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Effects of Aqueous Leaf-Extract of *Ocimum gratissimum* on Lipid Profile and Body Weight of Normal Male Rabbits

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Abstract: The inhabitants of south-east Nigeria are noted for their high consumption of vegetable and spices, which also form part of the ingredients of herbal medicine. In this study, the effects of the aqueous leaf-extract of *Ocimum gratissimum* (African basil) on body weight, serum lipid and some serum liver biomarkers of normal rabbits were investigated. The animals were randomly selected into four groups with five animals per group. A group was administered normal saline (control), while the other three groups were administered 75, 150 and 250mgkg^{-1} of aqueous leaf-extract of *Ocimum gratissimum*. The normal saline and extract were administered orally to the animals twice daily for 21 days. Samples of blood were collected from the ear vein of the animals on days 0, 7, 14 and 21 for determination of serum Alkaline phosphatases (ALP), Alanine Transaminase (ALT), Aspartate Transaminase (AST), Total Bilirubin, Total Cholesterol (TC), High Density Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C) and Triglyceride (TG). The extract showed increase in % changes in serum ALP, ALT and AST, while there was % reduction in serum bilirubin. The LDL-C, TG and body weight also showed % reduction. The results are suggestive of a potential hypolipidemic property of the aqueous extract of *Ocimum gratissimum*.

Key words: Body weight · Hypolipidemic · Lipoprotein · Transaminase · *Ocimum gratissimum*

INTRODUCTION

Poverty by every definition is a hitch to good global health care system. Africa is most hit by this, as the various governments have been unable to provide adequate and affordable health care system for its citizens. To that effect the larger population of Africa and Nigeria in particular hinge on their old companion plants, which has ever provided food, shelter, wealth and has helped to maintain a relatively disease free state on appropriate use as herbal medicine [1]. Southern Nigeria, particularly the south-eastern region is noted for their high consumption of vegetable and spices, which also form part of the ingredients of herbal medicine within the region because of their high secondary metabolite constituents [2]. One of such plants is the *Ocimum gratissimum* Linn which is also called African basil. *Ocimum gratissimum* is native to Africa and Asia belonging to the family lamiaceae. It is found in the tropical regions of Nigeria and described by different local names, but popularly known as 'Scent Leaf' in most part of the country [3]. Though this plant is mostly used as spice, it is also used widely in folk medicine for management of diabetes [4, 5], as anti-diarrhoea [6], mosquitocidal activity [7], as anti-oxidant [8], antifungal activities [9] and antibacterial activities [10].

In this work the effect of the aqueous leaf extract of *Ocimum gratissimum* on serum lipid and some liver serum biomarkers was investigated. Care was taken to monitor the changes in the control animals with a view to comparing them with the extract fed groups.

MATERIALS AND METHODS

Plant Material: Fresh leaves of *Ocimum gratissimum* were harvested from a local farm in Okada, Ovia North-East L.G.A of Edo State, Nigeria. They were sorted, washed, air-dried at room temperature and milled into powder. The cold extraction was carried out using water as solvents. The extract was concentrated to about 10%

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of the original volume using a rotary evaporator (BUCHI, type RE111, Rotavapor).

Experimental Animals: The experimental animals were all male New Zealand rabbits, which weighed between 1.3-1.6kg. The animals were obtained from the Central Animal Facility of Igbinedion University, Okada. The animals were housed in well ventilated cages and allowed 12 hours light and dark cycle for the period of experiment. They were given water and food *ad libitum* throughout the duration of the experiment.

Experimental Design: The animals were randomly selected and grouped. There were a total of four groups with five animals per group. One group was administered normal saline, while the other three groups were administered 75, 150 and 250mgkg^{-1} body weight of aqueous leaf extract of *Ocimum gratissimum*. The normal saline and extract were administered orally to the animals twice daily for the duration of the experiment (21 days). The normal saline group served as control. The body weights of the animals were taken and blood samples were collected from the ear vein before administration and at seven days intervals within the period of experiment.

The experiments and procedures employed in this study were reviewed and approved by the Animal Care Committee of the College of Health Sciences, Igbinedion Univerisity, Okada, Edo State, Nigeria.

Biochemical Assays: Alkaline phosphatases (ALP), Alanine Transaminase (ALT), Aspartate Transaminase (AST) and Total Bilirubin, in serum of investigated rabbits were evaluated using assay kits (Randox Laboratories LTD. United Kingdom BT29 4QY). The determination of serum Total Cholesterol (TC) was determined by method of Searcy and Berquist [11], High Density Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C) and Very Low Density Lipoprotein Cholesterol (VLDL-C) were determined by Friedwald *et al*. [12], while Triglycerides (TG) was estimated by method of Tiez [13].

Statistical Analysis: The results obtained in this work were expressed as mean ± Standard error. The comparison of mean values was analyzed by SPSS (version 16.0) using paired samples T-Test at $p<0.05$ level of confidence.

RESULTS

The results from table 1 show progressive increases in the body weights of rabbits administered different concentrations of aqueous extract of *O. gratissimum* leave and the control. The results were not significant (p□0.05) within the groups when compared with the day 0. The results in table 1 also showed decrease in the body weight of rabbits with increase in the concentration of extract administered that was not significant when compared with the control. When the percentage changes within the groups were considered after 21 days as seen in figure 1, it revealed a concentration dependent reduction in the body weight of 12.75, 7.85 and 4.19% in animals administered 75, 150 and 250mgkg^{-1} respectively when compared with the control which showed 6.00% increase.

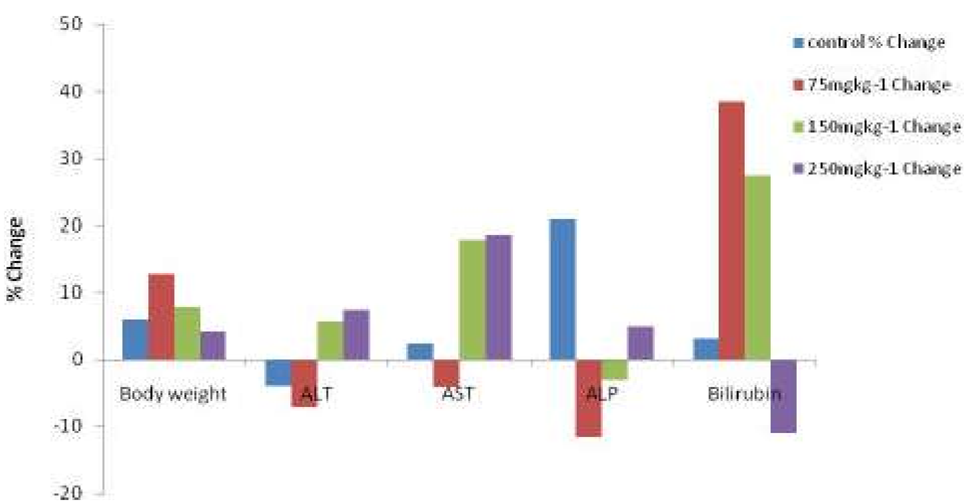

Fig. 1: Percentage changes in serum liver enzymes and body weight of the investigated rabbits administered different concentrations of aqueous extract of *O. gratissimum* after 21 days of experiment

Table 1: The effects of different concentrations of aqueous extract of *O. gratissimum* on body weight (Kg) of investigated rabbits

	Control	75mgkg^{-1}	150mgkg^{-1}	250mgkg^{-1}
Day 0	1.50 ± 0.09	1.49 ± 0.0	1.40 ± 0.08	1.43 ± 0.08
Day 7	1.53 ± 0.08	1.64 ± 0.06	1.55 ± 0.06	1.49 ± 0.08
Day 14	1.54 ± 0.11	1.65 ± 0.05	1.60 ± 0.09	1.41 ± 0.06
Day 21	1.59 ± 0.06	1.68± 0.06	1.51 ± 0.04	1.49 ± 0.05

Values given as mean ± standard error, significant differences (*), when compared with the control ($p < 0.05$).

Table 2: The effects of different concentrations of aqueous extract of *O. gratissimum* on serum ALT (mgdl^{-1}) of investigated rabbits

	Control	75mgkg^{-1}	150mgkg^{-1}	250mgkg^{-1}
Day 0	88.75 ±4.75	95.75 ±3.22	87.00 ±2.37	85.50 ± 3.36
Day 7	86.75±1.96	99.00 ± 2.43	82.25 ± 3.76	82.25 ± 2.21
Day 14	87.25 ± 4.19	94.50 ± 2.43	92.75 ± 1.37	92.25 ± 2.28
Day 21	85.25 ± 4.16	89.00± 3.36	92.00 ± 3.06	91.75 ± 2.67

Values given as mean ± standard error, significant differences (*), when compared with the control ($p < 0.05$).

Table 3: The effects of different concentrations of aqueous extract of O. gratissimum on serum AST (mgdl^{-1}) of investigated rabbits

	Control	75mgkg^{-1}	150mgkg^{-1}	250mgkg^{-1}
Day 0	55.50 ± 3.90	55.00 ± 2.35	56.00 ± 3.03	52.50 ± 3.50
Day 7	61.50 ± 2.30	68.25 ± 3.07	60.00 ± 3.76	63.25 ± 2.18
Day 14	60.50 ± 2.07	61.25 ± 3.99	63.00 ± 1.06	55.00 ± 2.24
Day 21	56.83 ± 3.78	52.75± 3.39	66.00 ± 2.02	62.25 ± 2.49

Values given as mean ± standard error, significant differences (*), when compared with the control ($p < 0.05$).

Table 4: The effects of different concentrations of aqueous extract of *O. gratissimum* on serum ALP (mgdl^{-1}) of investigated rabbits

	Control	75mgkg^{-1}	150mgkg^{-1}	250mgkg^{-1}
Day 0	15.50 ± 1.66	21.25 ± 2.39	20.75 ± 1.7	20.25 ± 1.03
Day 7	16.25 ± 1.25	24.50 ± 3.66	26.25 ± 2.28	27.50 ± 3.50
Day 14	18.50 ± 3.57	23.25 ± 2.69	19.60 ± 1.68	22.50 ± 3.23
Day 21	18.75 ± 2.39	18.75± 2.39	16.25 ± 1.25	21.25 ± 2.39

Values given as mean ± standard error, significant differences (*), when compared with the control ($p < 0.05$).

Table 5: The effects of different concentrations of aqueous extract of *O. gratissimum* on serum Total Bilirubin (mgdl^{-1}) of investigated rabbits

	Control	75mgkg^{-1}	150mgkg^{-1}	250mgkg^{-1}
Day 0	1.53 ± 0.15	1.48 ± 0.32	1.50 ± 0.24	1.80 ± 0.20
Day 7	1.58 ± 0.14	2.35 ± 0.62	1.80 ± 0.52	1.73 ± 0.11
Day 14	1.60 ± 0.30	2.25 ± 0.58	1.83 ± 0.14	1.63 ± 0.20
Day 21	1.58 ± 0.21	2.05± 0.46	1.91 ± 0.60	1.60 ± 0.53

Values given as mean ± standard error, significant differences (*), when compared with the control ($p < 0.05$).

Table 6: The effects of different concentrations of aqueous extract of *O. gratissimum* on serum TC (mgdl^{-1}) of investigated rabbits

	Control	75mgkg^{-1}	150mgkg^{-1}	250mgkg^{-1}
Day 0	55.57 ± 3.84	55.57 ± 3.06	68.08 ±2.61	62.88 ± 2.78
Day 7	51.90 ± 2.69	65.48 ± 3.57	65.78 ± 4.06	66.75 ± 2.91
Day 14	57.75 ± 4.75	64.85 ± 2.43	61.90 ± 3.18	60.15 ± 3.32
Day 21	51.70 ± 2.67	63.88 ± 3.51	63.85 ± 4.13	57.05 ± 3.59

Values given as mean ± standard error, significant differences (*), when compared with the control ($p < 0.05$).

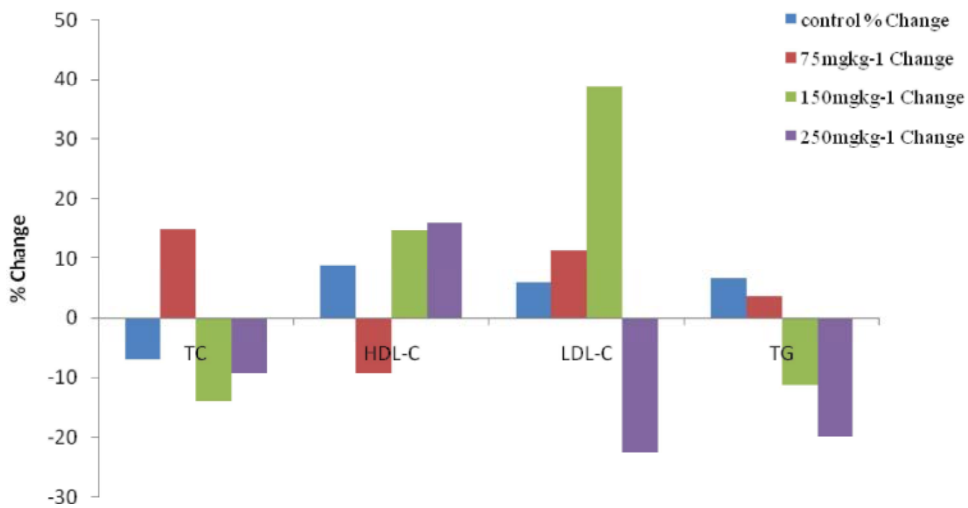

Fig. 2: Percentage changes in serum TC, HDL-C, LDL-C and TG of the investigated rabbits administered different concentrations of aqueous extract of *O. gratissimum* after 21 days of experiment

From table 2, the serum ALT levels did not show any significant (p□0.05) change when compared with day 0 within the groups though there were reductions in serum ALT in the control and 75 $mgkg^{-1}$ groups and increase in the 150 and 250$mgkg^{-1}$ groups. When the serum ALT levels of rabbits administered different concentrations of the extract was compared with the control, no significant change was also observed. The percentage change for each group after 21 days seen in figure 1 showed that the control and rabbits administered 75$mgkg^{-1}$ of the extract ALT was reduced by 3.94and 7.04% respectively. The rabbits administered 150 and 250$mgkg^{-1}$ of *O. gratissimum* leave extract showed concentration dependent increases of 5.75 and 7.31% respectively.

The results of serum AST levels in table 3 showed no significant change when compared within and across the groups. The results revealed a concentration dependent increase of 17.86 and 18.57% in the animals administered 150 and 250$mgkg^{-1}$ of extracts after 21 days of extract administration shown in Figure 1.

Also, the serum ALP levels did not show any significant ($p<0.05$) difference when compared with the control or day 0 as shown in table 4. The percentage changes in serum ALP of rabbits administered different concentrations of the extracts shown in figure 1 seem to be concentration dependent with reductions of 11.76 and 3.11% in animals administered 75and 150$mgkg^{-1}$, which then increased to 4.94% in the 250$mgkg^{-1}$ group.

The results of serum total bilirubin as shown in table 5 did not also show any significant change when compared with results of day 0 within the groups and when compared between extracts groups and control, but in figure 1, there was an initial percentage increase of 38.51% in the 75$mgkg^{-1}$ group. This increase dropped with increase in concentration of *O. gratissimum* leave extract administered at the end of 21 days.

The results of the serum total cholesterol (TC) represented in table 6 showed no significant change ($p<0.05$) when the extracts groups were compared with the control and when compared with day 0 within the groups. From figure 2, the percentage change within the groups after 21 days revealed a 6.96% decrease in serum TC of control. The administration of 75$mgkg^{-1}$ of extracts resulted in 14.95% increase in serum TC, which was reduced by 14.02% and 9.27% in a concentration dependent pattern with the administration of 150 and 250$mgkg^{-1}$ respectively.

The results of serum HDL-C levels in table 7 were found to be a reverse of the results of serum TC. There were no significant changes within and between the groups, but in figure 2, the percentage change after 21 days showed that the control had an 8.82% increase in serum HDL-C. While the 75$mgkg^{-1}$ was reduced by 9.30% and the administration of 150 and 250$mgkg^{-1}$ resulted in a concentration dependent increase in serum HDL-C of 14.81 and 16.03% respectively.

Table 8 represents the results of serum LDL-C levels of rabbits administered different concentrations of *O. gratissimum* leaves extract. This result showed no significant change (p□0.05) when the extract groups were compared with the control and when compared with day 0 within the groups. The percentage changes did not reflect any pattern for the serum LDL-C.

Table 7: The effects of different concentrations of aqueous extract of *O. gratissimum* on serum HDL-C (mgdl^{-1}) of investigated rabbits

	Control	75mgkg^{-1}	150mgkg^{-1}	250mgkg^{-1}
Day 0	29.97 ± 2.89	30.98 ± 1.57	26.13 ± 3.32	24.20 ± 2.44
Day 7	31.22 ± 2.13	29.03 ± 1.14	21.30 ± 1.60	24.97 ± 3.16
Day 14	32.70 ± 0.99	28.05 ± 2.91	25.90 ± 2.60	26.33 ± 3.35
Day 21	32.87 ± 3.31	28.10± 2.97	30.00 ± 2.58	28.08 ± 3.84

Values given as mean ± standard error, significant differences (*), when compared with the control ($p < 0.05$).

Table 8: The effects of different concentrations of aqueous extract of *O. gratissimum* on serum LDL-C (mgdl^{-1}) of investigated rabbits

	Control	75mgkg^{-1}	150mgkg^{-1}	250mgkg^{-1}
Day 0	27.10 ± 4.29	28.78 ± 2.42	26.68 ± 2.04	28.15 ± 3.07
Day 7	25.25 ± 0.95	36.15 ± 4.42	27.30 ± 2.32	25.75 ± 2.14
Day 14	25.75 ± 3.68	32.90 ± 3.53	34.65 ± 3.61	20.45 ± 3.47
Day 21	28.72 ± 2.21	32.05± 1.56	37.07 ± 4.25	21.77 ± 2.66

Values given as mean ± standard error, significant differences (*), when compared with the control ($p < 0.05$).

Table 9: The effects of different concentrations of aqueous extract of *O. gratissimum* on serum TG (mgdl^{-1}) of investigated rabbits

	Control	75mgkg^{-1}	150mgkg^{-1}	250mgkg^{-1}
Day 0	61.65 ± 3.98	69.73 ± 3.93	65.22 ± 2.71	78.08 ± 4.74
Day 7	68.68 ± 0.94	67.63 ± 3.98	68.68 ± 4.08	61.43 ± 3.94
Day 14	60.08 ± 3.19	70.05 ± 3.50	59.07 ± 3.28	63.45 ± 2.62
Day 21	65.80 ± 3.60	72.28± 2.87	57.90 ± 2.83	62.50 ± 3.67

Values given as mean ± standard error, significant differences (*), when compared with the control ($p < 0.05$).

The results of serum TG of rabbits administered different concentrations of aqueous extract of *O. gratissimum* leaves are represented in table 9. There were no significant changes comparing within and between groups. In figure 2, the control and 75mgkg^{-1}groups showed 6.73 and 3.66% increases respectively in serum TG levels after 21 days. The reverse was observed with the administration of higher doses of 150 and 250mgkg^{-1} of the extract resulting in 11.22 and 19.95% concentration dependent reduction respectively.

DISCUSSION

The results of ALT, AST, ALP and bilirubin can be an indicator of the state of health of the liver, defining its functionality and cellular integrity [11-14]. ALT is most prevalent in the liver relative to other tissues, while AST though found in the liver could also be found in the red cells, skeletal and cardiac muscles [15-18]. At lower concentration of 75mgkg^{-1} of administration of *Ocimum gratissimum* extract, serum ALT and AST decreased but increased none significantly with higher doses after 21 days. The concentration dependent percentage increase in serum ALT and AST observed in this work may have been as a result of the liver metabolism of the extract resulting in changes in biochemical processes [19] and or the presence of saponin in the extract would have also been responsible for the increase [20]. These results do not agree with the work of Ephraim *et al*. [15], where there was decrease in serum liver enzymes at higher concentration of the same extract administered.

Alkaline phosphatase is present in tissues throughout the entire body of the animal, but is particularly concentrated in the liver, bile duct, kidney, bone and the placenta [21-24]. ALP is a marker enzyme for the plasma membrane and endoplasmic reticulum and is often used to assess the integrity of plasma membrane [25]. In this work, there was a percentage reduction of the serum ALP levels with the administration of 75mgkg^{-1} after 21 days when compared with the control. The reduced serum ALP levels were alleviated with increase in the concentration (150mgkg^{-1}) of *O. gratissimum* administered to the animals. Upon further increase (250mgkg^{-1}) in concentration of extracts administered there was a further increase in the serum ALP levels. The increase in serum ALP is likely to result from disruption of the phospholipid bilayer of the membrane leading to leakage of the enzyme into the serum [26, 27]. The reduction ALP activity in the liver would hinder the adequate transportation of required ions or molecules across cell membrane and other metabolic processes such as the cleavage of phosphate esters, as well as the synthesis of nuclear proteins, nucleic acid and phospholipids [28-30]. Bilirubin is a catabolic intermediate of haem that is mopped up from plasma by the liver preventing increased plasma level which will result in jaundice [31]. The concentrations of total bilirubin in the serum of rabbits could indicate the state of the liver [32]. The initial increases in total bilirubin levels observed in this study might be an indication of impairment in the functional capacity of the liver as extensive liver damage can lead to an increase in serum bilirubin concentration. It could be a consequence of severe defects in bilirubin transport [31, 32]. It could be suggested that specific components in the plant extracts were capable of protecting the red cell integrity with increase in concentration of the extract administered. Lipid profile is the collective term given to the estimation of typically serum, TC, HDL-C, LDL-C and TG. This profile is a measure of the risk of cardiovascular disease state as demonstrated in conditions as diabetes [1-3]. Dyslipidaemia can range from hypercholesterolemia to hyperlipoproteinemia and is one of the many modifiable risk factors for coronary artery diseases, stroke and peripheral vascular disease [6].

HDL-C, LDL-C and VLDL-C in the plasma form the total cholesterol of the plasma. The total cholesterol does not always give the ideal arteriosclerotic status of an individual as the HDL-C might contribute to high TC [2]. The difference in serum TC levels in the control group and extract groups in this work lies in their percentage reductions after 21 days. This might be attributed to utilization of cholesterol for steroidogenesis [19] at higher doses.

High density lipoproteins (HDL-C) are very important as they possess antiatherosclerotic and anti-inflammatory properties [31]. High levels of expression of apoprotein A-1 (apoA-1) have been found to increase the concentration of HDL-C and protect transgenic mice against diet induced atherosclerosis [21, 25]. To this effect it might be suggestive that the extract may have induced the expression apoA-1 leading to the seeming increase in serum HDL-C at higher doses. Also there is a chance that the increase may have resulted from the ability of the extract components to influence the activities of Lecithin cholesterol Acyl transferase (LCAT), which plays key role in the maturation of HDL-C particles [17].

LDL transports cholesterol mainly to the arterial walls, resulting in the builds up of insoluble lipid on the walls arteries reducing blood flow and increased pressure on the wall as well as the heart. Epidemiological evidence suggests that increased levels of LDL-C and low concentration of high density lipoprotein are associated with atherosclerosis [18]. From the results obtained in this work, it could be inferred the reduction of TG at the highest dose of extract administration resulted in reduction in the LDL-C by inhibition of the activities of lipoprotein lipase responsible for hypertriglyceridemia [20, 24].

In conclusion, the results of this experiment are highly suggestive and revealing as seemingly none significant change across and within groups showed percentage changes that were concentration dependent after 21 day. The pattern shown by the percentage changes may suggest a relationship between body weight and lipid profile. To that effect, the results of this experiment are likely to be more revealing when higher concentrations of *O. gratissimum* extract are administered to animals.

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