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EFFECT OF ETHANOL LEAF EXTRACT OF *OCIMUM GRATISSMUM* (SCENT LEAF) ON LIPID PROFILE OF ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

The present study was undertaken to investigate the chemical composition and therapeutic effect of *Ocimum gratissimum* on alloxan-induced diabetes in albino rats. The vitamin and mineral compositions were determined using standard methods. Sixteen rats were divided into four groups made of four rats each. Group A served as the control normal rat, while groups B, C and D were induced with diabetes by intraperitoneal injection of alloxan monohydrate with a dose of 80mg/kg body weight. Group B (untreated diabetic control), while group C and D received 200 mg/kg and 400 mg/kg body weights of *Ocimum gratissimum*. The extract was administered through oral intubation. The results showed high contents of vitamin A 6.34±0.01, vitamin E 0.546±0.0052 and vitamin C 0.5267± 0.005 respectively in the ethanol leaf extract of *Ocimum gratissimum*. No death was recorded in the acute toxicity test. Blood samples were collected by vein puncture after fasting overnight and standard methods were used for the determination of fasting blood glucose and lipid profile. The results showed a significant ($P<0.05$) decrease in the blood glucose level when compared to the untreated diabetic control. There was a significant ($P<0.05$) increase in triglyceride, cholesterol and low density lipoprotein level of the diabetic control compared to all the treated groups and control while the HDL decreased significantly ($P<0.05$) compared to treated and normal control that increase significantly ($P<0.05$).

Keywords: *Ocimum gratissimum*, alloxan, diabetes and lipid profile.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder with numerous complications. It is characterized by chronic high blood glucose levels leading to increased morbidity and mortality. Diabetes if not controlled results in structural and functional changes in various target tissues and organs. The worldwide prevalence of DM has risen dramatically over the past two decades, from an estimated 30 million cases in 1985 to 177 million in 2000 [1]. Based on current trends, it is projected that ~435 million individuals will have diabetes by the year 2030. In modern medicine, the beneficial effects of standard medications on glycemic levels are well documented, however, the preventive activity of medications against the progressive nature of diabetes and its complications was modest but not always effective [2]. Insulin therapy affords glycemic control in type 1 diabetes, yet its shortcomings such as, short shelf life, the requirement of constant refrigeration, fatal hypoglycemia in event of excess dosage, reluctance of patients to take insulin injection and above all resistance due to prolonged administration limits its usage. Similarly, treatment of type 2 diabetes patients with sulfonylureas and biguanides is almost always associated with side effects [3]. Hence, the search for drugs with low cost, more potential and with fewer side effects is being pursued in several laboratories around the world.

Ocimum gratissimum L. is widely distributed in tropical and warm temperature regions. The plant is commonly used in folk medicine to treat different diseases upper respiratory tract infections, diarrhea, headache, ophthalmic, skin diseases, pneumonia, and also as a treatment for cough, fever, and conjunctivitis [4].

Ocimum gratissimum belongs to the family *Lamiaceae*. It is commonly called 'alfavaca' and is cultivated in many gardens around village huts in Nigeria for its medicinal and culinary uses [5].

It is believed to have originated in Central Africa and South East Asia [6]. Several species and varieties of the genus *Ocimum* have been reported to yield oils of

diverse nature; these are commonly called basilica oils. According to the literature, the oils produced from *O gratissimum* are active against several bacteria including *Staphylococcus aureus, Listeria monocytogenes* and *Escherichia coli* and fungi including *Trichophyton rubum, T mentagrophytes* [7]. The oils are used in the treatment of many ailments, including upper respiratory tract infections, diarrhea, headache, fever, eye problems, skin diseases, and pneumonia [8]. The oil is also a potent antidiabetic agent [8]. [9], have reported the use of *O. gratissimum* for flavoring foods and as an antimicrobial agent.

AIM AND OBJECTIVES

The aim of the project work was to investigate the anti diabetic effect of ocimum gratissimum extract on alloxan induced diabetic rats

MATERIALS AND METHODS

Determination of Minerals

Minerals were determined using the methods of Pearson (1976); AOAC (1995);

Determination of Vitamins.

Fat soluble and water soluble vitamins were assayed using the methods of AOAC (1990); Leo and Nollet, (2000).

ACUTE TOXICITY TEST

The acute toxicity tests were carried out as method described by Lorke (1983).

Determination of Lipid Profile parameters

Determination of total serum cholesterol:

The evaluation of total cholesterol was done by the method of Allain and Roschlain (1979).

Measurement of serum triacylglycerol concentration

The method of Tietz (1990) was used to determine triacylglycerol level in serum of the rats.

HDL cholesterol determination

High density lipoprotein cholesterol was determined using the method adopted by Jacob *et al.* (1990) and while cholesterol level was determined by the method of Allain *et al.* (1974).

LDL Cholesterol Determination

LDL Cholesterol was measured using the calculation below

$$\text{LDL cholesterol (mg/dl)} = \frac{\text{TotalCholesterol- Triacylglycerides-HDL Cholesterol}}{5}$$

STATISTICAL ANALYSIS

The data were expressed as mean ± standard deviation and analysed using statistical package for the social sciences (SPSS). Comparison was made between the test group and the control groups using the student's T-test and $p \leq 0.05$ was considered significance

RESULTS

Vitamin composition of ethanol leaf extract of *Ocimum gratissimum*

The results of the vitamin analysis of *Ocimum gratissimum* were represented in Table 1 The result showed the presence of Vitamins B1, B2, B3, B9, B12, A, E, and C. respectively.

Table 1: Vitamin Constituent of *Ocimum gratissimum*

Vitamins	Concentrations(mg/100g)
Vitamin E	0.546±0.0052
Vitamin A	6.34±0.01
Vitamin C	0.5267± 0.005

Vitamin B1	0.716±0.0052
Vitamin B2	0.433±0.0057
Vitamin B3	0.162±0.0046
Vitamin B9	0.272±0.0040
Vitamin B12	0.113±0.0030

Mineral composition of leaf ethanol extract of *Ocimum gratissimum*

The result of the mineral composition of *Ocimum gratissimum* extract showed the presence of K, Ca, Mg, Fe, Zn, Cu, P, Pb and Ar. Iron having the highest concentration and arsenic the lowest. The amount of arsenic present in *Ocimum gratissimum* extract is grossly insignificant.

Table 2: Mineral Constituent of *Ocimum gratissimum*

Minerals	Percentage composition (%)
Sodium	0.472±0.0046
Potassium	0.274±0.0036
Calcium	1.409±0.008
Magnessium	1.09±0.0036
Iron	2.416±0.0052
Zinc	1.415±0.0050
Copper	0.242±0.0045
Phosphorus	0.813±0.0057
Lead	0.132±0.0025
Arsenic	0.065±0.0032

Acute Toxicity of Ethanol Extract of *Ocimum gratissimum* Leaf in Albino Rats

There was a decrease in physical activities of the ethanol leaf extract of *Ocimum gratissimum* administered groups when compared to control that was administered with normal saline. No death was observed. The LD_{50} was found to be greater than 5000 mg/kg body weight of albino wistar rats.

Groups	Dose (mg/kg)	No of rats	No of rats at 24 hours	No of rats at 48 hours
Group 1	5 of normal saline	4	0	0
Group 2	100	4	0	0
Group 3	200	4	0	0
Group 4	1000	4	0	0
Group 5	1600	4	0	0
Group 6	2900	4	0	0
Group 7	5000	4	0	0

Effect of Ethanol Extracts of *Ocimum gratissimum* HDL levels of alloxan induced diabetic rats.

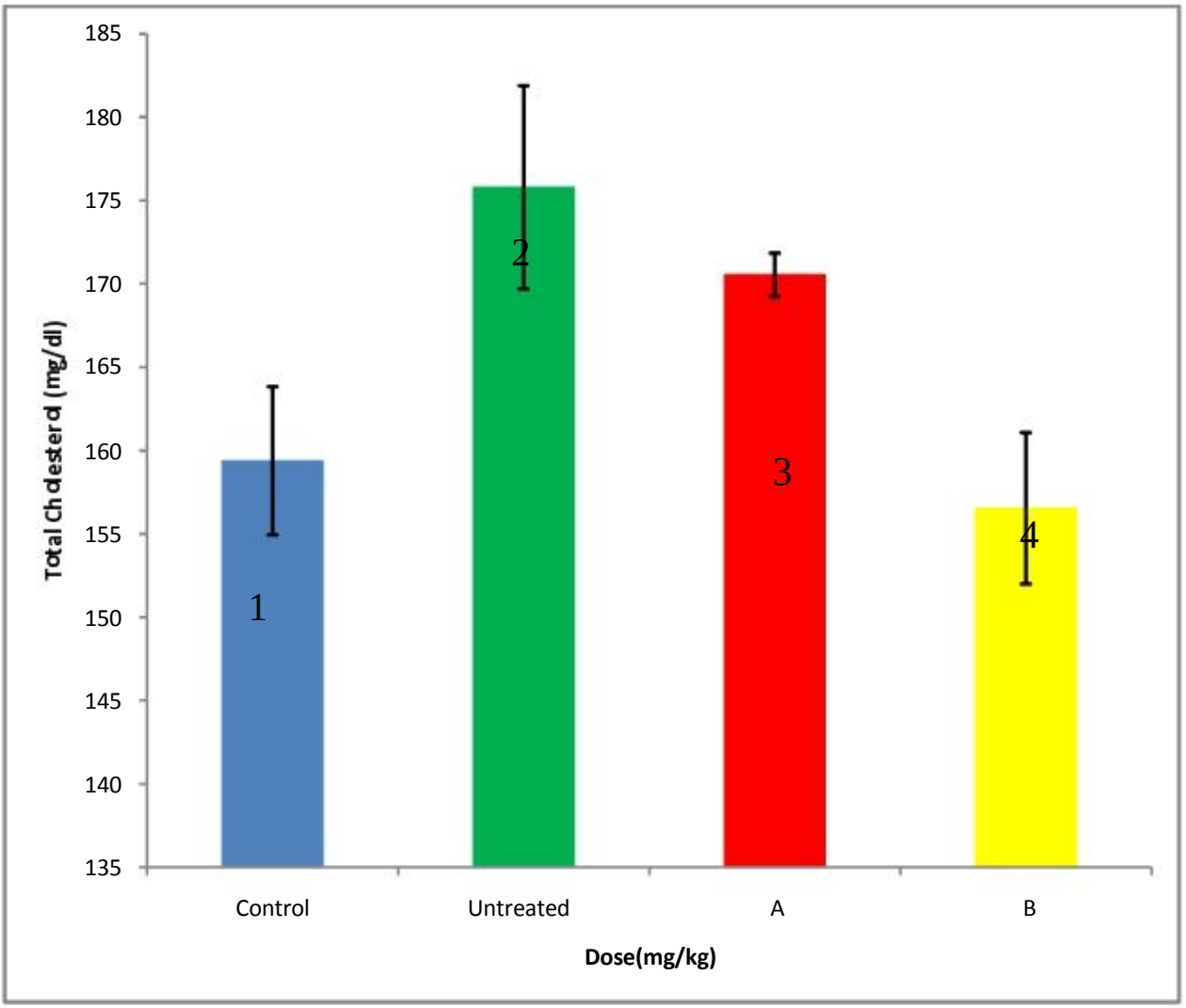
The results of effect of ethanol leaf extract of *Ocimum gratissimum* on alloxan induced diabetes in albino rat were presented in Figures 1, 2, and 3 respectively. There was a significant ($P<0.05$) decrease in HDL level in all the diabetic rats when compared to the control. However treatment with extract of *Ocimum gratissimum* at 200 and 400 mg/ kg body weight reversed the HDL level close to normal when compared to the diabetic untreated group. This reversal effect of the plant extract was dose dependent.

There was a significant ($P<0.05$) increase in LDL level in all the diabetic rats when compared to the control. However treatment with extract of *Ocimum gratissimum* at 200 and 400 mg/ kg body weight reduced the LDL level close to normal when

compared to the diabetic untreated group. This reversal effect of the plant extract was dose dependent

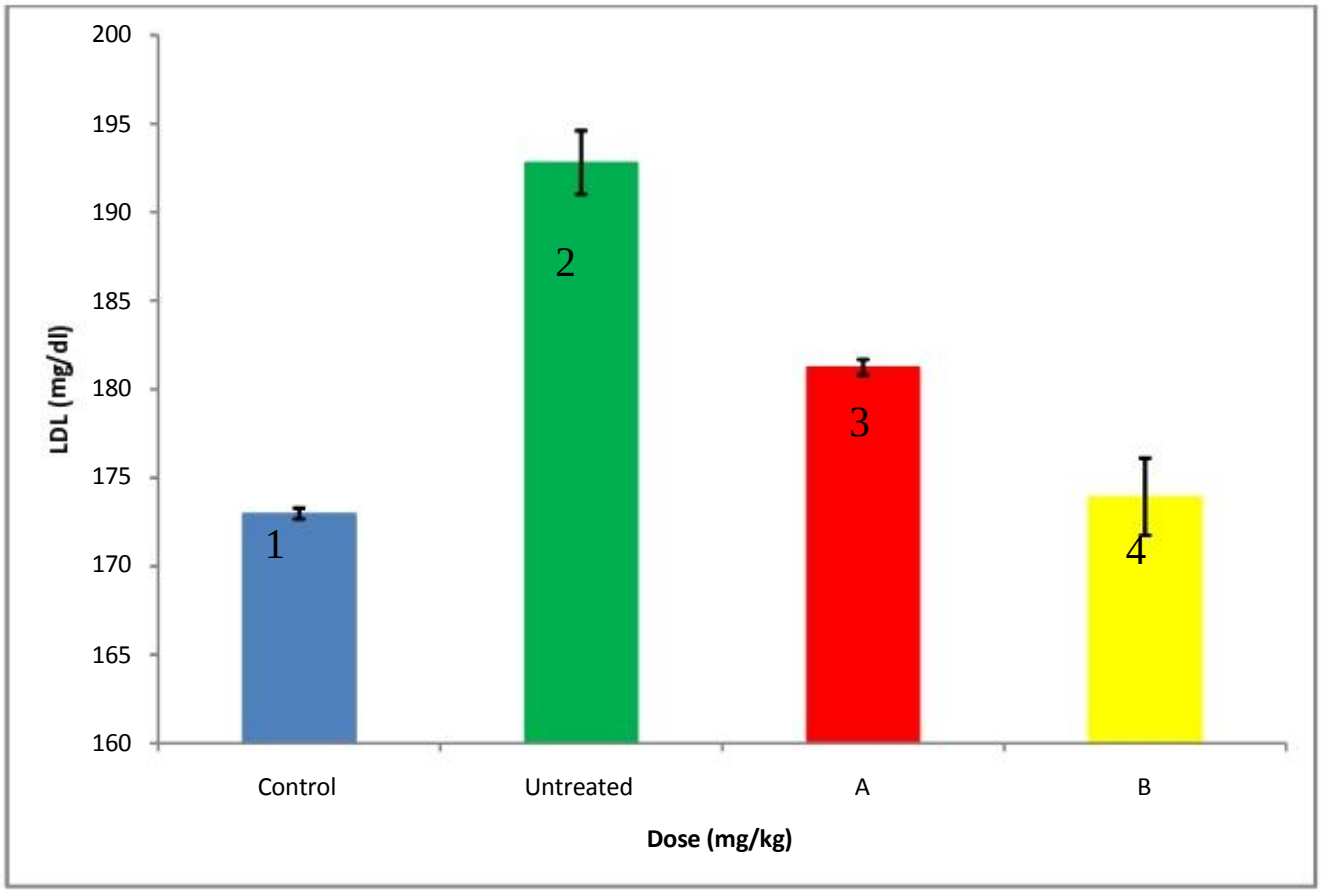
While in cholesterol there was a significant ($P<0.05$) increase in triglyceride and total cholesterol level in all the diabetic rats when compared to the control. However treatment with *Ocimum gratissimum* at 200 and 400 mg/ kg body weight reversed the total cholesterol level near normal when compared to the diabetic untreated rats.

Figure 1: Effect of ethanol leaf extract of *Ocimum gratissimum* on total cholesterol level of alloxan-induced diabetes in albino rats.



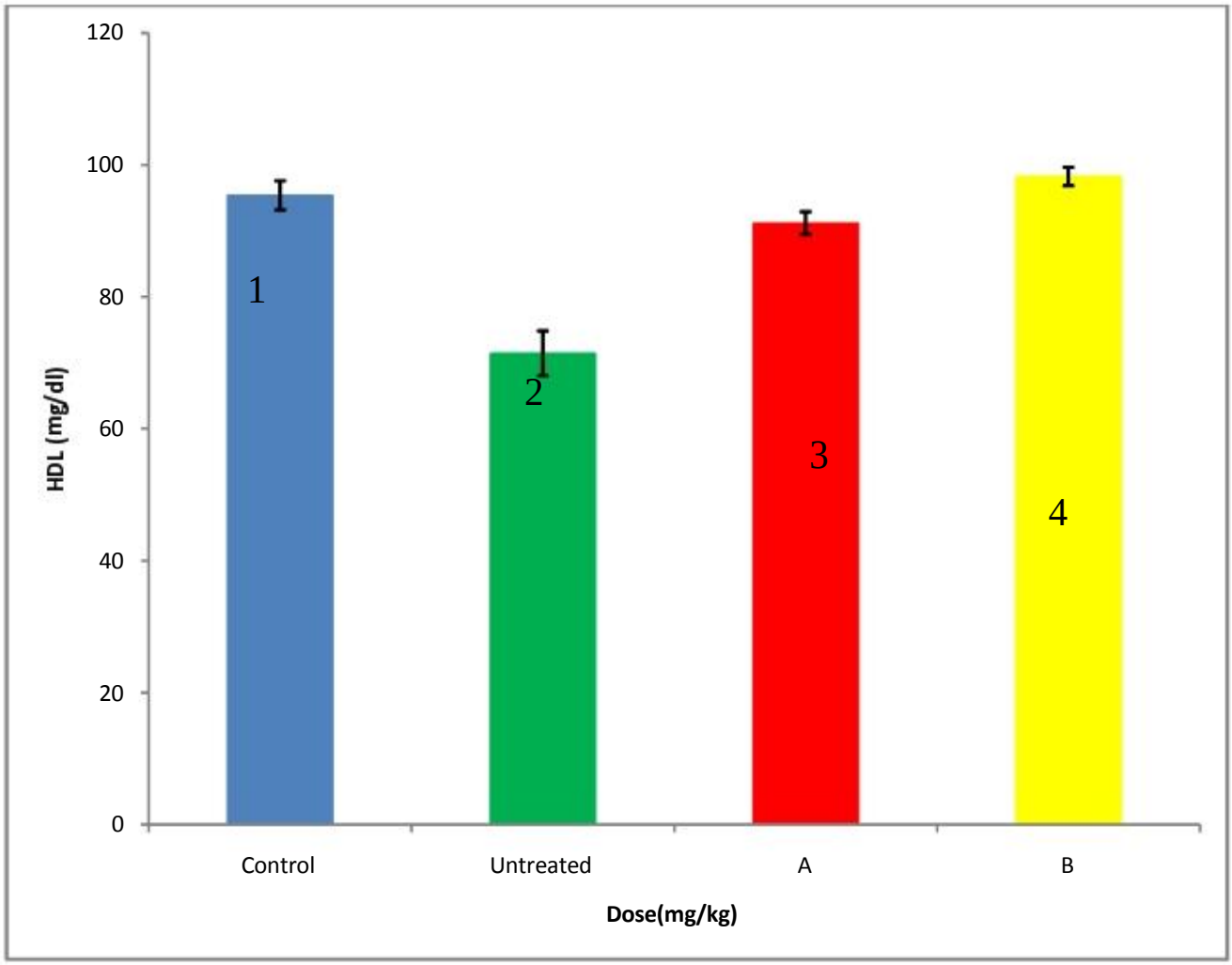
1= Control, 2= diabetic untreated, 3= 200 mg/kg body weight, 4= 400 mg/kg body weight of leaf extract

Figure 2: Effect of ethanol leaf extract of *Ocimum gratissimum* on high density lipoprotein cholesterol (LDL-C) of alloxan-induced diabetic albino rats.



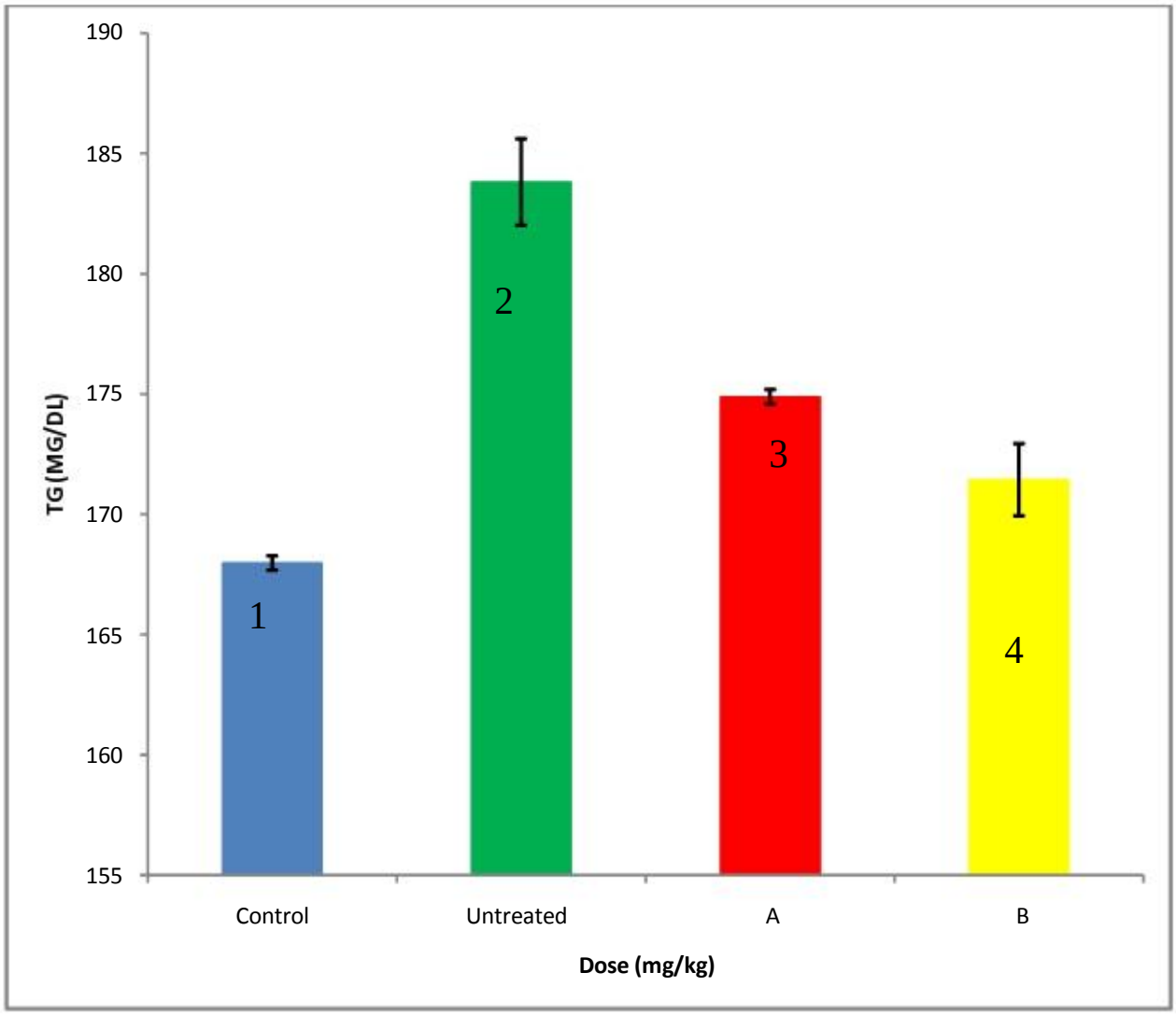
1= Control, 2= diabetic untreated, 3= 200 mg/kg body weight, 4= 400 mg/kg body weight of leaf extract

Figure 3: Effect of ethanol leaf extract of *Ocimum gratissimum* on high density lipoprotein cholesterol (HDL-C) of alloxan induced-diabetic albino rats.



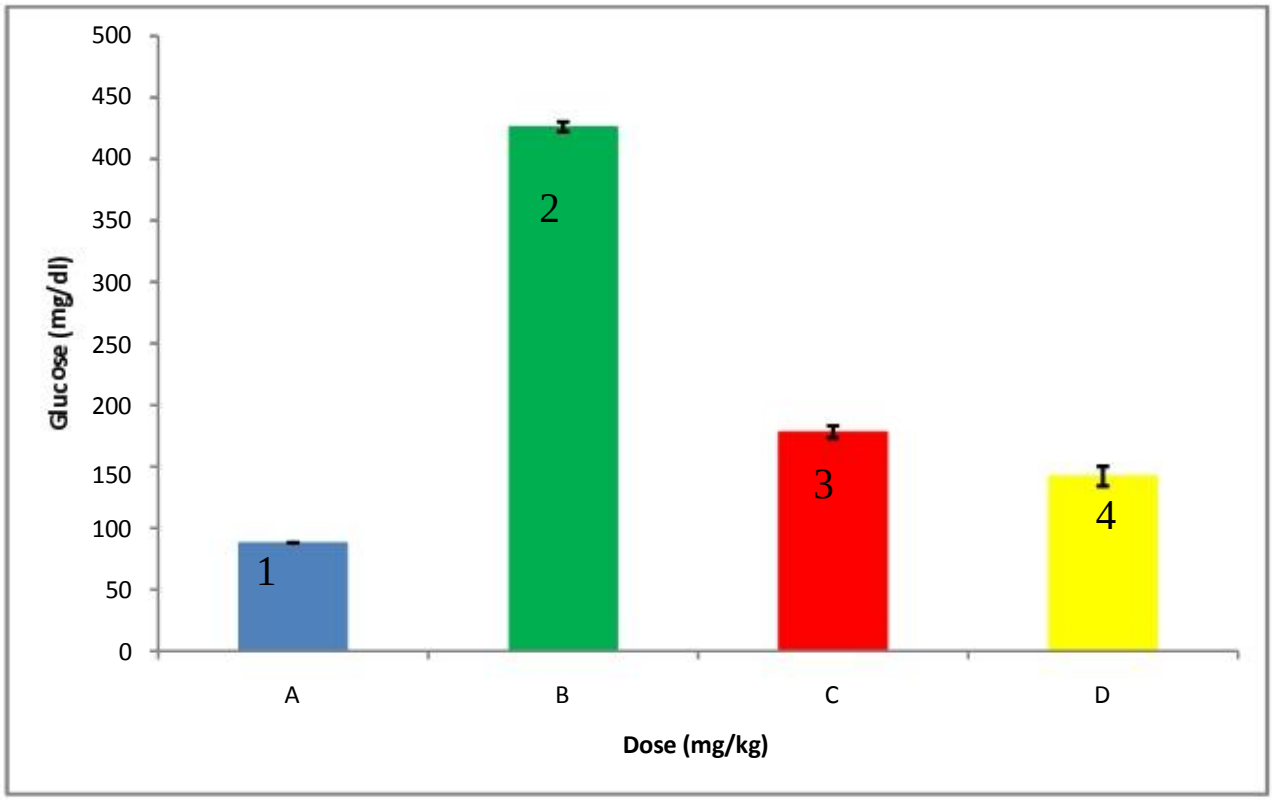
1= Control, 2= diabetic untreated, 3= 200 mg/kg body weight, 4= 400 mg/kg body weight of leaf extract

Figure 4: Effect of ethanol leaf extract of *Ocimum gratissimum* on triacyglycerol level of alloxan-induced diabetic albino rats.



1= Control, 2= diabetic untreated, 3= 200 mg/kg body weight, 4= 400 mg/kg body weight of leaf extract.

Figure 5: Effect of ethanol leaf extract of *Ocimum gratissimum* on glucose level of alloxan-induced diabetic albino rats.



1= Control, 2= diabetic untreated, 3= 200 mg/kg body weight, 4= 400 mg/kg body weight of leaf extract

DISCUSSION AND CONCLUSSION

Diabetes mellitus is a syndrome of impaired carbohydrate, fat and protein metabolism caused by either lack of insulin secretion or decreased sensitivity of the tissues to insulin (Guyton). Diabetes is a serious metabolic abnormality characterized with micro and macro vascular complications that result in significant

morbidity and mortality. This is due to chronic hyperglycemia with disturbance of carbohydrate, fats and protein metabolism resulting from defects in insulin secretion, insulin action or both (World Health Organization, 1999). Diabetes mellitus is a progressive disease and is one of the major killer diseases in recent times. The increasing proportion of aging population, consumption of caloric rich diet, obesity and sedentary lifestyle have led to tremendous increase on the number of diabetic worldwide (Wild *et al.,* 2004) there are over 171 million people or 2.8% of the population predicted to be suffering from this ailment as of 2000 (Wild *et al,* 2004). Some researchers reported that alcoholic leaf extract of *Cinnamomum tamala* produced hypoglycemic activity in alloxan induced diabetic rats when administered orally for two weeks at a dose of 250 mg/kg (Kar *et al.*, 2003). Also, aqueous and ethanolic extract of the fruit-pulp of *Eugenia jambolana* has been reported to produce antihyperglycemic effect in alloxan diabetic rats, and 24.4% raise in plasma insulin level in mild diabetic and 26.3% in severely diabetic rabbits. (Sharma *et al.*, 2006 and Kumar *et al.* 2007) also reported a significant reduction ($p < 0.05$) of the fasting blood glucose levels of alloxan-induced diabetic rats when given ethanolic extract of *Ficus microcarpa* leaves orally for two weeks. The results of the effect of *Ocimum gratissimum* leaf extract on alloxan -induced diabetes in albino rats are presented in Figures 1, 2, 3, 4, and 5 respectively. The levels of LDL-C, TG, and cholesterol in the group 2 was significantly higher ($P < 0.05$) than those in the untreated (group 1). The hypoglycemic action of the *Ocimum gratissimum* extract seen in this experiment may be attributed to a combination of the following: the active principle(s) may have enhanced the activity of residual insulin in the alloxan induced diabetic animals or promoted glucose uptake by peripheral tissues, by other means; it is also possible that the extract slowed down glucose absorption in the gastrointestinal tract (GIT) or regulated the metabolism of glucose by the liver which is in agreement with our work (Twaij and Al-Badr 1988 and Gupta 1994).

The increase in the total cholesterol, triglyceride (TG) and Low density lipoprotein cholesterol (LDL-C) levels of diabetic rats observed in this study are in accordance with earlier report documenting increased plasma TG, LDL and cholesterol level in

diabetic rats (Oberley 1988). Diabetes-induced hyperlipidemia has been reported to be attributable to excess mobilization of fat from the adipose due to underutilization of glucose (Nimenibo-Uadia 2003). The significant reduction in triacylglycerol, LDL-C and total cholesterol levels of the diabetic rats when treated with ethanol leaf extract of *O. gratissimum* in our study is in support of the findings that most hypoglycaemic plants have potentials of ameliorating diabetic lipid metabolism anomalies. This cholesterol lowering effect was earlier reported by some researchers when used as supplementary diet in normal rats for six months. The hypolipidemic effect of O. *gratissimum* could be related to its chemical composition, which shows the presence of alkaloids, flavonoids, saponin and cardiac glycosides. All these components are known to reduce serum lipid level in animals (Ezekwe and Obidoa, 2001). Saponins may lower cholesterol by binding with cholesterol in the intestinal lumen, preventing its absorption, and/or by binding with bile acids, causing a reduction in the enterohepatic circulation of bile acids and increase in its faecal excretion (Rotimi *et al* 2012) The increased bile acid excretion is offset by enhanced bile acid synthesis from cholesterol in the liver and consequent lowering of the plasma cholesterol.

Though the precise mechanism by which the ethanol leaf extract of *Ocimum gratissimum* exerts its hypolipidemic effect is not clearly known by our study, it could not be excluded that the control of glycaemia is a contributing mediator since control of glycaemia is a major determinant of total cholesterol and triglyceride levels (Lenzen 2008). Accordingly, the evolution of glycaemia was parallel to lipid parameters in both the normal control and the diabetic control rats of our study.

CONCLUSION

Administration of ethanol leaf extract of *Ocimum gratissimum* orally for twenty-one (3 weeks) days produced decreased blood glucose level and reduced the lipid imbalances associated with diabetes mellitus in albino rats. Owing to this, *Ocimum gratissimum* has a hypoglycemic effect and may be safe when taken orally.

Investigation of bioactive constituents *Ocimum gratissimum* will be highly recommended such as to produce a cheaper anti-diabetic drugs.

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