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# Comparative and correlative levels of copper, zinc, with ceruloplasmin and metallothionein in art-HIV infected and non-art-HIV infected subjects: A cross-sectional study.

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## Abstract

### Background:

Trace elements such as copper and zinc, along with their transport proteins ceruloplasmin and metallothionein, play crucial roles in immune function and oxidative balance. Human immunodeficiency virus (HIV) infection and antiretroviral therapy (ART) alter these micronutrient levels, influencing disease progression and metabolic complications.

### Objectives:

To compare and correlate copper, zinc, ceruloplasmin, and metallothionein levels between ART-HIV–infected and non-ART-HIV–infected subjects and evaluate associated biochemical and metabolic parameters.

### Methods:

A cross-sectional study was conducted on 200 HIV-infected subjects (100 on ART, 100 not on ART) attending a tertiary care center. Demographic, physical, lipid, glycaemic, and trace element parameters were assessed using standard biochemical methods. Statistical analysis was performed with SPSS software; $p < 0.05$ was considered significant.

### Results:

The mean age of participants was 55.5 ± 6.2 years, with a male-to-female ratio of 1.3:1. BMI was significantly higher in ART-HIV subjects ($p < 0.0001$), while blood pressure and age were comparable between groups. ART-HIV subjects exhibited lower total cholesterol and LDL but higher HDL compared with non-ART-HIV patients. Fasting blood sugar was significantly lower in the ART group ($p < 0.001$), though insulin levels showed no difference. Notably, serum zinc levels were markedly reduced in ART-HIV subjects ($p < 0.0001$), whereas copper, ceruloplasmin, and metallothionein were significantly elevated ($p < 0.05$). A positive correlation was observed between copper and ceruloplasmin, and a negative correlation between zinc and ceruloplasmin.

### Conclusion:

HIV infection and ART significantly alter trace element balance and related biomarkers. ART appears beneficial in improving lipid and glycaemic profiles but is associated with zinc depletion and compensatory increases in copper, ceruloplasmin, and metallothionein.

### Recommendations:

Routine monitoring of trace elements in HIV-infected patients is essential. Zinc supplementation and dietary counseling may mitigate long-term metabolic complications and improve immune resilience.

***Keywords:*** *HIV, antiretroviral therapy, zinc, copper, ceruloplasmin, metallothionein, lipid profile, glycaemic parameters*
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## Introduction

Human immunodeficiency virus (HIV) infection remains a major global health challenge, with an estimated 37.7 million people affected worldwide and more than 24 lakh cases reported in India alone [1]. Despite significant advances in antiretroviral therapy (ART) that have improved survival and quality of life, HIV continues to exert profound systemic effects on immunity, metabolism, and micronutrient balance [2]. The role of nutritional status and trace elements in modulating immune responses and disease progression among people living with HIV has therefore become an important area of investigation [3].

Trace elements, particularly zinc and copper, serve as essential cofactors for numerous enzymatic processes, including nucleic acid synthesis, antioxidant defense, and immune modulation [2,4]. Zinc deficiency impairs T-cell–mediated responses and increases susceptibility to opportunistic infections, while copper excess has been implicated in oxidative stress and tissue injury [2,4]. Regulatory proteins such as ceruloplasmin, a copper-binding glycoprotein, and metallothionein, a zinc-binding protein, play crucial roles in maintaining trace element homeostasis and oxidative balance. Altered expression of these proteins has been documented in HIV infection, contributing to immune dysregulation and disease progression [2,5].

ART itself has complex metabolic implications. While effective in suppressing viral replication, long-term use has been associated with dyslipidemia, insulin resistance, and glucose metabolism disturbances [2]. Moreover, recent studies indicate that ART and HIV infection can differently influence trace element levels, potentially predisposing patients to cardiovascular disease, metabolic syndrome, and accelerated aging [3–5].

Given the paucity of data from the Indian subcontinent, particularly regarding trace element interactions in HIV-positive individuals on ART compared with those not receiving therapy, the present study was undertaken. We aimed to evaluate and compare serum copper, zinc, ceruloplasmin, and metallothionein levels, alongside lipid and glycaemic parameters, in ART-HIV and non-ART-HIV subjects. Understanding these biochemical alterations may provide valuable insights into disease monitoring and guide adjunctive nutritional interventions to optimize long-term outcomes.

## Methodology

### Study Design and Setting

This was a cross-sectional, comparative study conducted in the Department of Pharmacology, Konaseema Institute of Medical Sciences and Research Foundation (KIMS & RF), Amalapuram, Andhra Pradesh, India. KIMS & RF is a tertiary care teaching hospital that caters to a large rural and semi-urban population of the Konaseema region. The hospital has well-equipped clinical, diagnostic, and research laboratories with an established Antiretroviral Therapy (ART) Centre and Integrated Counselling and Testing Centre (ICTC) under the National AIDS Control Programme, serving as a referral hub for HIV care. The study was carried out from January 2023 to December 2023 after obtaining institutional ethical clearance.

### Study Population and Sampling

A total of **200 adult HIV-infected individuals** were enrolled, comprising 100 subjects on ART (Group I) and 100 subjects not on ART (Group II). Participants were recruited consecutively from the ART Centre and ICTC units attached to the hospital.

### Inclusion Criteria

Age ≥ 18 years
Confirmed HIV infection as per National AIDS Control Organization (NACO) guidelines
CD4 count between 350–500 cells/mm³
ART duration less than 3 months at the time of enrolment (for the ART group)

### Exclusion Criteria

Pregnant or lactating women
Individuals on post-exposure prophylaxis
Patients on second-line ART
Subjects with known chronic systemic diseases (e.g., diabetes mellitus, hypertension, tuberculosis, or chronic liver disease)

### Study sample Size

The sample size of 200 (100 per group) was determined based on previous comparative studies evaluating trace element alterations in HIV-infected individuals [4,9]. Assuming an expected difference in serum zinc levels of 20 µg/dL between groups, a standard deviation of 40 µg/dL, 95% confidence level ($\alpha = 0.05$), and 80% power ($\beta = 0.20$), the minimum required sample size was calculated as 90 per group using the formula:

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$n = (Z_{1-\alpha/2})^2 \times 2\sigma^2 / d^2$

where $\sigma$ = standard deviation, $d$ = expected mean difference. Accounting for 10% non-response, a final sample size of 100 participants per group was chosen.



## Bias and Efforts to Minimize It

To minimize **selection bias**, all eligible participants attending the ART and ICTC centers during the study period were recruited consecutively. Measurement bias was reduced by using standardized operating procedures and calibrating all instruments before use. Information bias was limited by blinding the laboratory technicians to participants' ART status during biochemical estimations. Data entry was double-checked to ensure accuracy.

## Sample Collection and Biochemical Estimation

Fasting venous blood (5 mL) was collected under aseptic precautions. Plasma and serum were separated immediately.

**Plasma glucose:** Glucose oxidase–peroxidase method

**Serum insulin:** Enzyme-linked immunosorbent assay (ELISA)

**Lipid profile:** Total cholesterol, HDL, LDL, and triglycerides estimated using an automated analyzer

**Serum ceruloplasmin and metallothionein:** ELISA method

**Serum copper and zinc:** Atomic absorption spectrophotometry

## Statistical Analysis

Data were analyzed using IBM SPSS (latest version). Continuous variables were expressed as mean ± standard deviation (SD). Intergroup comparisons were performed using the unpaired Student's ***t***-test. Pearson correlation analysis was applied to assess relationships between biochemical variables. A ***p***-value < 0.05 was considered statistically significant.

## Ethical Consideration

The study protocol was reviewed and approved by the Institutional Ethics Committee for Human Studies, Konaseema Institute of Medical Sciences and Research Foundation, Amalapuram. Written informed consent was obtained from all participants before enrolment. Confidentiality and anonymity of participants were strictly maintained throughout the study in accordance with the Declaration of Helsinki.

## Results

During the twelve-month study period, a total of 236 individuals with confirmed HIV infection attended the Antiretroviral Therapy (ART) Centre and Integrated Counselling and Testing Centre (ICTC) at KIMS & RF, Amalapuram. Of these, 218 were examined for eligibility based on inclusion and exclusion criteria. Eighteen individuals were excluded, 7 due to advanced opportunistic infections, 5 with chronic systemic diseases (diabetes mellitus, hypertension, or tuberculosis), 3 who were on second-line ART, and 3 who declined participation.

Thus, 200 participants were confirmed eligible and enrolled in the study, comprising 100 ART-HIV subjects and 100 non-ART-HIV subjects. All enrolled participants completed the study procedures and laboratory investigations; there were no dropouts or missing data, and all 200 were included in the final analysis (Figure 1).

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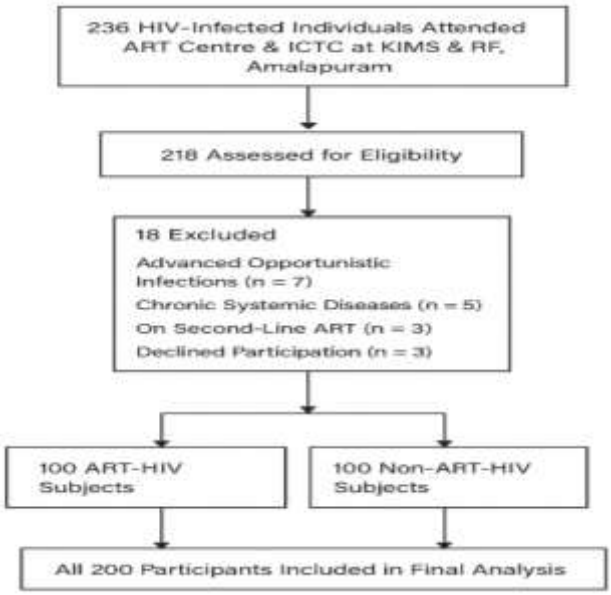


**Figure 1: Participant Flow Diagram**

## Physical Characteristics of the Study Population

The baseline physical parameters of the study groups are presented in **Table 1**. The mean age of participants was comparable between ART-HIV–infected subjects (54.4 ± 4.3 years) and non-ART-HIV–infected subjects (56.6 ± 8.1 years), with no statistically significant difference ($p > 0.05$). Similarly, systolic and diastolic blood pressures did not differ significantly between groups ($p > 0.05$). However, body mass index (BMI) was significantly higher among ART-HIV–infected subjects (31.4 ± 2.7 kg/m²) compared to non-ART-HIV–infected subjects (27.4 ± 2.8 kg/m²; $p < 0.0001$), indicating a marked difference in nutritional and metabolic status between the two groups (Table 1).

**Table 1. Physical parameters of ART-HIV and non-ART-HIV infected subjects**

| Variable | ART-HIV subjects (n=100) | Non-ART-HIV subjects (n=100) | p-value |
|---|---|---|---|
| Age (years) | 54.4 ± 4.3 | 56.6 ± 8.1 | >0.05 |
| BMI (kg/m²) | 31.4 ± 2.7 | 27.4 ± 2.8 | <0.0001 |
| Systolic BP (mmHg) | 148 ± 4.1 | 151.1 ± 7.2 | >0.05 |
| Diastolic BP (mmHg) | 89.2 ± 2.9 | 91.9 ± 4.2 | >0.05 |

## Lipid Profile

The lipid parameters are summarized in **Table 2**. A significant reduction in total cholesterol was observed in ART-HIV–infected subjects (156.7 ± 48.1 mg/dL) compared with the non-ART-HIV group (168.5 ± 8.3 mg/dL; $p < 0.0165$). High-density lipoprotein cholesterol (HDL) was notably higher in the ART-HIV group (45.1 ± 6.1 mg/dL) relative to the non-ART-HIV group (28.3 ± 2.6 mg/dL; $p < 0.0001$). Conversely, low-density lipoprotein cholesterol (LDL) levels were significantly lower in ART-HIV subjects (135.1 ± 41.4 mg/dL) than in non-ART-HIV individuals (149.8 ± 51.4 mg/dL; $p = 0.0271$). Serum triacylglycerol concentrations showed no statistically significant difference between the two groups ($p > 0.05$). These findings suggest that ART is associated with favorable modulation of HDL and LDL levels (Table 2).

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**Table 2. Lipid profile of study groups**

| Variable | ART-HIV subjects (n=100) | Non-ART-HIV subjects (n=100) | p-value |
|---|---|---|---|
| Total cholesterol (mg/dL) | 156.7 ± 48.1 | 168.5 ± 8.3 | <0.0165 |
| Triacylglycerols (mg/dL) | 174.8 ± 14.3 | 178.5 ± 24.3 | >0.05 |
| HDL (mg/dL) | 45.1 ± 6.1 | 28.3 ± 2.6 | <0.0001 |
| LDL (mg/dL) | 135.1 ± 41.4 | 149.8 ± 51.4 | 0.0271 |

## Glycaemic Parameters

As shown in **Table 3**, fasting blood sugar (FBS) was significantly lower in ART-HIV subjects (102 ± 22.1 mg/dL) compared to non-ART-HIV subjects (141 ± 13.2 mg/dL; $p < 0.001$). Insulin levels were similar across both groups (19.4 ± 11.3 pg/mL vs. 20.2 ± 12.1 pg/mL; $p > 0.05$). The homeostatic model assessment of insulin resistance (HOMA-IR) demonstrated a modest but statistically significant difference between ART-HIV (15.9 ± 2.5) and non-ART-HIV subjects (17.1 ± 4.1; $p = 0.0133$), indicating relatively better glycaemic control in the ART-treated group (Table 3).

**Table 3. Glycaemic parameters in study groups**

| Variable | ART-HIV subjects (n=100) | Non-ART-HIV subjects (n=100) | p-value |
|---|---|---|---|
| Fasting blood sugar (mg/dL) | 102 ± 22.1 | 141 ± 13.2 | <0.001 |
| Insulin (pg/mL) | 19.4 ± 11.3 | 20.2 ± 12.1 | >0.05 |
| HOMA-IR | 15.9 ± 2.5 | 17.1 ± 4.1 | 0.0133 |

## Trace Elements and Biomarkers

The comparison of trace element and biomarker levels is detailed in Table 4. ART-HIV subjects exhibited significantly lower serum zinc concentrations (70.5 ± 34.9 µg/dL) than non-ART-HIV individuals (121.5 ± 49.5 µg/dL; $p < 0.0001$). In contrast, serum copper (152.8 ± 22.9 µg/dL vs. 128.7 ± 30.5 µg/dL; $p < 0.05$), ceruloplasmin (64.8 ± 5.3 mg/dL vs. 42.1 ± 6.9 mg/dL; $p < 0.001$), and metallothionein (72.3 ± 9.7 ng/mL vs. 58.8 ± 10.7 ng/mL; $p < 0.001$) were all significantly elevated in the ART-HIV group compared with non-ART-HIV subjects. These alterations indicate profound ART-related shifts in trace metal homeostasis and associated biomarkers (Table 4).

**Table 4. Trace element and biomarker levels**

| Variable | ART-HIV subjects (n=100) | Non-ART-HIV subjects (n=100) | p-value |
|---|---|---|---|
| Serum zinc (µg/dL) | 70.5 ± 34.9 | 121.5 ± 49.5 | <0.0001 |
| Serum copper (µg/dL) | 152.8 ± 22.9 | 128.7 ± 30.5 | <0.05 |
| Ceruloplasmin (mg/dL) | 64.8 ± 5.3 | 42.1 ± 6.9 | <0.001 |
| Metallothionein (ng/mL) | 72.3 ± 9.7 | 58.8 ± 10.7 | <0.001 |

## Discussion

The present study compared physical, biochemical, and trace element parameters between ART-HIV–infected and non-ART-HIV–infected individuals. The findings demonstrate significant variations in nutritional status, lipid metabolism, glycaemic regulation, and trace element balance, reflecting the multifactorial metabolic influence of HIV infection and antiretroviral therapy (ART).

## Physical Parameters

Although age and blood pressure were comparable between groups, body mass index (BMI) was markedly higher among ART-treated individuals. This observation is consistent with

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prior evidence showing that ART initiation promotes immune recovery, appetite restoration, and reduction of catabolic stress, leading to weight gain [6]. However, excess weight gain, particularly in those on integrase inhibitor–based regimens, has been linked to metabolic syndrome and cardiovascular complications [7].

## Lipid Profile

The study revealed significantly higher HDL and lower LDL concentrations among ART-treated individuals, while untreated participants exhibited more atherogenic lipid patterns. These findings are consistent with multicentric analyses indicating that tenofovir-based regimens may improve HDL fractions but that long-term ART, depending on drug class and duration, can still cause dyslipidemia [8]. Protease inhibitor–based regimens, in contrast, are associated with increased triglycerides and atherogenic lipid changes [8]. Hence, continuous lipid monitoring remains essential in all individuals receiving ART [9].

## Glycaemic Parameters

Fasting blood sugar levels were significantly lower among ART-HIV subjects compared with untreated patients, though HOMA-IR values remained elevated in both groups. This suggests that ART partially mitigates hyperglycemia through viral suppression and reduction of inflammation, yet insulin resistance persists due to mitochondrial dysfunction and altered adipokine signaling [9,10]. These findings emphasize the need for routine metabolic screening and early lifestyle or pharmacologic interventions in HIV care.

## Trace Elements and Biomarkers

The most prominent differences were observed in trace element and biomarker profiles. ART-HIV subjects exhibited significantly reduced serum zinc and elevated copper, ceruloplasmin, and metallothionein levels. These results corroborate existing literature showing that HIV infection and ART disrupt trace element homeostasis by promoting oxidative stress and cytokine-mediated inflammatory responses [10]. Zinc deficiency impairs immune regulation, while increased copper and ceruloplasmin levels reflect acute-phase inflammation and oxidative burden [11]. Elevated metallothionein concentrations further indicate adaptive responses to redox imbalance and altered zinc–copper dynamics [11].

The positive correlation between copper and ceruloplasmin, along with the inverse association between zinc and ceruloplasmin, is biologically plausible and consistent with reports in other chronic infections, suggesting a compensatory antioxidant mechanism against free-radical–induced cellular injury [12].

## Clinical Relevance

Overall, these findings indicate that while ART confers partial metabolic benefits by improving lipid and glycaemic indices, it does not fully normalize and may exacerbate trace element imbalances. Persistent zinc deficiency and copper excess can impair immune recovery, elevate oxidative stress, and increase the risk of cardiometabolic disorders [12]. Integrating nutritional surveillance, zinc repletion, and antioxidant supplementation into ART follow-up protocols may enhance overall treatment outcomes and quality of life for individuals living with HIV.

## Generalizability

The findings of this study provide important insights into trace element alterations, lipid metabolism, and glycaemic regulation in HIV-infected individuals, with and without ART. However, as the study was conducted at a single tertiary care center in South India with a modest sample size, results may not be universally applicable. Variations in dietary practices, ART regimens, genetic background, and healthcare access could influence outcomes across different populations. Nonetheless, the observed trends are consistent with global evidence, supporting external validity. Larger, multicentric, and longitudinal studies are warranted to enhance generalizability and to establish standardized nutritional monitoring protocols in HIV care.

## Conclusion

This study demonstrates that HIV infection and antiretroviral therapy significantly influence nutritional and biochemical profiles. While ART was associated with improved BMI, favorable lipid modulation, and lower fasting glucose, persistent insulin resistance was evident. Importantly, ART-HIV subjects exhibited reduced serum zinc alongside elevated copper, ceruloplasmin, and metallothionein, reflecting disrupted trace element homeostasis and heightened oxidative stress. These findings suggest that, despite the therapeutic benefits of ART, micronutrient imbalance may predispose to long-term metabolic and immunological complications. Routine monitoring of trace elements, coupled with zinc supplementation and dietary interventions, could be

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valuable adjuncts in optimizing care and preventing complications in HIV-infected patients.

## Limitations

This study was limited by its cross-sectional design, which restricts causal inference, and by the lack of detailed dietary assessment or ART regimen stratification. Larger, multicentric, and longitudinal studies are warranted to validate these associations and clarify causal pathways.

## Recommendations

Based on the findings, it is recommended that routine monitoring of trace elements, particularly zinc and copper, should be integrated into HIV care alongside standard biochemical assessments. Nutritional counseling with emphasis on balanced diets and targeted supplementation, especially zinc, may help mitigate micronutrient deficiencies and improve immune resilience. Periodic screening for lipid and glycaemic alterations is essential to detect early metabolic derangements associated with HIV and ART. Multicentric, longitudinal studies are warranted to validate these observations and formulate evidence-based guidelines. Incorporating trace element evaluation into HIV management could optimize long-term outcomes and reduce secondary complications.

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## Abbreviations

HIV: Human Immunodeficiency Virus;
ART: Antiretroviral Therapy;
BMI: Body Mass Index;
BP: Blood Pressure;
HDL: High-Density Lipoprotein;
LDL: Low-Density Lipoprotein;
FBS: Fasting Blood Sugar;
HOMA-IR: Homeostatic Model Assessment of Insulin Resistance;
ICTC: Integrated Counselling and Testing Centre;
CD4: Cluster of Differentiation 4;
ELISA: Enzyme-Linked Immunosorbent Assay;
TAG: Triacylglycerol.

## Source of funding

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## Conflict of interest

The Author declares no conflict of interest.

## Author contributions

**TN**-Concept and design of the study, results interpretation, review of literature, and preparation of the first draft of the manuscript. Statistical analysis and interpretation, revision of manuscript. -Concept and design of the study, results interpretation, review of literature, preparing the first draft of the manuscript, and revision of the manuscript. Review of literature and preparing the first draft of the manuscript. Statistical analysis and interpretation. - preparing first draft of manuscript. Statistical analysis and interpretation, revision of the manuscript

## Data availability

Data available on request

## Author Biography

**Dr. T. Nageswararao** is currently serving as an Associate Professor of Pharmacology at Konaseema Institute of Medical Sciences and Research Foundation, Amalapuram, East Godavari District, Andhra Pradesh, India. He holds a doctoral degree in Medical Pharmacology from the prestigious Index Medical College and Research Institute, Madhya Pradesh. With over 16 years of teaching experience in various medical institutions, Dr. Nageswararao has made significant contributions to the field of pharmacology. His academic work includes nine research publications in reputed national and international journals, including one article indexed in PubMed. His dedication to research and education continues to enrich the academic and clinical landscape of medical pharmacology.**ORCID iD**:https://orcid.org/0009-0005-1638-6897

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Dr. Thomas has served in progressively senior academic roles, including her current position as Senior Lecturer, Department of Clinical Pharmacology, St Joseph College of Health and Allied Sciences, Dar es Salaam, Tanzania. Previously, she was Associate Professor and Head of Pharmacology at Kampala International University, Tanzania, where she played a pivotal role in elevating the Department of Pharmacy into a full-fledged Faculty of Pharmacy. She also held faculty and leadership positions at the International Medical and Technological University (IMTU), Tanzania.

Her earlier career includes valuable research and industry experience as a Research Assistant in the Department of Medicine and Therapeutics at the University of Aberdeen, UK, and as Project Leader in Genetic Toxicology at Charles River Laboratories, Edinburgh, UK. These roles provided her with extensive expertise in molecular pharmacology, toxicology, chemoprevention, and laboratory management under GLP and GCP standards.

With more than 10 peer-reviewed publications in national and international journals, Dr. Thomas's research contributions span cancer chemoprevention, polyamine metabolism, diabetes models, drug formulation, complementary medicine, and addiction studies. She has also contributed book chapters and presented at international conferences. In addition, she has supervised three master's students and nearly thirty undergraduates, reflecting her strong commitment to mentorship.

Dr. Thomas is a member of the British Pharmacological Society (UK) and the Kerala Pharmacy Council (India). She has also served as Editor of the Indian Journal of Mednodent and Allied Sciences (IJOMDAS). Her academic profile is enriched by international workshops and certifications in neuroscience, genetics, pharmacovigilance, GLP, and clinical research methods.

Beyond academia, Dr. Thomas is deeply engaged in current research projects on antimicrobial misuse, lifestyle influences on endometriosis, and sanitation-related child health issues in Tanzania. Her achievements highlight not only her scientific rigor but also her commitment to addressing pressing global health challenges.

In addition to her academic and research pursuits, she has a keen interest in literature, classical music, and creative writing, having won prizes in essay and elocution competitions. Her career exemplifies a blend of scholarship, leadership, and mentorship, making her a distinguished figure in the field of Pharmacology.

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