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EFFECT OF AQUEOUS EXTRACT OF *OCIMUM GRATISSIMUM* AND *GARCINIA KOLA* ON ALLOXAN-INDUCED DIABETIC WISTAR ALBINO RATS

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ABSTRACT

The effect of aqueous extracts ofOcimumgratissimum and Garciniakola on blood glucose concentration, lipid profile and liver enzymes activities of wistar albino rats was investigated. The study was carried out using fourty four (44) wistar albino rats, divided into eleven (11) groups with four (4) rats in each groups. The rats were administered different concentrations of aqueous extracts of O.gratissimum and G.kolaon daily basis for the period of twenty-one (21) days. Group 1 served as control and they were not administered with any extract. Group 2 and 3 were administered 10% and 15% aqueous extract of O.gratissmum, group 4 and 5 were administered 10% and 15% aqueous extracts of G.kola. Group 6 to 11 were induced with diabetes using a single dose of alloxan (120mg/kg body weight). Group 6 served as diabetic control. Group 7 was treated with metformin (300mg/kg body weight), group 8 and 9 were administered with 10% and 15% aqueous extract of O.gratissimum and group 10 and 11 were administered with 10% and 15% of G. kola. Results showed significant decrease ($P<0.05$) in glucose concentration of rats in group 6 to 11 from 8.75 $\pm$ 0.62 to 6.40 $\pm$ 0.33. The results of the lipid profile showed that there was significant decrease ($P<0.05$) in cholesterol from 3.40 $\pm$ 0.32 to 2.63$\pm$ 0.13. for groups 6, 9, 10 and 11. There was significant increase ($P<0.05$) in HDL from 0.38$\pm$ 0.10 to 0.53$\pm$ 0.05 for groups 6, 8, 9 and 10. at $P<0.05$. There was a significant difference ($P<0.05$) in all the liver enzymes (AST, ALT and ALP) except groups 7 and 8 AST. The study showed that O.gratissimum and G.kola possess hypoglycemic, hypolipidemic and hepatoprotective properties.

Key words: Aqueous extracts, Hepatoprotective effects.

INTRODUCTION

Experimental diabetes in animals has provided a considerable insight into the physiologic and biochemical derangements of the diabetic state (Magarinos and McEwen, 2000; Srinivassan and Ramarao, 2007). Studies have shown that individuals with diabetes have a higher incidence of liver and kidney function abnormalities, as well as formation of free radicals due to glucose oxidation, non enzymaticglycosylation of proteins and subsequent oxidative degradation of glycated proteins, leading to a decline in antioxidant defence mechanism and damage of cellular organelles and enzymes, increase lipid peroxidation and development of insulin resistance (Kangralkar*et al*., 2010; Arora, 2010). Diabetes is associated with profound alteration in serum lipid and

lipoproteinprofile with an increased risk in coronaryheart disease (Arora, 2010). Adequate treatment of diabetes is important. However, population increase, inadequate drug supply, exorbitant cost of treatment and side effects of several on conventional drugs have increased the dependence on plant materials as source of medicine for a variety of ailments, many of which are yet to be scientifically validated (Yaro*et al.*, 2007).

Medicinal plants like *Ocimumgratissimum*and *Garcinia kola* has been ascertained to provide various culinary and medical properties such as antidiabetic properties (Mohammed *et al*., 2007). Therefore, this work was designed to determine the effect of aqueous extract of *Ocimumgratissimum*and *Garciniakola* on liver enzymes, lipid profile and glucose level of alloxan-induced diabetic wistar albino rats. With respect to the antidiabetic properties of both plants, this study sought to know the effect on diabetics. The objective was to investigate the effect of aqueous *Ocimumgratissimum* and *Garciniakola* on liver enzymes, lipid profile and glucose level using the wistar albino rats as experimental model.

MATERIALS AND METHODS

Preparation of Aqueous Extracts

The samples of *Ocimumgratissimum*and *Garcinia kola* were obtained from Mile one market, Port Harcourt, Rivers State, Nigeria. They were properly identified and kept at the herbarium of the Plant Science and Biotechnology Centre of the University of Port Harcourt for reference purposes. The fresh leaves and seeds were carefully plucked, thoroughly washed and air dried. The dried leaves were made into powder by being ground with a ceramic pestle and mortar. 100g of the powder was soaked in 1000ml of distilled water for 48 hrs. The extract was sieved using a muslin cloth and then filtered under suction pressure with a Whatman's filter paper. The residue was discarded and the filtrate was collected concentrated in an evaporating dish using a water bath set at 50^0C. The dried extract was scraped out, weighed, stored in air tight sample bottles and kept in a refrigerator between 2 to 8^0C until required for use.

Induction of Diabetes Mellitus

A single dose of 120 mg/kg body weight of freshly prepared alloxan monohydrate was injected intraperitoneallyto rats in group 6 to 11 to induce type II diabetes. Prior to this, their blood glucose level wasmeasured. After 48 hours, rats that had blood glucose level above 200mg/dl were considered diabetic and used for the study. Thereafter, aqueous leaf and seed extracts of *Ocimumgratisimum*and *Garcinia kola* were resuspended in water and administered orally at concentrations of 150 and 200mg/kg (1:1) body weight rats per day for 21 days.

Experimental Animals

A total of forty four wistar rats **(***Rattusnorvegicus)* weighing 150-275g were obtained from the Department of Biochemistry, University of Port HarcourtAnimal House. They were housed in stainless steel cages (4 rats per cage) and kept in a well-ventilated room. The rats were fed with standard diet (standard rat pellet), (Top Feeds Nig. Ltd) and clean water *ad libitum*.They were marked for easy identification. The standard guidelines for the use of experimental animals (including applying humane actions during sacrifice) were adhered to.

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Effect of Extracts

The rats were divided into eleven groups. At the end of seven days following grouping to allow the rats get used to their new caging situation, 150 and 200mg/kg body weight of aqueous extract of *O.gratissimum*were orally administered to non-diabetic rats in groups 2 and 3 (group 1 was the control for non-diabetic animals) respectively; 150 and 200mg/kg body weight of aqueous extract of *G.kola*were orally administered to non-diabetic rats in groups 4 and 5 respectively. Groups 6 and 7 rats received alloxan (diabetic groups). Group 6 was diabetic control, while Group 7 was administered metformin (diabetic animals treated with standard drug). 150and 200mg/kg body weight of aqueous extract of*O.gratissimum*were administered to diabetic rats in group 8and 9 respectively, while 150 and 200mg/kg of aqueous extract of *G.kola* were administered to diabetic rats in groups 10 and 11 respectively.

Four (4) rats from each group weresacrificed at determined time intervals (24-hr for control and alloxan-treated rats, and 1-,2-,3- week for all groups) after anaesthetics with chloroform, fresh blood drawn by cardiac puncture and serum obtained.

Enzyme Assays

The determination of aspartate aminotransferase (AST) in the serum samples were performed at 37^0C using the Randox test kits by measuring the amount ofoxaloacetatehydrazone formed in the presence of L-aspartate, α-oxoglutarate and 2,4-dinitrophenyl hydrazine as reported by the Ibekwe*et al.,* (2007). For alanine aminotransferase (ALT), L-alanine replaced L-aspartate. The serum activity of ALT was determined using Randox test kits (Reitman *et al.*, 1957). The determination of alkaline phosphataseactivity used the Diagnosticum Rt. Kit and monitored the amount of inorganic phosphate released from p-nitrophenyl phosphate following the procedure of Haussament (1999). The glucose level in the serum was determined using Randox test kits for glucose. The cholesterol level in the blood was determined using the serum of the blood sample and Randox test kits for cholesterol.

The triglycerides in the blood sample was determined using the serum of the blood sample andRandox test kits for triglycerides. The HDL- Cholesterol level in the blood sample was determined using the serum of the blood sample and Randox test kits for HDL-Cholesterol. The serum LDL-Cholesterol (LDL-C) was calculated using Friedewald equation (Friedewald*et al.*, 1972).

Statistical Analysis

The data were statistically analyzed with the one way ANOVA,to test for differences between treatment groups using Statistical Package for Social Sciences (SPSS) version 16, at 95% confidence level. A p value of <0.05 was considered statistically significant. All data were expressed as means ± SD (standard deviation).

RESULTS

The results obtained for the serum levels of the biochemical parameters were represented in tables.

Table 1: Effect of *Ocimumgratissimum*and *Garcinia kola*extract on Liver enzyme activity of normal and diabetic induced wistar albino rats.

Group	**AST (1U/L)**	**ALT (1U/L)**	**ALP (1U/L)**
Normal control	45.25 ± 3.30a	41.25 ± 3.59a	161.50 ± 11.09a
Treated with 10% *Ocimumgratissimum*	42.25 ± 2.06b	38.50 ± 1.29b	149.25 ± 3.30b
Treated with 15% *Ocimumgratissimum*	41.25 ± 2.06b	36.00 ± 1.63a	146.75 ± 3.30b
Treated with 10% *Garcinia kola*	40.00 ± 1.63b	34.75 ± 1.71a	167.00 ± 3.56c
Treated with 15% *Garcinia kola*	39.00 ± 1.83a	34.00 ± 1.83a	166.50 ± 1.29c
Diabetic control	63.00 ± 4.69a	65.50 ± 3.87a	201.00 ± 18.78a
Diabetic and treated with antidiabetic drug (Metformin)	57.25 ± 2.50a	54.00 ± 2.45a	183.25 ± 4.19a
Diabetic and treated with 10% *Ocimumgratissimum*	58.00 ± 1.15a	53.50 ± 1.29a	178.25 ± 3.77c
Diabetic and treated with 15% *Ocimumgratissimum*	54.00 ± 1.63a	48.25 ± 1.26a	175.75 ± 3.30c
Diabetic and treated with 10% *Garcinia kola*	57.00 ± 1.83a	51.50 ± 1.29a	182.50 ± 2.08a
Diabetic and treated with 15% *Garcinia kola*	50.00 ± 1.63c	47.00 ± 0.82c	182.50 ± 2.89a

Values are means ± SD. Values with the different superscripts letter are significantly different at P<0.05 in a column.

KEY: AST = Aspartate Transaminase, **ALT=** Alanine Transaminase.

ALP = Alkaline Phosphatase.

Table 2: Effect of *Ocimumgratissimum*and *Garcinia kola*extract on Liver enzyme activity of diabetic induced wistar albino rats.

Group	**AST (1U/L)**	**ALT (1U/L)**	**ALP (1U/L)**
Diabetic control	63.00 ± 4.69a	65.50 ± 3.87a	201.00 ± 18.78a
Diabetic and treated with antidiabetic drug (Metformin)	57.25 ± 2.50a	54.00 ± 2.45a	183.25 ± 4.19a
Diabetic and treated with 10% *Ocimumgratissimum*	58.00 ± 1.15a	53.50 ± 1.29a	178.25 ± 3.77c
Diabetic and treated with 15% *Ocimumgratissimum*	54.00 ± 1.63a	48.25 ± 1.26a	175.75 ± 3.30c
Diabetic and treated with 10% *Garcinia kola*	57.00 ± 1.83a	51.50 ± 1.29a	182.50 ± 2.08a
Diabetic and treated with 15% *Garcinia kola*	50.00 ± 1.63c	47.00 ± 0.82c	182.50 ± 2.89a

Values are means ± SD. Values with the different superscripts letter are significantly different at P<0.05 in a column.

KEY: AST = Aspartate Transaminase, **ALT=** Alanine Transaminase.

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Table 3: Effect of *Ocimumgratissimum*and *Garcinia kola*extract on glucose level of normal and diabetic induced wistar albino rats.

Group	**Concentration (mmol/l)**
Normal control	5.33 ± 0.56a
Treated with 10% *Ocimumgratissimum*	4.30 ± 0.94b
Treated with 15% *Ocimumgratissimum*	3.88 ± 0.48a
Treated with 10% *Garcinia kola*	3.05 ± 0.59a
Treated with 15% *Garcinia kola*	4.08 ± 0.10a
Diabetic control	8.75 ± 0.62a
Diabetic and treated with antidiabetic drug (Metformin)	5.50 ± 0.43c
Diabetic and treated with 10% *Ocimumgratissimum*	6.80 ± 0.34a
Diabetic and treated with 15% *Ocimumgratissimum*	6.50 ± 0.37c
Diabetic and treated with 10% *Garcinia kola*	6.50 ± 0.34c
Diabetic and treated with 15% *Garcinia kola*	6.40 ± 0.33c

Values are means ± SD. Values with the different superscripts letter are significantly different at P<0.05 in a column.

Table 4: Effect of *Ocimumgratissimum*and *Garcinia kola*extract on glucose level of diabetic induced wistar albino rats.

Group	**Concentration (mmol/l)**
Diabetic control	8.75 ± 0.62d
Diabetic and treated with antidiabetic drug (Metformin)	5.50 ± 0.43a
Diabetic and treated with 10% *Ocimumgratissimum*	6.80 ± 0.34c
Diabetic and treated with 15% *Ocimumgratissimum*	6.50 ± 0.37b
Diabetic and treated with 10% *Garcinia kola*	6.50 ± 0.34b
Diabetic and treated with 15% *Garcinia kola*	6.40 ± 0.33b

Values are means ± SD. Values with the different superscripts letter are significantly different at P<0.05 in a column.

Table 5: Effect of *Ocimumgratissimum*and *Garcinia kola*extract on Lipid profile of normal and diabetic induced wistar albino rats.

GROUP	**CHOL**	**TG**	**HDL**	**LDL**
Normal control	2.43 ± 0.26a	0.73 ± 0.22a	0.58 ± 0.10a	1.48 ± 0.21a
Treated with 10% *Ocimumgratissimum*	2.40 ± 0.16b	0.75 ± 0.17c	0.65 ± 0.06c	1.40 ± 0.22b
Treated with 15% *Ocimumgratissimum*	2.25 ± 0.17b	0.80 ± 0.16c	0.63 ± 0.15c	1.20 ± 0.18b
Treated with 10% *Garcinia kola*	2.48 ± 0.25c	0.80 ± 0.16c	0.60 ± 0.08c	1.50 ± 0.29c
Treated with 15% *Garcinia kola*	2.20 ± 0.18b	0.65 ± 0.13b	0.50 ± 0.08b	1.35 ± 0.13b
Diabetic control	3.40 ± 0.32a	1.78 ± 0.17a	0.38 ± 0.10a	2.20 ± 0.18a
Diabetic and treated with antidiabetic drug (Metformin)	3.03 ± 0.13a	1.20 ± 0.16a	0.53 ± 0.05b	1.85 ± 0.13c
Diabetic and treated with 10% *Ocimumgratissimum*	2.95 ± 0.13a	1.20 ± 0.18a	0.63 ± 0.05c	1.80 ± 0.08c
Diabetic and treated with 15% *Ocimumgratissimum*	2.85 ± 0.13c	1.23 ± 0.13a	0.58 ± 0.05b	1.50 ± 0.48c
Diabetic and treated with 10% *Garcinia kola*	2.75 ± 0.13c	1.38 ± 0.10a	0.58 ± 0.05b	1.53 ± 0.10c
Diabetic and treated with 15% *Garcinia kola*	2.63 ± 0.13c	1.25 ± 0.10a	0.53 ± 0.05b	1.50 ± 0.08c

Values are means ± SD. Values with the different superscripts letter are significantly different at P<0.05 in a column.

KEY: CHOL= Cholesterol, **TG =** Triacylglycerol.**HDL =** HDL –Cholesterol and **LDL =** LDL – Cholesterol.

Table 1.6: Effect of *Ocimumgratissimum*and *Garcinia kola*extract on Lipid profile of diabetic induced wistar albino rats.

Group	**CHOL**	**TG**	**HDL**	**LDL**
Diabetic control	3.40 ± 0.32a	1.78 ± 0.17a	0.38 ± 0.10a	2.20 ± 0.18a
Diabetic and treated with antidiabetic drug (Metformin)	3.03 ± 0.13b	1.20 ± 0.16a	0.53 ± 0.05c	1.85 ± 0.13b
Diabetic and treated with 10% *Ocimumgratissimum*	2.95 ± 0.13b	1.20 ± 0.18a	0.63 ± 0.05a	1.80 ± 0.08b
Diabetic and treated with 15% *Ocimumgratissimum*	2.85 ± 0.13a	1.23 ± 0.13a	0.58 ± 0.05a	1.50 ± 0.48a
Diabetic and treated with 10% *Garcinia kola*	2.75 ± 0.13a	1.38 ± 0.10a	0.58 ± 0.05a	1.53 ± 0.10a
Diabetic and treated with 15% *Garcinia kola*	2.63 ± 0.13a	1.25 ± 0.10a	0.53 ± 0.05c	1.50 ± 0.08a

Values are means ± SD. Values with the different superscripts letter are significantly different at P<0.05 in a column.

KEY: CHOL = Cholesterol, **TG =** Triacylglycerol. **HDL =** HDL –Cholesterol and **LDL =** LDL – Cholesterol.

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DISCUSSION

The significant increase in blood glucose level upon administration with alloxan is evident that diabetes was induced in the rats. The diabetic effect of alloxan has been attributed to a specificcyto-toxic action mediated by hydroxyl radical generation in pancreatic ß-cell, which damages a large number of ß-cellsresulting in a decrease in endogenous insulin release (Szkudelski, 2001). These result in elevated blood glucose within a short period of time after alloxan administration (Milagro and Martinez, 2000, Haidari*et al.*, 2012).

The high concentration of TAG, TC, LDL-Cand HDL-C observed in diabetic rats compared to control rats in this study is consistent with reports of several studies (Pari and Latha, 2002;Nwanjo and Oze, 2007;Akah*et al.*, 2009; Ayinla*et al.*, 2011) demonstrating that a rise in glucose level on induction of diabetes, results in a corresponding increase in plasma lipids. It has been reported that elevated serum lipids in diabetes is due to the increased mobilization of free fatty acids from peripheral fat depots as a result of inhibition of the sensitive lipase (Sharma *et al.*, 2010).

Liver function tests (LFTs) are commonly used in clinical practice to screen for liver disease, monitor the progression of a known disease and determine the effects of potentially hepatotoxic drugs (Harris, 2005). The significant increase in the serum AST, ALP and ALT observed in diabetic rats is consistent with studies by Nwanjoand Oze (2007), indicating liver damage as a result of an increase in hepatic glucose output and a decline in hepatic insulin sensitivity (Harris, 2005). In conclusion, this study has demonstrated that the aqueous extract of *O.gratissimum and G. kola* induced significant reductions in the blood glucose, lipoprotein and liver enzymes of alloxan-induced diabetic rats. If treatment with both medicinal plants is prolonged, the levels of the three parameters will be reduced to levels as low as when antidiabetic drugs is used for treatment.

It is recommended that further research be carried out using *O.gratissimum*and *G. kola* to know the active ingredients that are responsible for the hypoglycemic, hypolipidemic and hepatoprotective effects.

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