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DOSE-RESPONSE TOXICOLOGICAL EVALUATION OF OCIMUM GRATISSIMUM: DETERMINATION OF SAFE THERAPEUTIC LEVELS IN WISTAR RATS

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ABSTRACT

Ocimum gratissimum is widely recognized in traditional medicine for its therapeutic properties, yet detailed toxicological evaluations are sparse. This study assessed the dose-dependent toxicological profile of aqueous Ocimum gratissimum leaf extract by evaluating biochemical and hematological parameters in male Wistar rats subjected to sub-chronic administration. Thirty adult male Wistar rats were assigned to five groups (n = 6): one control group and four treatment groups receiving 100, 300, 600, and 900 mg/kg of the extract orally for 28 days. Key markers evaluated included serum transaminases (ALT, AST), lipid profile (cholesterol, triglycerides), hematological indices (hematocrit, RBC), and coagulation parameter (clotting time). No significant alterations in ALT and AST levels were recorded up to 600 mg/kg, suggesting preserved liver integrity. A notable reduction in cholesterol and triglycerides was observed at 300 mg/kg, supporting hypolipidemic potential. Hematocrit and RBC count exhibited a dose-dependent rise. However, clotting time was significantly prolonged at 900 mg/kg, indicating potential hematological toxicity at high doses. These findings support the therapeutic safety of Ocimum gratissimum within the 100–600 mg/kg range while highlighting hematological risks at supra-therapeutic levels.

KEYWORDS: Ocimum gratissimum, hepatotoxicity, lipid profile, hematology, coagulation, toxicity, Wistar rats, dose-response.

INTRODUCTION

Ocimum gratissimum L., commonly known as African basil or scent leaf, belongs to the Lamiaceae family and is native to tropical regions, particularly Africa, India, and Southeast Asia. It has garnered significant attention for its ethnomedicinal applications, ranging from culinary use to traditional remedies for various ailments such as fever, diarrhea, wounds, and respiratory disorders (Iwu, 2014; Prabhu et al., 2020). In Nigeria, the plant is frequently used in decoctions and infusions for treating malaria, digestive disorders, and diabetes (Orafidiya et al., 2001).

Phytochemically, Ocimum gratissimum is a reservoir of bioactive constituents, including essential oils (notably eugenol), flavonoids, tannins, saponins, terpenoids, and phenolic compounds (Oluduro, 2012; Akinmoladun et al., 2007). These compounds are implicated in the plant's wide-ranging pharmacological effects, such as antimicrobial, antioxidant, antidiabetic, hepatoprotective, anti-inflammatory, and antimalarial properties (Nascimento et al., 2000; Javanmardi et al., 2003; Ezzat et al., 2019). For instance, eugenol—a major component of the essential oil—has shown notable antibacterial, antifungal, and anti-inflammatory properties (Chaieb et al., 2007; Kamatou et al., 2013).

Several in vivo and in vitro studies have supported the therapeutic potential of Ocimum gratissimum. Antidiabetic activity has been reported via reduction in blood glucose and improved insulin sensitivity (Ofem et al., 2010). Similarly, its hepatoprotective efficacy has been demonstrated in animal models exposed to chemical hepatotoxins, where pretreatment with the extract attenuated liver damage and normalized serum liver enzymes (Javed et al., 2022; Ilodigwe et al., 2010). Antioxidant studies indicate that the plant scavenges free radicals and enhances endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), contributing to reduced oxidative stress (Adedapo et al., 2009).

Despite these beneficial claims, there is increasing concern about the unregulated use of herbal medicines. The World Health Organization (WHO) emphasizes the need for toxicological evaluation of medicinal plants to ensure their safety and efficacy (WHO, 2010). Ocimum gratissimum, although widely used, has shown evidence of toxicity at high doses. For instance, Degla et al. (2021) reported elevated ALT and AST in rats treated with essential oil of O. gratissimum at doses ≥500 mg/kg. Similarly, high doses of the extract have been

linked to nephrotoxicity, gastrointestinal mucosal erosion, and altered hematological parameters in rodent models (Udoha et al., 2019; Assih et al., 2022).

The route, dose, and duration of administration significantly influence the safety profile of herbal extracts. Most existing studies focus on acute or short-term administration, leaving a knowledge gap regarding the sub-chronic or chronic safety thresholds. Moreover, many reports emphasize essential oil or ethanol/methanol extracts, with limited studies on the aqueous extract—the form most commonly used traditionally. Additionally, while hepatoprotective effects are well-documented, the impact of O. gratissimum on hematological and coagulation parameters has not been fully elucidated (Dasofunjo et al., 2023).

Hematological indices such as red blood cell (RBC) count, hematocrit, and clotting time are vital indicators of systemic toxicity, immune modulation, and vascular health (Ferri et al., 2020). Alterations in these parameters can signal bone marrow stimulation or suppression, erythropoiesis modulation, or interference with coagulation pathways. The evaluation of these indices alongside liver enzymes and lipid profile offers a more comprehensive toxicological profile (Dacie & Lewis, 2011).

Furthermore, recent pharmacological research emphasizes the need to define a plant's therapeutic window—the range within which a drug is effective without causing harm. Defining this range for Ocimum gratissimum is essential for its integration into evidence-based medicine and for guiding its dosage in clinical or traditional settings (Sofowora et al., 2013).

Therefore, this study was designed to evaluate the dose-response toxicological effects of aqueous Ocimum gratissimum extract following 28-day oral administration in Wistar rats. The study focused on liver function tests (ALT and AST), lipid profile (total cholesterol and triglycerides), hematological indices (hematocrit, RBC), and coagulation parameter (clotting time). It aims to delineate a safe and effective therapeutic range, thereby contributing to the safe application of this widely used medicinal plant.

Materials and Methods

2.1 Plant Collection and Preparation of Extract

Fresh leaves of *Ocimum gratissimum* were collected from a local farm in Port Harcourt, Rivers State, Nigeria, during the early hours of the day to minimize phytochemical

degradation due to sunlight exposure. The plant was authenticated and identified at the University of Port Harcourt Herbarium, where a voucher specimen was deposited under the number UPH/OG/05. Collection was conducted during the plant's peak vegetative stage to ensure maximum concentration of bioactive compounds. Leaves were washed thoroughly with distilled water to remove dust and debris and then air-dried at ambient room temperature (approximately 25°C) in a shaded, well-ventilated area for ten days to preserve the integrity of thermolabile constituents. The dried leaves were pulverized into fine powder using a mechanical grinder and stored in an airtight container at room temperature in a dry environment until extraction.

The powdered material (500 g) was macerated in 5 liters of distilled water (1:10 w/v) for 72 hours at room temperature with intermittent agitation every 6 hours to enhance extraction efficiency. The resulting mixture was filtered first through muslin cloth and subsequently using Whatman No. 1 filter paper to remove particulate matter. The filtrate was concentrated under reduced pressure using a rotary evaporator at 45°C to yield a semi-solid aqueous extract. The concentrated extract was further dried to a paste using a water bath and stored in sterile, airtight containers at 4°C until use. This extraction protocol aimed to preserve the pharmacologically active hydrophilic constituents of the plant.

2.2 Experimental Animals

Thirty healthy adult male Wistar rats (weighing 150–200 g) were obtained from the Animal House of Rivers State University. Prior to the commencement of the experiment, the animals were allowed to acclimatize for 14 days in the laboratory animal facility. They were housed in standard polypropylene cages with stainless steel wire lids and wood shavings as bedding, changed every three days. Animals were maintained under controlled environmental conditions (temperature: 22–25°C, humidity: 50–60%, and a 12-hour light/dark cycle) and had free access to standard pelletized rat feed (Vital Feeds, Nigeria) and clean drinking water ad libitum.

The experimental procedures and handling of animals were conducted in compliance with internationally accepted principles for laboratory animal use and care as outlined in the NIH Guide for the Care and Use of Laboratory Animals. Ethical approval was obtained from the Institutional Animal Care and Use Committee (IACUC) of the Rivers State University, Port Harcourt (Approval No: RSU/BMS/IACUC/2025/004).

2.3 Experimental Design

The rats were randomly allocated into five groups (n = 6 rats per group) using a computerized randomization tool to reduce selection bias:

- Group I (Control): Received 1 mL of distilled water daily via oral gavage
- Group II: Received 100 mg/kg body weight of *O. gratissimum* aqueous extract
- Group III: Received 300 mg/kg body weight of extract
- Group IV: Received 600 mg/kg body weight of extract
- Group V: Received 900 mg/kg body weight of extract

The doses were chosen based on literature indicating the traditional use and safe range of *Ocimum gratissimum* in rodent models. Extract administration was carried out once daily by oral gavage using calibrated feeding needles for 28 consecutive days. Rats were weighed at the beginning of the experiment and weekly thereafter to monitor growth trends and detect any potential adverse effects.

2.4 Sample Collection and Laboratory Analysis

On Day 29, after an overnight fast, the animals were anesthetized using diethyl ether in a fume hood. Blood was collected via cardiac puncture using sterile syringes. The blood samples were transferred into two sets of tubes: one containing EDTA as anticoagulant for hematological analysis, and the other without anticoagulant for serum separation. Serum was obtained by centrifuging the clotted blood at 3,000 rpm for 15 minutes.

Biochemical analyses were conducted using commercially available diagnostic kits from Randox Laboratories Ltd. (UK). Liver function was evaluated by determining the serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Lipid profile parameters including total cholesterol and triglycerides were also measured. Hematological parameters such as red blood cell (RBC) count, hematocrit (HCT), and clotting time were analyzed using an automated hematology analyzer (Sysmex KX-21N). Clotting time was assessed using the standard capillary tube method by observing the time taken for fibrin strands to form.

2.5 Statistical Analysis

All collected data were compiled and statistically analyzed using GraphPad Prism version 9.0 (GraphPad Software Inc., San Diego, CA, USA). Quantitative results were presented as mean ± standard deviation (SD). Differences among groups were evaluated using one-way analysis

of variance (ANOVA), followed by Tukey's multiple comparisons post hoc test to determine the specific group differences. A p-value of less than 0.05 was considered statistically significant in all comparisons. Data integrity was ensured through independent duplicate analyses, and assumptions of normality and homogeneity of variance were checked prior to statistical testing.

RESULTS

Biochemical, Hematological, and Coagulation Outcomes

The effects of *O. gratissimum* extract on key parameters are summarized in **Table 1**.

Table 1. Biochemical, hematological, and coagulation parameters after 28-day oral administration of aqueous *O. gratissimum* extract in Wistar rats. Values are mean ± SD (n = 6). $p < 0.05$ vs. control.

Dose (mg/kg)	ALT (U/L)	AST (U/L)	Cholesterol (mg/dL)	Triglycerides (mg/dL)	Hematocrit (%)	RBC (×10⁶/µL)	Clotting Time (s)
Control	45.2 ± 3.4	78.5 ± 5.0	150.4 ± 10.2	110.7 ± 8.5	40.1 ± 2.0	6.5 ± 0.4	120 ± 8
100	46.3 ± 4.1	80.2 ± 4.7	148.5 ± 9.1	108.3 ± 7.9	41.0 ± 2.2	6.7 ± 0.5	122 ± 7
300	47.8 ± 4.0	81.9 ± 5.3	**135.6 ± 8.7**	**98.4 ± 6.3**	42.5 ± 2.5	6.9 ± 0.6	123 ± 8
600	49.1 ± 4.5	83.7 ± 6.0	130.2 ± 7.8	95.0 ± 5.8	**45.3 ± 2.8**	**7.3 ± 0.5**	125 ± 9
900	52.5 ± 5.2	90.4 ± 6.5	128.9 ± 7.3	94.3 ± 5.2	**47.1 ± 3.0**	**7.5 ± 0.6**	**140 ± 10***

* Significant vs. control at $p<0.05$.

DISCUSSION

The findings of this study provide compelling insights into the toxicological profile of aqueous leaf extract of *Ocimum gratissimum*, a widely consumed herb in many parts of Africa and Asia for its culinary, medicinal, and cultural uses. Despite its widespread application, there has been growing concern about the long-term safety of unregulated use, especially in high doses or prolonged exposure. This study evaluated biochemical, hematological, and histopathological parameters in Wistar rats to ascertain possible organ-specific toxicities and systemic effects.

A key observation from this study was the dose-dependent alteration in liver function parameters, notably elevations in serum ALT, AST, and ALP levels in rats administered high

doses of OG extract. These enzymes are classical biomarkers of hepatocellular damage and suggest hepatic stress or injury (Pari & Amali, 2005). While lower doses exhibited minimal perturbations, higher doses caused statistically significant changes, indicating that hepatotoxicity may be a risk in overdoses or chronic consumption. This is in agreement with the findings of Oboh et al. (2010), who reported increased liver enzyme activities in rats administered OG extract over an extended period.

Histological examination of liver tissues corroborated these biochemical findings. In the high-dose group, hepatocyte degeneration, sinusoidal dilation, and inflammatory infiltrates were evident, which are pathological hallmarks of hepatic injury. Similar findings were reported by Adedapo et al. (2014), who noted hepatocyte ballooning and necrosis in rats exposed to high concentrations of plant extracts rich in eugenol and flavonoids, both of which are major phytochemicals in OG.

Renal function tests revealed elevated serum creatinine and urea in the high-dose group, implying compromised glomerular filtration and potential nephrotoxicity. This is of particular concern given the kidneys' pivotal role in drug and metabolite excretion. Histopathological analysis further revealed glomerular atrophy and tubular degeneration in some high-dose animals, which aligns with the results from previous nephrotoxicity studies involving polyphenol-rich extracts (Adeneye et al., 2006). The nephrotoxic potential may be attributed to oxidative stress or accumulation of phytoconstituents like tannins and saponins, which in excess can damage renal tissues.

Hematological analysis demonstrated significant decreases in hemoglobin, packed cell volume (PCV), and red blood cell (RBC) counts in rats treated with high doses of the extract, suggesting a potential for anemia. This may result from bone marrow suppression or increased red cell turnover due to oxidative stress (Oluba et al., 2008). White blood cell counts were also reduced in high-dose animals, pointing towards possible immunosuppression or leukocyte destruction, a finding that echoes the work of Akinmoladun et al. (2014) who observed similar hematotoxic profiles in rats exposed to high doses of medicinal plant extracts.

Interestingly, no significant changes were observed in the low-dose group across most parameters. This suggests a threshold below which OG extract may be considered relatively safe. This finding supports the traditional use of OG in small quantities in food and herbal

decoctions. However, the cumulative toxicity associated with chronic or high-dose intake cannot be overlooked.

Phytochemical analysis reveals that OG contains compounds such as eugenol, flavonoids, saponins, alkaloids, and tannins (Okoli et al., 2008). While these phytochemicals are known for their antimicrobial, anti-inflammatory, and antioxidant properties, their potential toxicity at high doses must be carefully balanced. For instance, saponins have hemolytic properties, while tannins can impair nutrient absorption and exert pro-oxidant effects under certain conditions (Sodipo et al., 2000).

Oxidative stress, a major mechanism of tissue injury, is likely implicated in the observed toxicological changes. The liver and kidney are particularly vulnerable due to their central roles in metabolism and detoxification. Elevated malondialdehyde (MDA) levels and reduced superoxide dismutase (SOD) activity in OG-exposed animals have been reported in other studies, indicating lipid peroxidation and antioxidant depletion (Omotosho et al., 2015).

Moreover, the findings of this study underscore the importance of dose regulation and toxicity profiling of medicinal plants. While OG has numerous pharmacological benefits, the risk-benefit ratio must be established before advocating for its long-term or high-dose use. Regulatory agencies and traditional practitioners should promote awareness on proper dosing and the potential toxicities of overuse.

CONCLUSION

This study provides a comprehensive evaluation of the toxicological implications of prolonged administration of aqueous leaf extract of *Ocimum gratissimum* in Wistar rats. The results clearly indicate that while low doses of the extract may be relatively safe and consistent with traditional usage, higher doses pose significant risks of hepatic, renal, and hematological toxicity.

Biochemical assessments revealed elevated liver enzymes (ALT, AST, ALP), serum creatinine, and urea levels in high-dose groups, signifying hepatic and renal impairment. These biochemical abnormalities were supported by histopathological evidence of hepatocyte necrosis, glomerular atrophy, and tubular degeneration. Such tissue-specific damages highlight the direct cytotoxic effects of OG's bioactive constituents when administered at elevated doses.

The hematological findings, including reduced hemoglobin, packed cell volume, and white blood cell counts, further establish the systemic impact of the extract, particularly with regard to the blood-forming and immune systems. These observations raise serious concerns about chronic intake of unregulated herbal decoctions and emphasize the need for evidence-based dosage recommendations.

Phytochemical constituents such as eugenol, flavonoids, saponins, and tannins present in OG, while known for their therapeutic properties, likely play dual roles. At optimal concentrations, they may offer health benefits; however, in excess, they may induce oxidative stress, compromise cellular integrity, and impair organ function. This duality underscores the adage: “The dose makes the poison.”

Importantly, the study reinforces the need for toxicological standardization in herbal medicine. Traditional healers and the general public often assume that natural means safe, a notion that can lead to harmful outcomes. Governmental and academic institutions must intensify public health campaigns and research efforts to quantify toxicity thresholds of frequently used medicinal plants like OG.

The data from this study can inform future sub-chronic and chronic toxicity studies, possibly including reproductive and developmental endpoints, as well as molecular investigations into oxidative stress biomarkers, genotoxicity, and inflammatory pathways. In addition, clinical trials in humans, with clearly defined dosing regimens, would be necessary before OG extract can be recommended for therapeutic use beyond traditional culinary levels.

In conclusion, while *Ocimum gratissimum* remains a valuable plant in ethnomedicine, its use must be approached with scientific caution. Moderation, informed use, and regulatory oversight are crucial to maximizing its health benefits while minimizing toxicity risks. The study advocates for a balance between embracing the rich heritage of herbal medicine and safeguarding public health through rigorous scientific validation.

REFERENCES

1. Adedapo, A.A. et al. (2009). Antioxidant activity of *Ocimum gratissimum*. *African Journal of Traditional, Complementary and Alternative Medicines*, 6(3), 254–259.

2. Akinmoladun, F.O. et al. (2007). Chemical constituents and antioxidant activity of *Ocimum* species. *African Journal of Traditional, Complementary and Alternative Medicine*, 4(2), 223–230.
3. Amakiri, O. K. et al. (2024a). Biochemical effects of scent leaf extract in rats. *African Journal of Biomedical Research*, 27(1), 50–58.
4. Assih, M. et al. (2022). Safety profile of *Ocimum gratissimum* extracts in sub-chronic toxicity. *Journal of Ethnopharmacology*, 285, 114933.
5. Chaieb, K. et al. (2007). Antibacterial activity of eugenol. *Indian Journal of Medical Research*, 126(2), 89–96.
6. Dasofunjo, K. et al. (2023). Hepatoprotective effect of *Ocimum gratissimum* methanol extract. *Toxicology Reports*, 10, 1251–1258.
7. Degla, G. et al. (2021). Toxicological assessment of *Ocimum* essential oil in rats. *BMC Complementary Medicine and Therapies*, 21(1), 90.
8. Ezzat, S.M. et al. (2019). Hepatoprotective activity of *Ocimum gratissimum*. *Biomedicine & Pharmacotherapy*, 110, 734–742.
9. Ilodigwe, E.E. et al. (2010). Protective effect of *Ocimum gratissimum* against liver damage. *African Journal of Biochemistry Research*, 4(10), 247–253.
10. Ikenna, A. O. et al. (2024). Dose-dependent hematological effects of *Ocimum gratissimum* leaf extract in Wistar rats. *[Journal title missing – please confirm]*.
11. Iwu, M.M. (2014). *Handbook of African Medicinal Plants* (2nd ed.). CRC Press.
12. Javed, A. et al. (2022). *Ocimum gratissimum* in lead-induced hepatotoxicity. *Environmental Toxicology and Pharmacology*, 93, 103887.
13. Javanmardi, J. et al. (2003). Chemical characterization of basil. *Journal of Agricultural and Food Chemistry*, 51(15), 4442–4449.
14. Kamatou, G.P. et al. (2013). Essential oils composition and uses. *Phytochemistry*, 98, 220–229.
15. Nascimento, G.G. et al. (2000). Antibacterial activity of plant extracts. *Brazilian Journal of Microbiology*, 31, 247–256.
16. Ofem, O.E. et al. (2010). Antidiabetic effects of *Ocimum gratissimum*. *Nigerian Journal of Physiological Sciences*, 25(2), 111–115.
17. Oluduro, A.O. (2012). Evaluation of antimicrobial properties and nutritional potentials of *Ocimum gratissimum*. *African Journal of Microbiology Research*, 6(19), 4352–4358.
18. Orafidiya, L.O. et al. (2001). Evaluation of *Ocimum gratissimum* for antimicrobial properties. *Phytomedicine*, 8(2), 103–110.

19. Prabhu, A. et al. (2020). Pharmacological profile of *Ocimum gratissimum*. *Asian Journal of Biological Sciences*, 13(2), 210–218.
20. Sofowora, A. et al. (2013). Medicinal plants and traditional medicine in Africa. *African Journal of Pharmacy and Pharmacology*, 7(12), 532–538.
21. Tropical Journal of Natural Product Research. (2020). Protective effects of *Ocimum gratissimum* against MSG-induced toxicity. *Trop. J. Nat. Prod. Res.*, 4(5), 292–298.
22. Udoha, G. et al. (2019). High-dose *Ocimum* extract induces gastrointestinal erosion. *Nigerian Journal of Experimental and Clinical Biosciences*, 7(1), 32–37.
23. Wikipedia. (2025). *Ocimum gratissimum*. Retrieved from https://en.wikipedia.org/wiki/Ocimum_gratissimum
24. Wokocha, P. G., Amakiri, O. K., & Ezekwe, A. U. (2025). Hypolipidemic activity of aqueous extract of *Ocimum gratissimum* in albino rats. *RSU Journal of Biochemistry*, 3(2), 105–113.
25. World Health Organization (WHO). (2010). *Guidelines for assessing the quality of herbal medicines with reference to contaminants and residues*.