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Original Research Article

Anti-obesity Effect of *Ocimum gratissimum leaf* Powder Supplementation *on* High Fat Diet-induced Obesity in Male Wistar Rats

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ABSTRACT

Obesity has been long known as a medical problem that needs to be tackled effectively and efficiently. Obesity raises the chance of acquiring several illnesses, including type 2 diabetes, heart attack, hypertension, hypercholesterolemia, and stroke. This research sought to ascertain the anti-obesity effects of *Ocimum gratissimum* leaf powder supplementation on obesity induced by high fat diet (HDF)in male Wistar rats. Twenty-four male Wistar rats (120 ± 20g) were housed in cages with 6 rats in each cage. Rats were maintained at room temperature during a two-week acclimatization period. Obesity was induced by feeding Groups 2, 3, and 4 with HFD for twelve weeks, while group 1 was fed a normal pellet diet (NPD). After that, the normal control group represented by Group 1 and obese control group by Group 2 were not treated. However, Groups 3 and 4 received HFD supplemented with 10% and 20% of the leaves powdered from *Ocimum gratissimum* respectively for 4 weeks. Feed intake, body weight, adiposity index, total fat mass, and low-density lipoprotein cholesterol were all considerably decreased ($p < 0.05$) by the plant, while high-density lipoprotein cholesterol levels were increased ($p < 0.05$) when compared to the HFD obese control. The *O. gratissimum* leaves contained moderate amounts of some nutrients (magnesium, iron, zinc, and copper) and it decreased the number and size of fat deposit on rat liver. The study indicated that *Ocimum gratissimum* leaves have the potential to reduce body weight and feed intake in animals thus suggesting potent anti-obesity properties.

Keywords: *Supplementation, blood glucose, Ocimum gratissimum, obesity, body weight, appetite*

Introduction

Obesity has been long known as one of the main medical problems that needs to be tackled effectively and efficiently. Obesity is commonly known as the excessive body fat that could be harmful to one's health. Globally, the rate of obesity has almost tripled for adults, and increased five times for adolescent and children since 1975.[1] Obesity makes one susceptible to high blood pressure and abnormal cholesterol levels, which are risk indicators for cardiovascular disease and stroke. Other conditions include type 2 diabetes, a few cancers, digestive problems, gynecological and sexual problems, insomnia, osteoarthritis, dyslipidemia, fatty liver, and heart problems, which have become epidemics in this new civilization.[2] As a medical health disease of abnormal or excessive fat deposition in adipose tissue, overweight and obesity are frequently described in this manner. Foods that are found to contain a high amount of fat are also high in calorie content (energy), and excessive consumption of such foods can lead to an increase in weight and subsequently obesity.[3] This medical condition -as with many others- has been approached in various ways. Drugs such as Sibutramine and Orlistat, medicinal plants, diet, surgery, exercise, and many others are methods used in solving the global problem.[4]

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Considering the negative effects connected with the synthetic medications being generated for the treatment of obesity, there has been a persistent effort to assess the natural substances for their appetite-suppressing activity.[5] Medicinal plants are now in high demand due to their specific qualities as a vast source of medicinal phytochemicals that may lead to the creation of innovative medications.[6]
Traditional medicinal herbs are frequently affordable; (either in raw form or as straightforward therapeutic formulations) it is locally accessible and easily digested. Most of the phytochemicals from plant sources, such as phenolics and flavonoids, have been identified as having a beneficial effect on health and cancer prevention, as well as additional beneficial effects such as antiobesity, anti-diabetic, anti-malarial, antioxidant characteristics, and so on.[7, 8] The Lamiaceae family includes *Ocimum gratissimum*, an aromatic medicinal herb, typically found in the tropics, it is a widely utilized plant species that is used as a food spice.[9] The term "scent leaf" is frequently used to describe it. *Ocimum gratissimum* has been found to contain the phytochemicals alkaloid, tannin, flavonoids (rutin, quercitrin, and luteolin), phytates, oligosaccharides, and steroids.[10]
In other reviews, scent leaves have also been tested for their pharmacological use against diabetes mellitus and hyperglycemia.[11,12] Research was done on extracts of *Ocimum gratissimum* that showed antibacterial activity.[13] According to a study, the natural oil of *O. gratissimum* causes quick, significant hypotension and bradycardia

when administered intravenously, suggesting that the hypotensive action of the oil results in vasodilatory effects directly upon vascular smooth muscle.[14]

In streptozotocin-induced diabetic rats, it was observed that *O. gratissimum* leaf extract has antidiabetic effects.[15] *Ocimum gratissimum* has been found to show anti-pancreatic lipase activity *in vitro*,[10] which has prompted further study of the plant *in vivo* for the treatment of obesity in this study to solve the global problem in a healthier, more efficient, and effective way. Also, because of the unfavorable effects of existing anti-obesity drugs, some of which have been withdrawn from the market, the need for safer, less expensive, and more effective drugs has stimulated studies aimed at using *Ocimum gratissimum* as an anti-obesity medication.

Materials and Methods

Collection and Identification of Plant Material

The source of the plant sample was from Suleja market, Diko, Niger state, Nigeria within February-March, 2020. Its authenticity was determined by Namadi Sanusi at Ahmadu Bello University, Zaria, Kaduna State, Nigeria with Abu01884 allocated as the voucher specimen holding number and a sample was placed in the herbarium. The fresh plant leaves of *Ocimum gratissimum* were dried at 23±2 degree Celsius. Afterward, the dried leaves were blended into powder using an electric blender, then measured, and preserved in a clean glass container.

Experimental Animals

Exactly 24 male Wistar rats (120 ± 20g) were used. They were first obtained from Nitel quarters animal care unit, Sabo, Kaduna, and then transferred to the animal shelter of Bingham University, Nasarawa State, Nigeria. Each cage included six rats kept at 23°C, which is normal temperature with adequate ventilation. The rats were given full access to water and a typical pellet diet (NPD) for the two-week acclimatization period before being divided into separate groups. At Bingham University in Nigeria, the study was executed in accordance with the regulations for using animals (approval No.- BHU/BCH/20/01-28).

Animal Feed Composition

Two sets of feed were given to the experimental animals. A normal pellet diet (NPD) made up the first, while a high-fat diet (HFD) made up the second. The HFD had a higher percentage of fat, whereas the NPD had a higher percentage of carbohydrates (Table 1).

Induction of Obesity

Obesity can be induced in animals by feeding them feeds rich in fat.[16] This feed in particular which was fed to the animals was composed of a majorly normal pellet diet, with the inclusion of butter and fish for the obese groups (groups II, III, and IV). A HFD feeding for 12 weeks made the rats in the obese groups obese, as shown by significant increase in body weight compared to the normal control.[16]

Group I (control) and Group II (obese control) received no treatment (Table 2). Groups III and IV (test groups) were served a high-fat meal containing 10% and 20% of the plant, respectively. All animals were given unlimited access to drinking water and food for the duration of the trial, which lasted four weeks (28 days).

Anthropometric and Biochemical Parameters Determination

Using a weighing balance, the rats' daily food consumption was calculated (with a three-day interval). By deducting the amount of food remainder in every cage from the quantity that was served the previous day (g/day/cage), it was possible to determine the amount of food consumed. In g/day/group, the average daily caloric intake was displayed. After the abdominal incision, each rat had its visceral and retroperitoneal fat (two distinct white adipose depots) removed.[17] The weight in grams (g) of the white adipose tissues after drying on various filter sheets was measured.

Blood and Tissue Sample Collection

At the conclusion of the investigation, the rats underwent fasting over 12 to 14 hours, were sacrificed, and their blood was drawn from their heart chambers or arterial blood vessels into blood-collecting plain bottles. The blood was then centrifuged for 15 minutes at 3500 rpm to extract serum, which was then used for liver function and lipid profile assays. Portions of the liver were removed, blotted dry, weighed, and put in 10% neutral buffered formalin until utilized for histopathology examinations.

Biochemical assays

At days 0, 7, 14, 21, and 28, blood was drawn from the rat tail to test the blood's level of glucose using an Accu-Check glucometer. The levels of triglycerides,[18] total cholesterol,[18] high-density lipoprotein cholesterol (HDL-C),[19] and low-density lipoprotein cholesterol (LDL-C),[20] were measured. The serum levels of very low lipoprotein were determined by applying the formula of Friedewald. The equation is triglycerides/5 used to represent very low-density lipoprotein (VLDL-C).[19] The methods described by Reitman *et al.*[21] was used to measure the enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Histopathological bioassay

For histological analysis, a liver segment that had been cut into pieces was placed in 10% neutral buffered formalin. Hematoxylin and eosin were used to create liver sections, which were then fixed. Light microscopy at x40 magnification was used to examine the permanent mounts.[22]

Elemental Analysis

Ocimum gratissimum leaves were analyzed using atomic absorption spectrophotometry (AAS) at Chemistry Department, Ahmadu Bello University, Zaria. The following elements were quantified: copper, nickel, iron, zinc, manganese, lead, cobalt, and cadmium. Samples were prepared for analysis using the method described by Jackson.[23]Briefly, ash samples in beakers were digested over a steam water bath by adding 10 ml of 6M HCl for 15 minutes, followed by the addition of 1 ml of concentrated HNO_3. Then, the solutions were cooled, filtered, and placed in 50-ml sample bottles for analysis using AAS.

Table 1: Feed composition for experimental rats

Composition	Normal pellet diet (%)	Estimated high-fat diet (%)
Carbohydrate	65.00	35.00
Fat	8.00	50.00
Protein	13.00	12.00
Fiber	12.75	1.75
Vitamin & Mineral mix	1.25	1.25

Table 2: Experimental design

Group	Nutrition	Treatment
I	Normal pelleted diet	Normal control
II	High-fat diet	HFD obese control
III	High-fat diet	10% *O. gratissimum* leaf powder
IV	High-fat diet	20% *O. gratissimum* leaf powder

Results and Discussion

Effect of Ocimum gratissimum on Feed Intake and Body Weight

The feed consumption of rats measured in grams at different time intervals are shown on Table 3. On the fourth week of treatment (day 28), there was a significant decrease ($p < 0.05$) in food intake for only the HFD + 20% of *O. gratissimum* group in comparison to both

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normal and obese control groups. The body weight of HFD fed rats were significantly increased ($p < 0.05$) when compared to the NPD fed rats (Figure 1). In experimental animals, obesity is defined as a significant elevation in body weight and/or a significant rise in Body Mass index (BMI).[24] Thus, the high-fat diet adopted in this study created by 50% fat induced obesity in the test rats. This is similar to earlier documented research whereby obesity is being induced in rats with hypercaloric meals compounded by adding extra fat.[25] Several studies have reported that rats given HFD increased body weight, fat weight, glucose intolerance and insulin resistance in comparison to rats administered a normal diet.[26,27,28]Experimental results revealed that the body weight of the rats in the treated groups (10% and 20% of *O. gratissimum* leaves) were decreased significantly ($p < 0.05$) in contrast to the obese control group. This shows that *O. gratissimum* assists in the reduction of body weight.

Effect of Ocimum gratissimum on Fat Mass, Adiposity Index, and Liver Mass

Table 4 shows the fat mass, adiposity index, and liver mass of the animals. A significant decrease ($p < 0.05$) in the amount of fat of the treated rats in comparison to the obese control was recorded, however, when compared to the normal control it was a significant increase ($p < 0.05$). As for the adiposity index, there was significant decrease ($p < 0.05$) for the rats receiving both 10% and 20% of *O. gratissimum* leaves when compared to the obese control. In terms of liver mass, there was no distinction between the treated rats and the obese control group but when compared to the normal control, the liver mass of the rats receiving treatment was significantly ($p < 0.05$) decreased. The administration of high fat diet to rats led to a significantly higher ($p < 0.05$)white adipose mass and adiposity index of the obese group relative to the normal control group. This phenomenon has been corroborated by other studies.[29] Although, the group of rats treated with 10% of *O. gratissimum* showed a significant reduction in the adiposity index and total fat mass in contrast to the obese control, the group treated with 20% of *O. gratissimum* demonstrated a more positive result. The statistically significant higher adiposity index and fat mass of the obese control in comparison to the normal control validates the results found in other studies.[29, 30]

Effect of Ocimum gratissimum on lipid profile, AST, ALT, and blood glucose

The triglyceride, total cholesterol, LDL-C, and VLDL-C levels in the HFD-obese rats were significantly decreased ($p > 0.05$) compared to the NPD fed rats (Table 6). The *O. gratissimum* treated groups of rats had significantly lower LDL-C and cholesterol levels than the obese control group ($p < 0.05$), but there was no significant change for triglyceride and VLDL-C levels. The HDL-C levels for the 20% *O. gratissimum* leaves was increased significantly ($p < 0.05$) when assessed with respect to the obese and control groups. The *O. gratissimum* leaves had a positive effect on the lipid profile of the animals in a dose-dependent manner. This finding was also demonstrated in a study by Oboh and Ademiluyi to, who found that *O. gratissimum* extract reduced triglycerides, total cholesterol, and LDL-C levels within the blood at the same time as increasing HDL-C levels in a manner that is dose dependent[31]. These changes in lipid profile were attributed to the suppression of inflammation, oxidative stress, and endoplasmic reticulum stress in the liver.

Table 5 shows the activity of ALT and AST in the rats. For ALT activity, the results showed no significant variance ($p > 0.05$) in the rats administered 10% of *O. gratissimum* when compared to obese control but a substantial decrease ($p < 0.05$) in rats treated with 20% of *O. gratissimum* in comparison to the normal control and the obese control. Regarding AST activities, no significant ($p > 0.05$) change was recorded between the groups.

AST and ALT are enzymes proven to be good markers for hepatotoxicity evaluation.[32] Elevated ALT and AST activities are connected to the hepatic accumulation of fat, which contributes to obesity.[33, 34] The group that ingested 20% of *O. gratissimum* revealed significantly decreased ($p < 0.05$) ALT activity which indicated increased metabolism of fat. However, the ALT levels of the obese group were elevated in comparison to the normal control group.

Figure 2 illustrates the glucose levels of experimental animals which were measured at different time intervals. In comparison to the normal control, the glucose levels of the obese untreated rats increased ($p < 0.05$) significantly, but the blood glucose levels of the 10 and 20% *O. gratissimum* treated rats were significantly ($p < 0.05$) decreased compared to the obese control rats. The reduction in the levels of glucose of the treated rats might indicate that glucose tolerance was enhanced by *O. gratissimum*. Some studies have shown that *O. gratissimum* leaves may have hypoglycemic effects and might be advantageous for those with diabetes or at risk of acquiring the illness.[35, 36, 37]

Effect of O. gratissimum on Liver Histopathology

Few small fat deposits were present on the livers of the rats in the normal control group (Figure 3). Large and numerous fat deposits were discovered in the rats' liver of the obese control. This finding shows similar conclusion to what was obtained in a study done in 2019. [30] However, rats given 10% and 20% *O. gratissimum* leaf supplementations had tiny and few fat deposits in their livers indicating a possible reversal of fat deposits.

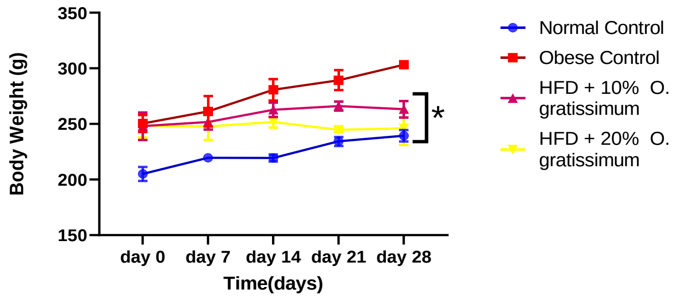

Figure 1: Weekly body weight of the animals. Values are expressed as means ± SEM. The symbol (*) shows that the other groups are significantly different from the obese control.

Table 3: Weekly feed intake of the animals (g) of rats

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
Normal control	87.33± 1.73[a]	90.00± 1.15[a]	86.13± 1.15[b]	87.03± 1.15[b]	89.20± 0.58[a]
Obese control	82.00± 1.15[ab]	85.27± 1.15[ab]	94.07± 1.15[a]	87.03± 1.15[b]	90.33± 1.15[a]
HFD + 10% *O. gratissimum*	80.00± 1.15[b]	85.03± 2.89[ab]	88.20± 1.15[a]	86.27± 1.15[a]	87.07± 1.73[a]
HFD + 20% *O. gratissimum*	83.13± 1.73[ab]	79.37± 1.73[b]	75.40± 1.73[b]	79.47± 1.15[b]	80.37± 1.15[b]

Data is presented as Mean ± SEM. Means having different letter(s) in the same column are significantly different ($p < 0.05$).

Table 4: Fat mass, adiposity index, and liver mass (g) of rats

Groups	Fat mass (g)	Adiposity index	Liver mass (g)
Normal control	2.24 ± 0.37[c]	0.94 ± 0.16[c]	8.78 ± 0.23[a]

Obese control	8.43 ± 0.55[a]	2.78 ± 0.15[a]	7.27 ± 0.22[b]
HFD + 10% *O. gratissimum*	4.78 ± 0.54[b]	1.81± 0.18[b]	6.55 ± 0.33[b]
HFD + 20% *O. gratissimum*	4.64 ± 0.16[b]	1.89 ± 0.10[b]	7.27 ± 0.21[b]

Data is presented as Mean ± SEM. Means having different letter(s) in the same column are significantly different ($p < 0.05$).

Ocimum gratissimum has been reported to consist of flavonoids and saponins [10]which have pancreatic lipase-inhibiting action that prevents the conversion of fats into absorbable loose fatty acids and monoglycerides. Since less of the ingested fat is absorbed, there is a corresponding overall decrease in calories being absorbed, and fats being deposited on the liver, and consequently results in weight loss. The absence of any noticeable damage of the liver of treated rats attests to the hepatoprotective properties of *O. gratissimum.*[38]

Elemental Analysis

Table 7 shows the elemental analysis results of *O. gratissimum* leaves. The *O. gratissimum* leaves contained lower amounts of some heavy metals (lead, cobalt, and nickel) and moderate amounts of some nutrients (magnesium, iron, zinc, and copper) in comparison to the WHO limits for food substances. However, cadmium was not detected in the plant. The absence of heavy metals in the plant under investigation suggests that the animals consuming it were not at risk of heavy metal toxicity. Our study found that the plant did not contain detectable levels of heavy metals, including lead, cadmium, and mercury, which are known to accumulate in the food chain and pose a health risk to animals.[39]

Iron was, however, found to be present in relatively large amounts in the *O. gratissimum* leaves. Iron is an essential mineral that has a major role in several physiological functions, including oxygen transport, energy synthesis, and immunological function. Also, research has shown that a high-fat diet supplemented with iron declines body weight gain and both plasma and hepatic lipid accumulation in mice.[40]

Table 5: Effect of *O gratissimum* on liver function status

Groups	ALT (U/I)	AST (U/I)
Normal control	65.33 ± 1.20[b]	43.33 ± 1.86[a]
Obese control	76.50 ± 9.36[a]	46.67 ± 3.18[a]
HFD + 10% *O. gratissimum*	72.33 ± 0.88[a]	41.00 ± 3.21[a]
HFD + 20% *O. gratissimum*	45.17 ± 0.73[c]	47.00 ± 0.58[a]

Data is presented as Mean ± SEM. Means having different letter(s) in the same column are significantly different ($p < 0.05$).

Conclusion

In conclusion, *Ocimum gratissimum* has proven to have the capacity to lower body fat and weight, blood glucose levels as well as improving lipid profile and reversing fatty liver. Further research is required to know the mechanism by which *O. gratissimum* leaves could function as a weight-reduction agent.

Conflict of interest

There were no conflicts of interest that could have influenced the design, implementation, or interpretation of the study results.

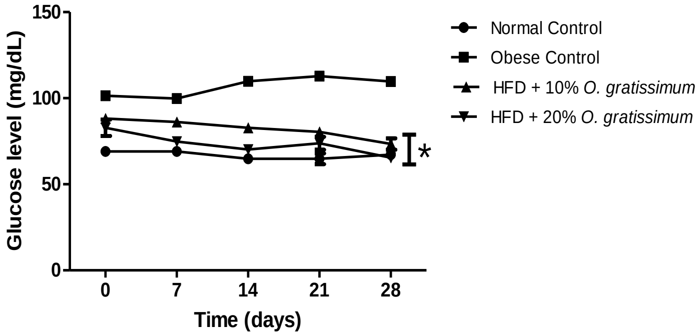

Figure 2: Effect of *O. gratissimum* on blood glucose levels
NB: The * shows that the other groups are significantly different ($p < 0.05$) from the obese control

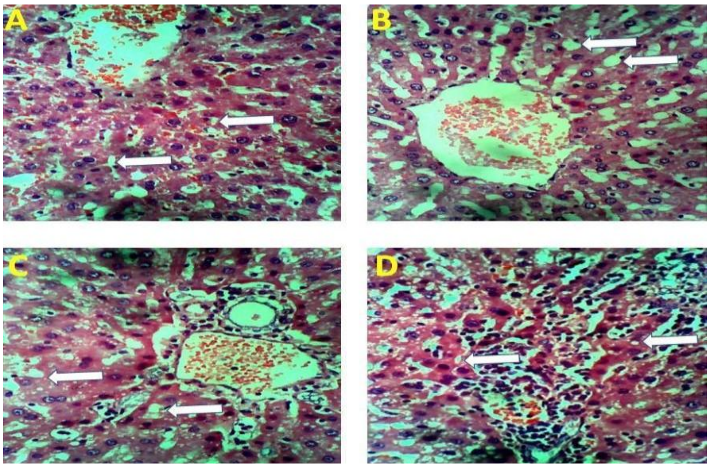

Figure 3: The photomicrographs of liver samples of the animals. (A) Normal Control - few and small fat deposits; (B) Obese Control –numerous and large fat deposits; (C) HFD + 10% of *O. gratissimum* - numerous and small fat deposits; (D) HFD + 20% of *O. gratissimum* – few and small fat deposits. Magnification X40.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

Table 6: Effect of *O. gratissimum* on lipid profile

Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-C (mg/dl)	VLDL-C (mg/dl)	LDL-C (mg/dl)
Normal control	135.56 ± 6.40[b]	105.16 ± 2.61[b]	27.17 ± 4.61[b]	21.03 ± 0.52[b]	84.52 ± 9.56[b]
Obese control	188.69 ± 6.15[a]	134.27 ± 4.43[a]	26.85 ± 0.03[b]	26.85 ± 0.89[a]	141.37 ± 7.02[a]
HFD + 10% *O. gratissimum*	148.07 ± 8.85[b]	134.08 ± 0.65[a]	27.49 ± 1.51[b]	25.62 ± 0.33[a]	90.99 ± 11.49[b]
HFD + 20% *O. gratissimum*	134.70 ± 4.45[b]	128.08 ± 1.64[a]	40.30 ± 11.45[a]	26.82 ± 0.13[a]	81.04 ± 4.36[b]

Data is presented as Mean ± SEM. Means having different letter(s) in the same column are significantly different ($p < 0.05$).

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Table 7: Concentration of elements in *O. gratissimum* leaves

Name of elements	**Concentration of elements in plant (mg/kg)**	**WHO standards for food substances (mg/kg)**
Heavy metals		Max limit for food substances
Cd	ND	0.06
Pb	0.76 ± 0.0025	1.4
Co	0.13 ± 0.0010	0.5
Ni	0.206 ± 0.0015	1.7
Nutrients		Recommended daily intake(mg/kg)
Fe	8.49 ± 0.0056	5.0
Mn	2.09 ± 0.0007	-
Zn	0.81 ± 0.0015	0.38-23.00
Cu	0.53 ± 0.0011	0.40-6.20

ND- not detected; Values are expressed as means ± SD.

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