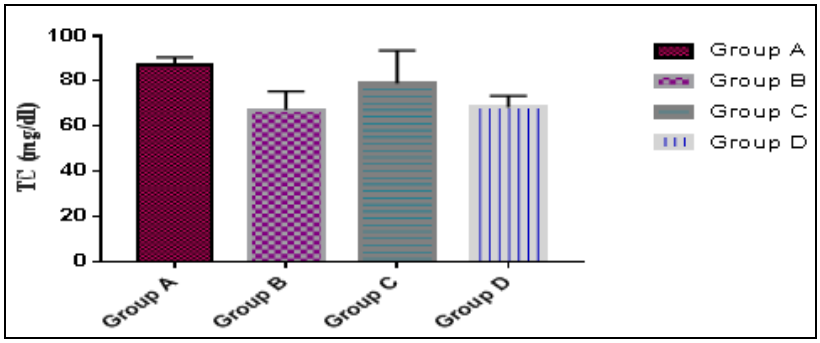

FIG. 3: EFFECT OF *AJU MBAISE* POLYHERBAL EXTRACT ON TOTAL CHOLESTEROL (TC). (n=5)

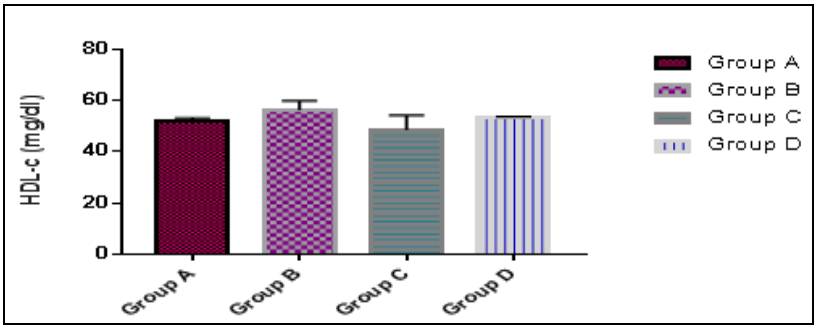

FIG. 4: EFFECT OF *AJU MBAISE* POLYHERBAL EXTRACT ON HIGH DENSITY LIPOPROTEIN CHOLESTEROL (HDL-C). (n=5)

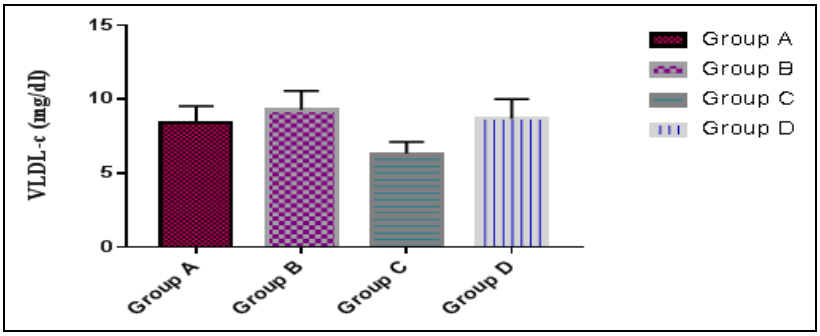

FIG. 5: EFFECT OF *AJU MBAISE* POLYHERBAL EXTRACT ON VERY LOW DENSITY LIPOPROTEIN CHOLESTEROL (VLDL-C) (n=5)

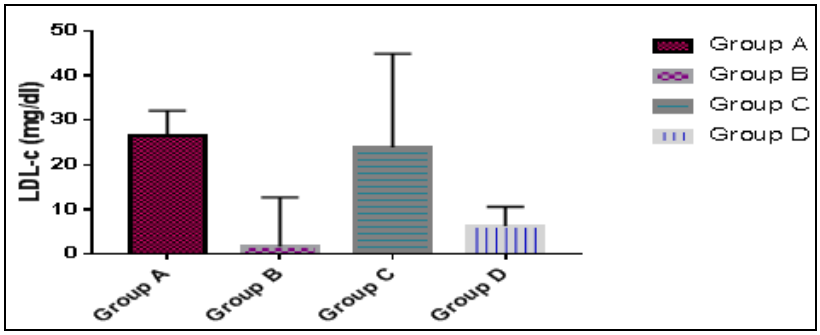

FIG. 6: EFFECT OF *AJU MBAISE* POLYHERBAL EXTRACT ON LOW DENSITY LIPOPROTEINCHOLESTEROL (LDL-C) (n=5)

DISCUSSION: Diabetes Mellitus is the most common endocrine disorder and a pathological state which leads to long term complications causing damage of different tissue and organs as heart and blood vessels [3]. It is one of the most prevalent metabolic disorders in both developed and developing countries. The use of phyto-chemicals and dietary agents have gained popularity in the management of this disorder [2]. This is because treatments with plant extracts are easily accessible, mostly safe, and there is global interest in the use of natural products. Hyperlipidemia is an associated complication of diabetes mellitus. Lowering of serum lipid levels seems to be associated with a decrease in the risk of vascular disease and related complications [4]. In the present study, experimentally-induced diabetic rats showed significant increase in plasma glucose levels when compared to normal rats. Increased fasting blood glucose levels are a major indication in diabetes mellitus, and accordingly, a trend of significantly elevated blood glucose levels was found in the alloxan-induced diabetic rats, which continued until the end of the experimental period. Alloxan-induced diabetes is one of the widely used model to induce Type I diabetes mellitus in experimental animals [18]. It induces a multiphasic blood glucose response when injected into an experimental animal, which is accompanied by corresponding inverse changes in the plasma insulin concentration followed by sequential ultrastructural beta cell changes ultimately leading to necrotic cell death. The diabetogenic effects of alloxan are underlined by its selective inhibition of glucose-induced insulin secretion *via* its ability to inhibit the beta cell glucose sensor glucokinase and selective necrosis of the beta cells via induced reactive oxygen species [19]. The result of this study also showed that *Ajụ mbaise* polyherbal extract had no effect on the glucose levels of experimentally induced diabetic rats at doses of 200 and 400 mg/kg. Accordingly, our finding disagrees with the reports of [20], who stated that the extract exhibited a hypoglycemic effect on diabetic rats. The results of this study also showed that there were no significant changes in the lipid parameters of all the alloxan-induced diabetic rats treated with *Ajụ mbaise* polyherbal extract. This is in disagreement with the report of [11], who reported that the extract exhibited non-significant hypolipidemic effect on serum TC and LDL-c concentration of female rat treated with high doses of *Ajụ mbaise* polyherbal extract dose-dependently for 28 days; and also reported a dose dependent increase in HDL-c level. According to the report, it suggests that could have cardio-protective potential.

It is possible that these variations may be related to species differences and/or the dose of the extract used and the duration of treatment.

CONCLUSION: On the basis of the results exhibited by this study, it can be concluded that *Ajụ mbaise* polyherbal extract has no effect on the glucose level and lipid parameters of the experimentally induced diabetic rats. We therefore, recommend increased treatment duration and further research to include studies at the cellular level to determine the mechanisms of improvement in the glucose levels and lipid profile more specifically.

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