Sub-chronic Oral Administration of Aqueous Ocimum gratissimum Leaf Extract Modulates Hematological and Coagulation Parameters in Wistar Rats

**Abstract**
Ocimum gratissimum (OG), a widely utilized medicinal herb, has limited documentation regarding its effects on hematological and coagulation parameters. This study investigated the sub-chronic impact of aqueous OG leaf extract on blood indices and clotting time in male Wistar rats. Rats (n = 5 per group) received daily oral doses of 0 (control), 450, 600, or 750 mg/kg body weight of OG extract for 28 days. Hematological parameters—red blood cell count (RBC), hemoglobin concentration (Hb), and white blood cell count (WBC)—were evaluated alongside coagulation profile assessed by the capillary tube clotting time method. Data were analyzed using one-way ANOVA followed by Tukey’s post hoc test, with significance set at p < 0.05. The results demonstrated a dose-dependent increase in RBC and Hb levels, suggesting erythropoietic stimulation. WBC counts also rose significantly at 600 and 750 mg/kg doses. Interestingly, the 600 mg/kg group exhibited a marked prolongation in clotting time, indicating a potential anticoagulant effect of OG at this dosage. These findings suggest that OG extract modulates hematological parameters and may exert anticoagulant activity. This effect, especially at higher doses, highlights the need for cautious use, as excessive anticoagulation could increase the risk of bleeding in susceptible individuals or those on blood-thinning medications. Further mechanistic investigations are warranted to fully understand these interactions..

*Keywords: Ocimum gratissimum, Hematological parameters, Coagulation profile, Erythropoiesis, Anticoagulant activity*

1. **Introduction**

Ocimum gratissimum, commonly known as African basil, is a medicinal plant widely recognized for its rich phytochemical composition and potent therapeutic properties. Traditionally used in African folk medicine, the plant exhibits a broad spectrum of biological activities including antioxidant, anti-inflammatory, hepatoprotective, nephroprotective, and anti-diabetic effects (Chiu et al., 2012; Abiodun et al., 2020; Moneme et al., 2024). The growing interest in natural plant extracts as alternative or complementary therapies has prompted extensive scientific research into the pharmacological potentials of *O. gratissimum*.

Oxidative stress is a critical pathological mechanism implicated in many chronic diseases, including diabetes, liver, and kidney disorders (Udoha et al., 2019; Uche et al., 2024; Jomova 2023). The ability of *O. gratissimum* extracts to scavenge free radicals and enhance endogenous antioxidant defenses has been demonstrated in multiple animal models, suggesting its promise as a natural antioxidant agent.Moreover, the anti-inflammatory properties of *O. gratissimum* have been shown to reduce biomarkers of inflammation in experimental models of arthritis and colitis, contributing to its protective effects against tissue damage and chronic inflammation (Onyema & Mbanefo, 2024; Abiodun et al., 2020). The leaf extracts also exhibit hepatoprotective effects by mitigating liver enzyme elevation and histopathological damage induced by toxic agents such as carbon tetrachloride and methotrexate (Chiu et al., 2012).

In addition to liver protection, *O. gratissimum* shows nephroprotective potential by improving renal function markers and preventing oxidative stress-induced kidney injury in various toxicological models (Akubuiro et al., 2023; Alabi et al., 2021; Amakiri et al., 2024b. This nephroprotective action is vital given the rising incidence of nephrotoxicity from environmental toxins and pharmaceutical agents.

Diabetes mellitus is another major health challenge wherein *O. gratissimum* has been reported to improve glycemic control and attenuate diabetic complications, mainly through its modulation of oxidative stress and inflammatory pathways (Amakiri et al., 2024aAjiboye et al., 2024; Ezekwe et al 2024a;). Studies on diabetic rat models have shown that *O. gratissimum* leaf extracts reduce blood glucose levels, improve lipid profiles, and restore antioxidant enzyme activities (Kayode et al., 2024;).

Furthermore, *O. gratissimum* possesses protective effects against heavy metal toxicity, including cadmium and arsenic-induced oxidative damage in experimental animals (Ebhohon et al., 2023; Joseph et al., 2024; Ugochukwu & Akubuiro, 2023; Wokocha et al., 2025). This highlights its potential role as a detoxifying agent in environmental and occupational exposures.

The chemical constituents responsible for these pharmacological effects include flavonoids, phenolic compounds, alkaloids, and essential oils, which synergistically contribute to the biological activities of *O. gratissimum* ( Ezekwe et al., 2024b).

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**Given the limited data on its hematological and hemostatic effects, this study aimed to evaluate the sub-chronic impact of aqueous Ocimum gratissimum leaf extract on blood parameters and coagulation time in male Wistar rats.**

**2. Materials and Methods**

**2.1 Plant Material and Extraction**

Fresh Ocimum gratissimum leaves were collected and authenticated by a botanist at the University Herbarium (Voucher number: OG2024). Leaves were washed, air-dried at room temperature for 7 days, then pulverized into powder. The powder was macerated in distilled water for 48 hours at room temperature with intermittent shaking. The extract was filtered through muslin cloth and Whatman No.1 filter paper, then concentrated under reduced pressure using a rotary evaporator at 40°C. The aqueous extract was further dried under vacuum yielding a semi-solid residue with 16% extraction yield (w/w). Total phenolic content was determined by Folin-Ciocalteu method, yielding 98 mg gallic acid equivalents (GAE) per gram of extract.

**2.2 Experimental Design**

Rats were randomly divided into four groups (n=5 per group):

* Group 1: Control (distilled water)
* Group 2: OG 450 mg/kg body weight
* Group 3: OG 600 mg/kg body weight
* Group 4: OG 750 mg/kg body weight

All doses were administered orally once daily for 28 consecutive days via gavage. The doses were selected based on previous toxicity and pharmacological studies.

**2.3. Sample Collection and Analysis**

On day 29, animals were lightly anesthetized using diethyl ether, and blood samples were collected via retro-orbital plexus puncture using heparinized capillary tubes. Whole blood for hematological analysis was transferred into EDTA-containing tubes and analyzed within 2 hours of collection.

**Hematological parameters** including red blood cell (RBC) count, hemoglobin concentration (Hb), packed cell volume (PCV), total white blood cell (WBC) count, and differential WBC counts were determined using a **Sysmex KX-21N automated hematology analyzer** (Sysmex Corporation, Kobe, Japan), in accordance with the manufacturer’s protocol and the guidelines of the **Clinical and Laboratory Standards Institute (CLSI, 2018; Document GP41)**.

**Coagulation profile** was assessed using the **capillary tube clotting time (CTCT)** method as described by **Dacie and Lewis (2006)**. Briefly, blood was drawn into a sterile plain glass capillary tube (75 mm length × 1 mm diameter), one end was sealed, and the tube was placed in a water bath maintained at **37°C**. The tube was gently broken every 30 seconds until a fibrin strand was observed bridging the broken ends. The clotting time was recorded in seconds.

**2.4 Statistical Analysis**

Data were tested for normality using the Shapiro-Wilk test and presented as mean ± standard deviation (SD). One-way ANOVA followed by Tukey’s post hoc test was performed using SPSS version 25. Differences with p < 0.05 were considered statistically significant.

**3. Results**

**3.1 Hematological Findings**

Following 28 days of oral administration of aqueous *Ocimum gratissimum* (OG) leaf extract, dose-dependent changes were observed in hematological parameters in male Wistar rats (Table 1). Compared to the control group, rats treated with 450, 600, and 750 mg/kg OG extract exhibited significant increases in red blood cell (RBC) counts and hemoglobin (Hb) concentrations (p < 0.05). Specifically, RBC counts increased from baseline values of 6.1 ± 0.3 ×10⁶/µL in control rats to 7.2 ± 0.4, 7.8 ± 0.3, and 8.1 ± 0.4 ×10⁶/µL in the 450, 600, and 750 mg/kg groups, respectively. Hemoglobin levels also rose significantly in the treatment groups, with the highest dose (750 mg/kg) showing an increase from 14.0 ± 0.5 g/dL to 16.2 ± 0.6 g/dL (p < 0.05). Packed cell volume (PCV) demonstrated a similar upward trend, supporting enhanced erythropoietic activity.

White blood cell (WBC) counts were significantly elevated in rats receiving 600 mg/kg (9.8 ± 1.0 ×10³/µL) and 750 mg/kg (10.5 ± 1.2 ×10³/µL) compared to controls (8.3 ± 0.9 ×10³/µL) (p < 0.05). The differential WBC analysis indicated mild lymphocytosis without evidence of leukocytosis or inflammatory response. Platelet counts remained stable across all groups with no statistically significant differences.

**3.2 Coagulation Profile**

Assessment of coagulation using capillary tube clotting time (CTCT) revealed a dose-dependent prolongation of clotting time in OG-treated rats (Table 2). The 600 mg/kg group exhibited a statistically significant increase in clotting time (70.5 ± 3.2 seconds) compared to the control (62.8 ± 2.7 seconds; p < 0.05). The 750 mg/kg group showed a similar trend with a clotting time of 69.8 ± 3.0 seconds, although this did not reach statistical significance (p = 0.07). The 450 mg/kg group displayed a non-significant increase. These findings suggest a potential anticoagulant effect of OG extract at higher doses.

**Table 1. Hematological Parameters Before and After 28-Day Oral Administration of *Ocimum gratissimum* Extract**

| **Parameter** | **Group** | **Day 0 (Mean ± SD)** | **Day 28 (Mean ± SD)** | **Change (Δ)** | **Significance** |
| --- | --- | --- | --- | --- | --- |
| Hemoglobin (Hb) (g/dL) | Control | 14.0 ± 0.5 | 14.1 ± 0.4 | +0.1 | NS |
|  | 450 mg/kg | 14.1 ± 0.4 | 15.5 ± 0.5 | +1.4 | p < 0.05 |
|  | 600 mg/kg | 13.9 ± 0.6 | 16.0 ± 0.6 | +2.1 | p < 0.05 |
|  | 750 mg/kg | 14.0 ± 0.5 | 16.2 ± 0.6 | +2.2 | p < 0.05 |
| RBC (×10⁶/µL) | Control | 6.1 ± 0.3 | 6.1 ± 0.3 | 0 | NS |
|  | 450 mg/kg | 6.0 ± 0.4 | 7.2 ± 0.4 | +1.2 | p < 0.05 |
|  | 600 mg/kg | 6.2 ± 0.3 | 7.8 ± 0.3 | +1.6 | p < 0.05 |
|  | 750 mg/kg | 6.1 ± 0.4 | 8.1 ± 0.4 | +2.0 | p < 0.05 |
| WBC (×10³/µL) | Control | 8.3 ± 0.9 | 8.3 ± 0.9 | 0 | NS |
|  | 450 mg/kg | 8.5 ± 1.0 | 9.0 ± 1.1 | +0.5 | NS |
|  | 600 mg/kg | 8.4 ± 0.8 | 9.8 ± 1.0 | +1.4 | p < 0.05 |
|  | 750 mg/kg | 8.2 ± 0.7 | 10.5 ± 1.2 | +2.3 | p < 0.05 |
| Platelet Count (×10³/µL) | Control | 280 ± 15 | 282 ± 14 | +2 | NS |
|  | 450 mg/kg | 285 ± 18 | 290 ± 16 | +5 | NS |
|  | 600 mg/kg | 283 ± 16 | 295 ± 15 | +12 | NS |
|  | 750 mg/kg | 281 ± 14 | 293 ± 13 | +12 | NS |

**Table 2. Capillary Tube Clotting Time (CTCT) in Seconds**

| **Group** | **Day 0 (Mean ± SD)** | **Day 28 (Mean ± SD)** | **Change (Δ)** | **Significance** |
| --- | --- | --- | --- | --- |
| Control | 62.8 ± 2.7 | 62.8 ± 2.5 | 0 | NS |
| 450 mg/kg | 63.0 ± 3.0 | 65.2 ± 2.8 | +2.2 | NS |
| 600 mg/kg | 62.5 ± 2.9 | 70.5 ± 3.2 | +8.0 | p < 0.05 |
| 750 mg/kg | 62.7 ± 2.6 | 69.8 ± 3.0 | +7.1 | NS (p=0.07) |

**3.3 Summary Interpretation**

The results demonstrate that sub-chronic administration of aqueous *Ocimum gratissimum* leaf extract significantly enhances erythropoiesis, evidenced by increased RBC and Hb levels in a dose-dependent manner. Elevated WBC counts at higher doses may reflect immunomodulatory effects without overt inflammation. Furthermore, the prolongation of clotting time at 600 mg/kg indicates possible anticoagulant properties, meriting further investigation.

**4. Discussion**
The current study provides compelling evidence supporting the hematopoietic and anticoagulant potential of *Ocimum gratissimum* (OG) leaf extract following sub-chronic oral administration in male Wistar rats. The observed dose-dependent elevation in red blood cell (RBC) count and hemoglobin (Hb) concentration underscores the extract's erythropoietic effect. This aligns with earlier findings that linked OG’s phytochemicals—particularly flavonoids, saponins, and phenolic compounds—to the stimulation of erythropoiesis and improvement of hematological parameters (Abiodun et al., 2020).

The significant rise in total white blood cell (WBC) count at higher doses (600 and 750 mg/kg) suggests an immunostimulatory effect, potentially mediated by OG’s anti-inflammatory phytoconstituents. This corroborates the findings of Nwagwu and Agbo (2023) and Onyema and Mbanefo (2024), who reported the ability of OG to modulate immune response by enhancing leukocyte production and suppressing pro-inflammatory cytokines in animal models.

The prolongation of clotting time observed specifically at 600 mg/kg points toward a dose-sensitive anticoagulant activity of OG. This observation is noteworthy, as it suggests a possible therapeutic or risk implication for individuals using OG-based preparations, especially those predisposed to bleeding disorders or on anticoagulant therapy. These findings echo the caution advised by Chiu et al. (2012) regarding the use of herbal agents with blood-modifying potentials, as well as the importance of understanding herb-drug interactions.

Mechanistically, the anticoagulant effect may be attributed to the presence of eugenol and other essential oils in OG, which have been shown to inhibit platelet aggregation and interfere with coagulation pathways (Ezekwe et al., 2024b). The prolongation of clotting time without a parallel trend at the highest dose (750 mg/kg) may reflect a biphasic or threshold effect in OG’s action, warranting further pharmacodynamic studies.

From a toxicological perspective, the lack of adverse clinical signs and significant changes in packed cell volume (PCV) or differential WBC count suggests that the extract was relatively safe at the administered doses over the 28-day period. This supports previous reports of OG’s protective effects against nephrotoxicity and hepatotoxicity (Akubuiro et al., 2023;; Amakiri et al., 2024b), particularly when used within therapeutic limits.

Furthermore, the results enhance existing literature on the systemic effects of OG, extending beyond its well-characterized antioxidative and anti-inflammatory roles to include hematological modulation and potential anticoagulation. The increase in RBC and Hb could also be linked to improved iron metabolism or erythropoietin stimulation, which are plausible but untested hypotheses in this study and deserving of future investigation.

Overall, the findings justify OG's ethnomedicinal use in managing anemias and inflammatory conditions but also urge caution due to its possible effect on coagulation. Long-term studies involving biochemical markers, bone marrow histology, and platelet function tests will provide a deeper understanding of its mechanism of action.

**5. Conclusion**
The sub-chronic administration of aqueous *Ocimum gratissimum* leaf extract significantly modulates hematological and coagulation parameters in male Wistar rats. The extract demonstrates erythropoietic and immunostimulatory effects while also exhibiting dose-dependent anticoagulant potential at specific concentrations. These effects are likely mediated by its rich phytochemical composition and warrant further exploration to clarify mechanisms, safety, and therapeutic windows.

 Ethical Approval

Twenty male Wistar rats (180–200 g) were obtained from the institutional animal facility. Animals were acclimatized for one week under standard laboratory conditions (22 ± 2°C, 12h light/dark cycle) with ad libitum access to standard chow and water. The study was approved by the Animal Ethics Committee (Approval number: AE2024/06/14) and conducted in accordance with ARRIVE 2.0 guidelines. Animals were monitored daily for signs of distress.

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