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The Synergistic Effect of *Ocimum gratissimum* and *Gongronema latifolium* Extracts on Lipid Profile of hyperlipidemic induced rats.

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Abstract

Accumulation of excess fat has great adverse health implications, including tissue damage and disorders affecting the liver, brain, bone marrow, and other vital organs. Cardiovascular diseases are known to be strongly associated with increased level of blood lipids. To mitigate this risk, there is a growing interest in exploring natural interventions, such as herbal extracts. This study aimed to evaluate the synergistic effects of *Ocimum gratissimum* and *Gongronema latifolium* extracts on the lipid profile of hyperlipidemic rats, addressing the increasing concern of hyperlipidemia-related health issues. This research involved the collection of fresh leaves from *Ocimum gratissimum* and *Gongronema latifolium*, which were subsequently processed to obtain plant extracts. The experiment had five groups: Group A was the normal control, Group B represented the hyperlipidemic control, Group C received *Ocimum gratissimum* extract, Group D was given *Gongronema latifolium* extract, and Group E received a combination of both extracts. These extracts were administered orally to hyperlipidemic rats, and their effects on lipid parameters were closely monitored. The lipid profile parameters

were expressed as mean ± standard deviation, analyzed using one-way ANOVA. A post Hoc test was employed to separate the comparison of mean. The study included key lipid indicators including low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TC), and triglycerides (TG). The findings revealed substantial improvements in the lipid profiles of rats treated with the combined extracts of *Ocimum gratissimum* and *Gongronema latifolium*. Notably, there was a significant reduction ($p<0.05$) in LDL, TC, and TG levels, while HDL levels were elevated. The results suggest that these plant extracts possess the potential to ameliorate hyperlipidemia and consequently promote cardiovascular health. In conclusion, this research highlights the promise of *Ocimum gratissimum* and *Gongronema latifolium* extracts as natural interventions for managing hyperlipidemia. However, further studies with larger sample sizes, extended treatment durations, and dose-response investigations are warranted to provide a more comprehensive understanding of the effects and mechanisms of these extracts.

Keywords: Synergistic, extract, hyperlipidaemic, induced, Latifolium.
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Introduction

According to research cited by Natesan and Kim (2021), the accumulation of excess fat (lipids) has far-reaching health implications, including tissue damage and disorders affecting various organs such as the liver, brain, bone marrow, peripheral nervous system, and spleen. Dyslipidemia, characterized by abnormal lipid levels, is linked to impairments in the immune, respiratory, and cardiovascular systems, as well as elevated levels of proinflammatory cytokines (Sun *et al*., 2020). Moreover, dyslipidemia is causally associated with an elevated risk of thrombotic complications, endothelial dysfunction, and increased platelet activity (Sorokin *et al*., 2020). It stands as a significant and modifiable risk factor for cardiovascular disease (Dong *et al*., 2021).

Lipids, including phospholipids, cholesterol, triglycerides (TG), and fatty acids, are vital components of cell membrane and therefore play crucial roles in the human body as highlighted by de Freitas *et al*. (2013). They constitute the fundamental structure of cell membranes, serve as precursors to important compounds like steroid hormones, bile acids, and vitamin D, among other essential functions (de Freitas *et al*., 2013). Maintaining a balance of lipid levels in the bloodstream is imperative for overall health, as emphasized by Ahmed *et al*. (2023). Dyslipidemia, characterized by abnormal blood lipid levels, leads to the deposition of fats in artery walls, instigating vascular complications (Natesan and Kim, 2021). Notably, diabetes mellitus, kidney disorders, liver diseases, alcoholism, stress and hypothyroidism are key players in the pathogenesis of hyperlipidemia (Natesan and Kim, 2021). Excessive lipids readily adhere to the inner walls of blood vessels, fostering the development of atherosclerosis-related conditions such as stroke or heart attack (Nelson, 2013). The rise in lipid metabolism-associated diseases has prompted global concern, necessitating further research into their diagnosis and treatment approaches (Monnerie *et al*., 2020).

Furthermore, the utilization of medicinal herbs in traditional medicine for prevention and treatment of various ailments dates to the dawn of humanity (Ekweogu *et al*., 2020). It's estimated that roughly 80% of the global population primarily relies on ethnomedicine or herbal medicine to address a multitude of ailments worldwide (Pant, 2014). Interestingly, herbal medicine use has tremendously increased over the conventional medicine, probably due to their less toxic active organic contents as well as their affordability and availability. (Ijioma *et al*., 2021). Moreover, medicinal herbs have garnered considerable attention from researchers probably due to their bioactive contents and their efficacies in prevention and management of chronic and life-threatening diseases (WHO, 2019). Presently, these medicinal plants are not only utilized for treating various conditions but also serve as a reservoir of novel drugs for traditional or mainstream medicine (Ugbogu *et al*., 2021). Examples of such plants include *Ocimum gratissimum* and *Gongronema latifolium*, both of which are analyzed in this study.

Ocimum gratissimum, commonly known as scent leaf, has been identified as a medicinal plant with the potential to offer an alternative therapy for various ailments or to serve as a source for new drug development (Ugbogu *et al*., 2021; Enyievi *et al*., 2021). Belonging to the Lamiaceae family, this plant is indigenous to Africa, Asia, and South America (Akara *et al*., 2021) and is commonly cultivated in gardens around village huts in Nigeria for its medicinal and culinary uses.

In culinary practices, *O. gratissimum* serves as a natural spice and flavoring agent for food preparation (Akara *et al*., 2021). Medicinally, it is utilized in traditional medicine to treat numerous conditions including ailments of viral, bacterial and mycotic origins. (Akara *et al*., 2021). Scientific studies have revealed that *O. gratissimum* contains a wide array of bioactive compounds including flavonoids and polyphenols (Irondi *et al*., 2016), as well as essential oils with multiple beneficial effects (Melo *et al*., 2019; Obianime *et al*., 2011).

On the other hand, *Gongronema latifolium*, commonly known as "utazi" in Igbo tribe of Nigeria, is a perennial edible plant with a pliable stem and nutritious leaves, full of vitamins, essential minerals and protein (Ugochukwu and Babady, 2003; Eleyinmi, 2007). It is frequently used in soups as a vegetable, dried and applied as a powdery spice, or consumed fresh and incorporated into salad preparations (Balogun *et al*., 2016). In Nigeria, it is employed in the treatment of cough, dyspepsia, viral hepatitis, mycotic and other microbial infections (Morebise, 2015). Widely utilized in the West African sub-region, *Gongronema latifolium* serves various medicinal and nutritional purposes (Adaramola-Ajibola *et al*., 2017).

Currently, the treatment landscape for dyslipidemias is broadening, with emerging options such as monoclonal antibodies targeting proprotein convertase subtilisin/kexin type 9 (PCSK9) for managing hypercholesterolemia (Berberich and Hegele, 2019). In Europe, volanesorsen, which focuses on apo C-III, has gained approval for managing familial chylomicronemia syndrome (FCS) (Berberich and Hegele, 2019). In North America, bempedoic acid, evinacumab, and inclisiran have received approval for various indications related to LDL-C reduction. Additionally, numerous other agents are in advanced clinical development, including those targeting novel markers like Lp(a) and angiopoietin-like protein 3 (ANGPTL3) (Berberich and Hegele, 2019). Given these advancements, it is pertinent to explore treatment avenues for dyslipidemia, extending into the realm of medicinal plants. This approach would expand the spectrum of options for managing dyslipidemia and help mitigate associated complications. Therefore, the present study aims to assess the combined anti-lipidaemic effects of *O. gratissimum* and *G. latifolium* against this backdrop.

Materials and Methods

Preparation of plant extracts

The leaves of the plant samples underwent washing, followed by air-drying at room temperature, and were subsequently processed into a pulp form. The ground pulp of both plant leaves (80g each) was soaked separately in 250ml of methanol for 72 hours to extract the active components. Whatman No. 1 filter paper purchased from Cjay reagent store, Abakaliki was used to filter the resulting mixture. These filtrates were then concentrated at low temperature (37˚C - 40˚C) to approximately one-tenth of their original volumes. The concentrates were left to evaporate completely in an open water bath (40˚C) and then stored refrigerated at 2˚C - 8˚C until needed.

Experimental Animals

A total of twenty-five (25) healthy Wistar rats were recruited for the experiment and were housed and maintained in metal cages under standard conducive laboratory conditions. They were all fed with rat chow and were allowed to be acquainted with the environment for seven days before starting the experiment.

Induction of Hyperlipidaemia

Hyperlipidaemia was induced following the descriptions of Karam *et al*., 2018. Commercially available high-fat diet (comprising 41.5% lipids, 40.2% carbohydrates and 18.3% proteins (kcal)) were fed to the rats, except the normal group. After the first 7 days of feeding with a high-fat diet, hyperlipidaemia was confirmed by collecting blood samples from the rat tail after having fasted for 12 hours prior to blood sample collection. The serum levels of LDL, HDL, TC and TG before induction and 7 days after induction were compared to confirm hyperlipidaemia.

Experimental Protocol

According to the protocol by Enyievi *et al.*, 2020, dimethylsulphoxide (DMSO) of 20% concentration was used to dissolve the plant extract before use, and treatment was administered via oral intubation at a dose of 400 mg/kg of body weight twice daily. The animals were divided into five groups, each consisting of five rats, as described below: Group A served as the normal control group, where animals were not fed a high-fat diet; instead, they were provided with rat chow and water.

Group B represented hyperlipidemic control, receiving a daily administration of 2.0ml of distilled water.

Group C consisted of hyperlipidemic rats receiving daily doses of *Ocimum gratissimum* extract.

Group D comprised hyperlipidemic rats receiving daily doses of *Gongronema latifolium* extract.

Group E included hyperlipidemic rats administered a combined dose of 200 mg/kg of *Ocimum gratissimum* and 200 mg/kg of *Gongronema latifolium* extracts daily.

Measurement of Body weight

Using an electronic top loading balance, the rats were weighed and rounded off to the nearest whole number. With the formulae, final weight – initial weight, the body weight gain and loss in grams were determined.

Blood Sample collection and Analyses

The administration of the extracts and the animal experiment continued for a duration of 28 days. At the conclusion of the administration period, the animals underwent a 12-hour fast with unrestricted access to water before being euthanized. Using a 5 ml syringe, blood samples were gotten through cardiac puncture. Serum from collected samples were obtained by centrifugation for 10 min at 3,000 rpm. The serum was carefully pipetted into clean sample containers and stored in a refrigerator at 4°C until it was ready for biochemical analysis.

Lipid Profile estimation

Using Randox Analytical kits made in UK, parameters such as low-density lipoprotein-cholesterol (LDL-c) and very low-density lipoprotein-cholesterol (VLDL-c): Total cholesterol, Triacylglyceride and High-density lipoprotein cholesterol (HDL-c) were estimated following the manufacturer's protocol.

Data Analysis

The lipid profile parameters were expressed as mean ± standard deviation, analyzed using one-way ANOVA. A post Hoc test was employed to separate the comparison of mean.

Results

The objective of the study was to examine the combined effects of *Ocimum gratissimum* and *Gongronema latifolium* extracts on lipid profiles in a hyperlipidemic rat model. In the experimental setup, Group A functioned as the normal control group and was provided with a standard rat chow and water diet without any high-fat content. Group B served as the hyperlipidemic control and received a daily dose of 2.0ml of distilled water. Group C consisted of hyperlipidemic rats receiving daily doses of *Ocimum gratissimum* extract. Group D comprised hyperlipidemic rats treated with *Gongronema latifolium* extract daily. Group E involved hyperlipidemic rats receiving a combined treatment of 200 mg/kg of both *Ocimum gratissimum* and *Gongronema latifolium* extracts every day for a duration of 28 days.

Table 1: Effects of the Extracts on Weight

Group	Mean Initial Weight (g)	Mean Final Weight (g)	Mean Weight Difference (g)	P-Value
A (Normal Control)	250 ± 6.8	255 ± 7.2	5 ± 2.0	-
B (Hyperlipidemic Control)	275 ± 5.1	280 ± 5.4	5 ± 3.3	-
C (*Ocimum gratissimum* Extract)	260 ± 3.3	245 ± 5.2	- 15 ± 4.1	0.001*
D (*Gongronema latifolium* Extract)	263 ± 4.1	250 ± 3.8	-13 ± 5.3	0.005*
E (Combined Extracts)	258 ± 3.6	240 ± 4.6	- 18 ± 6.6	0.001*

$p < 0.05$ is considered statistically significant

* p-values are presented in comparison to hyperlipidaemic control (Group B)

Table 2: Effects of the Extracts on LDL Levels

Group	Mean LDL (mg/dL)	Standard Deviation	P-Value
A (Normal Control)	107.50	5.01	-
B (Hyperlipidemic Control)	171.25	6.19	-
C (*Ocimum gratissimum* Extract)	138.25	4.20	0.001*
D (*Gongronema latifolium* Extract)	143.50	3.04	0.005*
E (Combined Extracts)	132.50	3.31	0.001*

$p < 0.05$ is considered statistically significant

* p-values are presented in comparison to hyperlipidaemic control (Group B)

Table 3: Effects of the Extracts on HDL Levels

Group	Mean HDL (mg/dL)	Standard Deviation	P-Value
A (Normal Control)	53.50	2.44	-
B (Hyperlipidemic Control)	31.50	1.13	-
C (*Ocimum gratissimum* Extract)	43.75	2.04	0.003*
D (*Gongronema latifolium* Extract)	38.75	2.22	0.012*
E (Combined Extracts)	47.00	1.50	0.002*

$p < 0.05$ is considered statistically significant

* p-values are presented in comparison to hyperlipidaemic control (Group B)

Table 4: Effects of the Extracts on Total Cholesterol Levels

Group	Mean TC (mg/dL)	Standard Deviation	P-Value
A (Normal Control)	162.50	2.50	-
B (Hyperlipidemic Control)	211.25	2.75	-
C (*Ocimum gratissimum* Extract)	178.25	2.41	0.007*
D (*Gongronema latifolium* Extract)	184.25	2.36	0.019*
E (Combined Extracts)	172.50	1.75	0.004*

$p < 0.05$ is considered statistically significant

* p-values are presented in comparison to hyperlipidaemic control (Group B)

Table 5: Effects of the Extracts on Triglyceride Levels

Group	Mean Triglyceride (mg/dL)	Standard Deviation	P-Value
A (Normal Control)	85.75	3.25	-
B (Hyperlipidemic Control)	126.25	3.50	-
C (*Ocimum gratissimum* Extract)	98.75	3.08	0.008*
D (*Gongronema latifolium* Extract)	101.25	2.75	0.014*
E (Combined Extracts)	93.01	2.35	0.003*

$p < 0.05$ is considered statistically significant

* p-values are presented in comparison to hyperlipidaemic control (Group B)

Discussion

The main aim of this investigation was to explore the potential synergistic effects of *Ocimum gratissimum* and *Gongronema latifolium* extracts on lipid profiles in a hyperlipidemic rat model. Our results unveiled notable alterations in key lipid profile parameters, including LDL, HDL, total cholesterol, and triglycerides, highlighting the therapeutic potential of these plant extracts.

One significant finding was the reduction in LDL levels (Table .2) observed within the treatment groups (Groups C, D, and E) compared to the hyperlipidemic control group (Group B). These reports confirmed the previous research by Enyievi et al., on the LDL-lowering effects of *Ocimum gratissimum* (Enyievi *et al*., 2020) in diabetic rats, and the decrease in LDL concentrations following the administration of *Gongronema latifolium* extracts, as noted by Beshel *et al*. (2019), consistent with Onwudiwe *et al*.'s (2023) findings.

Of particular interest was the substantial LDL reduction observed in Group E (Table 2), where the combination of *Ocimum gratissimum* and *Gongronema latifolium* extracts was administered, suggesting a potential synergistic effect. The significant differences in LDL levels between Group E and the hyperlipidemic control group underscore the therapeutic promise of this combination in managing hyperlipidemia. While both individual extracts (Groups C and D) showed beneficial effects, the combination appeared to yield more pronounced results.

Additionally, a noteworthy increase in HDL levels, particularly in Group E (Table 3), was observed. Elevated HDL levels are associated with reduced cardiovascular disease risk, which aligns with previous research showing increased HDL concentrations in extract-treated groups, as reported by Akara *et al*. (2021) and Ugbogu and Akara (2020).

Total cholesterol levels, crucial in assessing cardiovascular health, exhibited significant reductions in the treatment groups (Table 4), consistent with prior studies. Group E displayed the most notable decrease, indicating that the combination of extracts may effectively manage total cholesterol levels. Triglyceride levels, another important component of lipid profiles, showed a significant decrease in all treatment groups, with Group E exhibiting the most substantial reduction (Table 5), consistent with previous research.

One of the most striking observations was the apparent synergistic effect of the combined extracts, resulting in significant reductions in LDL, triglycerides, and total cholesterol levels, along with an increase in HDL levels in Group E. This suggests complementary mechanisms of action between the two plant extracts, potentially influencing different aspects of lipid metabolism.

Overall, the observed changes in lipid profiles, including lower TC, TG, LDL, and VLDL, alongside higher HDL, are generally considered favorable for cardiovascular health. Elevated levels of TC, TG, LDL, and VLDL are typically associated with an increased risk of heart disease, while higher HDL levels are associated with a lower risk. Therefore, our study suggests that *Gongronema latifolium* extract may have a positive impact on lipid profile parameters and may be beneficial for managing conditions related to high lipid levels in the blood, such as hyperlipidemia.

Conclusion

This study has revealed the potential synergistic effects of *Ocimum gratissimum* and *Gongronema latifolium* extracts on lipid profiles in a hyperlipidemic rat model. In general, the observed alterations in lipid profiles, characterized by decreased TC, TG, LDL, and VLDL levels, along with increased HDL levels, suggest an enhancement in cardiovascular health. The findings imply that *Ocimum gratissimum* and *Gongronema latifolium* extracts may hold promise in positively modulating lipid profile parameters, offering a potentially valuable intervention for conditions associated with elevated blood lipid levels, such as hyperlipidemia. This research contributes to the understanding of the potential benefits of these plant extracts in managing hyperlipidemia and promoting cardiovascular well-being. Further studies are needed to delve into the underlying mechanisms and establish the clinical efficacy of these extracts as potential therapeutic agents in lipid-related conditions.

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