Lipid Profile of Alloxan-Induced Diabetic Albino Wistar Rats Pre-treated with *Curcuma Longa* Rhizome Powder, Crude Aqueous Extract and Both

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***Abstract*:- The pre-treatments with turmeric powder, extract and their mixture was evaluated in this paper to unriddle their effects on the lipid profile of diabetic rats after daily feeding and administration of the crude aqueous extract of turmeric rhizome powder for three weeks. A significantly (P≤ 0.05) decrease in weight and plasma level of High-Density Lipoprotein Cholesterol (HDL-C) were observed in alloxan-induced- diabetic rats in comparison with normal and pretreated groups.There was however a significant increase (*P<0.05*) in the blood glocuse level, plasma levels of total cholesterol, triglycerides, Low-Density Lipoprotein Cholesterol (LDL_C) of alloxan induced-diabetic rats (group B) when compared with normal (group A) and pre-treated groups (group C, D and E).The decrease in plasma triacylglycerol(TG) level, wheight gained and normal blood glucose levels observed in normal rats (group A) and pre-treated alloxan-induced diabetic rats in group E and others (C and D is an indication that turmeric rhizome in diet and as extract does not increase blood triglycerides. This suggests that *it* is unlikely to pose a health hazard as a high triglyceride level increases the risk of cardiovascular disease.The weight gained with normal blood glucose levels observed in group C, D and E might implies that pretraetment with turmericpowder or extract appears to be protective against pathological effects inflicted on alloxan induced-diabetic rats.**

I. INTRODUCTION

Diabetes mellitus has been described as one of the major complex and chronic disorders of protein metabolism ,carbohydrate.It is usualllipid usually characterized by persistent elevation of blood glucose, resulting from a partial or complex cessation of insulin secretion , or peripheral resistance to insulin action (Longe*et al*., 2015). In recent literature, diabetics have been found to show abnormal lipid disorders such as hyperlipidemia, atherosclerosis (Asanga*et al*., 2013).Plasma lipids assayed for includes total cholesterol (TC), TG, LDL-C, and HDL-C. Lipid metabolism disorder has been found to be one of the reasons for premature atherosclerosis in patients with diabetes mellitus (Khanna*et al*., 1996).

Turmeric (*Curcuma longa*) , which belong to the family of ginger is a rhizomatous herbaceous perennial plant . It is called by different names in different cultures and widely cultivated in the tropics. In some states in Nigeria, like Ekiti, turmeric is commonly called "Osun"and "Ajo" in Ondo State. Furthermore, it is called "Hald" in North India, while in South, turmeric is known as "Manjal". It is known as "*terremerite*" in French and simply as "yellow root" in many languages. In many cultures, its name is based on the Latin word *Curcuma* (Prasad and Aggarwal, 2011). Preliminary phytochemical screening by Akpanabiat (2005) showed that it contains alkaloids, saponins, and polyphenols.This study will help to unriddle the effect of turmeric formulated diet, its aqueous extract, and both pre-treatments on the lipid profile ofalloxan-induced diabetic rats.

II. MATERIALS AND METHODS

2.1 Materials

2.1.1 Sample collection and preparation

The rhizome part of turmeric was purchased locally from Oja Oba's market, Ado Ekiti, Nigeria. Identification of the rhizome was carried out in the Herbarium Unit of the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria (Voucher specimen number: Bot/Her./1910). The sample was dried, ground into powder, and stored inside a polythene bag until required for analysis. Both the powder and aqueous extract of the sampleswere used in this work.

2.1.2 Sample Preparation/ Treatment

The clean sample was dried and ground into a powdery form using a mixer grinder and stored in an airtight sample bottle before analysis.

2.1.3 Preparation of the Crude Extract of the Sample

The aqueous extract of the powdered rhizome plant part (10 g) of the sample was prepared thus: 10g of the sample was weighed and poured inside a glass measuring cylinder. Distilled water was added and made up to 100ml which is equivalent to 10% aqueous extract of the sample. The mixture was shaken and kept for 24h after which it was then filtered using a muslin cloth.

All chemicals used were of analytical grade, while all-glass distilled water was used in the analysis. Alloxan used was obtained from Sigma/Aldrich.

2.1.4 Reagents

Standard Erba kits were used to determine Total Cholesterol, HDL-Cholesterol,Triglycerides, and LDL-Cholesterol.

2.2 Animals Selection and their Care

Forty (40) matured female Wistar rats with weights ranging from (90 to 100g) were used for this experiment. The animals were acclimatized for a period of one week to the laboratory conditions before the experiment at the Animal House of College of Medicine, Ekiti-State University, Ado-Ekiti, Nigeria. After acclimatizing them, they were fed with a specially prepared diet. The individual weights of the rats were taken after withdrawing food and water for 13hours prior to the commencement of the experiment. The rats were fed *ad libitum* for 21days with this special diet and housed in cages at room temperature with 12hours light and dark cycle.

2.3 Experimental design

For the experiment, the weights and fasting blood sugar levels of the rats were measured and randomly allocated into five groups ($n = 8$ group) as follows:

- **Group A**: Non-diabetic rats were maintained on a formulated diet containing 5% turmeric powder daily for 3 weeks
- **Group B**: Rats were fed with normal rat chow for 3 weeks and then induced with a single dose of alloxan (150mg/kg) intraperitoneally
- **Group C**: Rats were fed with 5% turmeric formulated diet daily for 3 weeks and then challenged with a single dose of alloxan (150mg/kg) intraperitoneally
- **Group D**: Rats were gavaged by intubation with 0.5ml of 10% turmeric crude aqueous extract daily for 3 weeks and then received a single dose of alloxan (150mg/ kg) intraperitoneally.
- **Group E**: Rats were gavaged by oral intubation with 0.5ml of 10% turmeric crude aqueousextract and fed with 5% turmeric formulated diet daily for 3 weeks after which they were injectedintraperitoneally with a single dose of alloxan(150 mg/kg)

2.4 Induction of Diabetes Mellitus

This was done by intraperitoneal administration of a single dose of freshly prepared alloxan of 150 mg/kg body weight, dissolved in 0.15M normal saline to rats in group B to E to induce type II diabetes. Prior to this, their fasting blood glucose levels were determined. Within 48 hours, rats' blood glucose levels were determined and blood glucose levels above 150mg/dL were considered being challenged especially from group B (negative control) and used for the study.

2.5 Determination of Whole Blood Glucose

Glucose concentration in mg/dl of the animals was measured with the aid of ON-CALL PLUS Glucometer using compatible glucose test strips according to prescribed instructions.

2.6 Collection of Blood Sample

At the end of the feeding experiment, rats in all the groupswere anesthetized and sacrificed. Blood was collected by cardiac puncture into sodium citrate and plain tubes and centrifuged at 3000Xg for 15 minutes. The supernatant was collected using a Pasteur pipette. The plasma obtained was appropriately labeled and stored in a freezer at -5^0C until required for further analysis.

2.7 Isolation and Homogenization of Tissues

The rats were sacrificed after three weeks of diet and aqueous administration in order to isolate tissue of interest (liver, heart, and kidney). The isolated tissues were cleansed with cotton wool to remove blood stains, weighed, and immediately stored in an ice-cold 7.4M Phosphate buffer solution. The liver, heart, and kidney were cut with a clean scalpel and were homogenized using Teflon homogenizer in ice-cold 7.4M phosphate buffer solution. The homogenates were stored in the freezer (-50^0C) until further analysis.

For the experiment, the weights and fasting blood sugar levels of the rats were measured and randomly allocated into five groups ($n = 8$ group) as follows:

Bodyweight of all pre-treated rats (3 groups), normal control, and diabeticcontrol groups were taken before and during the treatment by electronic balance. For blood glucose level estimation, blood samples were collected from the overnight fasted rats of all the five groups prior to the pre-treatment (of 4 groups) with turmeric formulated diet, crude aqueous extract,and their mixture, measured at the interval and finally measured using glucometer strips after pre-treatment with turmeric formulated diet, aqueous extract and their mixture for 3 weeks. This follows the procedure of Ojo*et al*., (2012). At the end of the experiment, the rats were sacrificed under sodium pentobarbitone anesthesia (Nafisa*et al*., 2007).

After the experimental period, all rats were sacrificed and blood samples were collected. Plasma was separated in sodium citrate bottles and centrifuged at $3000X_g$ for 15 minutes. After centrifugation, the supernatant was collected using a Pasteur's pipette. The plasma obtained was appropriate labeled and stored in a freezer at -50^0C till used for estimation of lipid profile which was carried using Erba kits: total cholesterol (Deeg, 1983), triglyceride (Fossati, 1982), LDL-C (Friede*et al*., 1972) and HDL-C (Burestin*et al*., 1970). Then the abdomen and the thorax were opened and the liver, heart, and kidney were removed and bottled individually for preparation of homogenates. These were used for the estimation of tissue GSH levels, the activity of CAT and SOD enzymes.

2.8 Statistical Analysis

All the grouped data were statistically evaluated and the significance of changes caused by various treatments was determined using one-way analysis of variance ANOVA (Chou, 1975). The results have been expressed as mean ± SD from four rats in each group. The level of statistical significance was set at P≤0.05.

III. RESULT

Table 3.1: Effects of turmeric formulated diet and aqueous extract pre-treatments on the body weight of alloxan-induced diabetic rats

Groups	Weight (before feeding in kg)	Weight (after feeding in kg)
A	95±2.91[a]	129± 4.35[abc]
B	96±2.40[a]	93.0±1.97[d]
C	95±5.28[a]	144±8.09[a]
D	95.1±3.26[a]	119±4.89[c]
E	95.4±2.26[a]	136±12.42[b]

Results are expressed as means ±SD of four determinations (n =4); values in the same row with different superscripts are significantly different at P ≤ 0.05.

Table 3.2: Effects of turmeric formulated diet and aqueous extract pre-treatments on blood glucose of alloxan-induced diabetic rats

Groups	Glucose level (before alloxan challengein g/dl)	Glucose level (after alloxanchallenge in g/dl)
A	103±4.79[ab]	67.0±3.16[d]
B	112±21.92[a]	144±10.61[a]
C	86.3±7.55[bc]	80.5±1.73[c]
D	80.5±13.48[c]	68.3±5.91[d]
E	84.8±2.63[c]	94.3±7.04[b]

Results are expressed as means ±SD of four determinations (n =4); values in the same row with different superscripts are significantly different at P ≤ 0.05.

A=Non-Diabetic control group, B= Diabetic control group

C = Pre-treated group with dietary turmeric rhizome before induction of alloxan

D = Pre-treated group with crude aqueous extract of turmeric before induction of alloxan

E = Pre-treated group fed with dietary turmeric rhizome and also given crude aqueous extract before induction of alloxan

Table 3.3:Effects of turmeric powder formulated diet and its aqueous extract on the plasma lipid profile concentrations in alloxan-induced diabetic rats

Groups	TC (mmol/l)	HDL (mmol/l)	TG (mmol/l)	LDL (mmol/l)
(Group A)	177.93±19.87[c]	319.04 ±45.56[e]	621.43 ±19.63[e]	133.08±19.02[b]
(GroupB)	258.56±55.17[d]	134.34 ±3.54[a]	842.70 ±4.33[b]	318.65±54.92[d]
(GroupC)	187.39 ± 14.41[a]	165.75 ±72.87[b]	859.93 ± 24.62[c]	164.71±14.35[a]
(GroupD)	130.63 ±61.09[b]	171.17 ±27.[18c]	858.60 ±39.90[d]	170.16±60.80[a]
(GroupE)	186.49±12.53[a]	194.46 ±56.[43d]	695.63 ±128.18[a]	193.74±12.07[c]

Results are expressed as mean±SD of four determinations (n =4); values in the same row with different superscript are significantly different at P ≤ 0.05.

A=Non-Diabetic control group

B= Diabetic control group

C = Pre-treated group with dietary turmeric rhizome before induction of alloxan

D = Pre-treated group with crude aqueous extract of turmeric before induction of alloxan

E = Pre-treated group with both dietary turmeric rhizome and crude aqueous extract before induction of alloxan

IV. DISCUSSION

4.1. Effect of Turmeric Formulated Diet and Extract on Average Bodyweight and Blood Glucose Level

Table 3.1 and 3.2 show the effects of turmeric formulated diet and extract on the weights and blood glucose levels of pretreated alloxan-induced diabetic rats. It was observed in this study that alloxan induced diabetic rats (group B) had a significant decrease ($p \leq 0.05$) in body weight when compared with normal and pre-treated rats (groups A, C, D and E). However, there are significant increase in the body weight of alloxan induced diabetic rats that were pre-treated with 5% turmeric formulated diet (group C) from 94.77±5.28 to144.03±8.09, 10% aqueous extract (group D) and those pretreated with both 5% formulated diet and 10% aqueous extract (group E) ($p \leq 0.05$) . However, there was no significant difference between the increase in body weight of normal control rats (group A) when compared with pretreated alloxan induced diabetic rats in group C, D and E. This is in line with Eleazu *et* al., (2010) that reported weight loss in diabetic rat before the administration of unripe plantain. Reports show that insufficiency in insulin level in the body prevents the body from getting glucose from the blood into the body's cells to use as energy in people with diabetes. Therefore, the body starts burning fat and muscle for energy, causing a reduction in overall body weight (Sunyer, 2005).This might account for the significant decrease in body weight of alloxan-induced diabetic rats (group B) observed in this study. The current study showed significant increases in blood glucose levels of rats that were not pre-treated with turmeric rhizome powder after induction of diabeteswhich is also in agreement with the report of Basma *et al*., (2017). Etuk and Muhammed (2010) and Adeyi *et al*., (2015)attributed this increase in glucose levels to the reactive oxygen species induced by alloxan; this is in conjunction with a simultaneous massive increase in cytosolic calcium concentrations led to rapid destruction of pancreatic islet cells and a concomitant reduction in synthesis/release of insulin (Basma *et al*.,, 2017).

The disorder of glucose metabolism in diabetes is mainly attributed to diabetic oxidative stress by several mechanisms such as increased production of radicals especially reactive oxygen species (ROS) and proteins glycosylation (West, 2000). *Curcuma longa* pretreatment to rats before induction in this study resulted in significant decreases in blood glucose levels. This agrees with an earlier report (Basma *et al*., 2017) and the possible contributing mechanisms is hypothesized to be due to the potentiation of insulin secretion from β-islet cells and this consequently enhanced the transport of blood glucose to peripheral tissues. Alloxan is known to destroy the cells of the pancreas that function in the regulation of insulin secretion and thus leads to an increase in the concentration of blood glucose. According to Edoga *et al., (*2013),there is deficiency of insulin resulting into an increased synthesis ofketone bodies which are excreted in urine due to the destruction of the pancreatic cells by alloxan.

4.1.Plasma lipid profile

In alloxan-induced diabetes, elevated glucose levels are usually associated with an increase in plasma cholesterol, triglycerides, LDL, and VLDL and reduction in hormone-sensitive lipase (HSL) which leads to the release of free fatty acids from adipose tissue. Thus, the production of excess fatty acids in the blood due to Alloxan-induced diabetes promote the conversion of excess fatty acids into phospholipids and cholesterol in the liver. Excess triglycerides formed in the liver with these two substances (phospholipids and cholesterol) may be discharged into the blood in the form of lipoproteins (Garber, 2000). Therefore, the marked hyperlipidemia that characterizes the diabetic state may be due to the uninhibited actions of lipolytic hormones on fat depots. However, pretreatment with turmeric formulated diet, crude aqueous extract, and their mixture for three weeks produced a significant decrease ($P \leq 0.05$) in the concentration of total cholesterol and low-density lipoprotein observed in groups A,C,D, and group E when compared with group B, There was also a significant decrease ($P \leq 0.05$) in the level of triglyceride observed in group A,D, and E when compared with group B but the non-significant difference ($P \leq 0.05$) between the triglyceride level in the plasma of group C and group D.The decrease in triglyceride level in the plasma of normal rats in group A and pre-treated alloxan-induced diabetic rats in group E is an indication that turmeric rhizome in diet and as extract does not increase blood triglycerides. This suggests that *it* is unlikely to pose a health hazard as a high triglyceride level increases the risk of cardiovascular disease. The hypolipidemia effect of turmeric rhizome could be as a result of increase in uptake of LDL from blood by the liver, inhibition of hepatic cholesterol biosynthesis, and stimulation of receptor-mediated catabolism of LDL-cholesterol and increased fecal bile acid secretion and (Dewayne *et al*., 2013).

A significant increase ($P \leq 0.05$) in the level of triglycerides was observed in group C and group D compared with group B but there was no significant difference between group C and D. A very high significant difference ($P \leq 0.05$) was observed in the level of high-density lipoprotein diabetic control (group) rats compared with alloxan-induced diabetic groups (B,C, D, and E).In addition, a significant increase ($P \leq 0.05$) was observed in the level of high-density lipoprotein in the groups pre-treated with turmeric formulated diet (group C), crude aqueous extract only (group D), and their mixture (group E) when compared with the diabetic control group (B). The significant decrease in the T C and LDL values observed for the groups A, C, D, and E with the significant reduction of triglycerides in group A and E indicate that turmeric rhizome prevented accumulation of cholesterol that may cause plaques in the blood vessels. This agrees with a previous study byAsanga*et al*., (2013) that the significant reduction in TG, LDL-C, VLDL, total cholesterol levels as well as the significant increase ($P \leq 0.05$) in HDL-C levels of some of the lipoprotein fractions may be either through the scavenging of the reactive oxygen metabolites due to the presence of antioxidant compounds in the extracts of Latifolia leave. Alterations in the concentration of major lipids can give useful information on the lipid metabolism and predisposition of an animal to atherosclerosis (Yakubu, 2008). It has been revealed that altered lipid concentrations and qualitative changes of the lipoprotein fractions in diabetes are associated with an increased risk of diabetes (Noeman, 2011). In addition, lipid alterations have been considered as contributory factors to oxidative stress in diabetes.

V. CONCLUSION

The pretreatment with dietary turmeric and extract may hinder the damages induced by preventing hyperglycemia and hyperlipidemia. This was revealed in the significant reduction (≤ 0.05) of TC, TG, and LDL-C levels as well as the significant increase ($P \leq 0.05$) in HDL-C levels of plasma of pretreated rats. Therefore, it may be suggested that turmeric rhizome powder at the level used in this study can be useful for the prevention ofhyperlipidemia induced by diabetes mellitus;which may be either through the scavenging of the reactive oxygen metabolites due to the presence of antioxidant compounds in the sample.

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