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Original Research Article

Investigating the Effects of Aqueous Leaf Extracts from *Moringa oleifera* and *Carica papaya* on Chloramphenicol-Induced Anaemia in Wistar Rats

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ABSTRACT

This study examined the hematinic effect of the aqueous leaf extracts of *Moringa oleifera* (MOAE) and *Carica papaya* (CPAE). Thirty-six physically fit and disease-free rats in adulthood were partitioned into six groups, consisting of six rats per group. Group 1 acted as the non-anemic control group and was given 5ml/kg of distilled water. Anemia was generated in rats in groups 2 to 6 with the oral administration of chloramphenicol at a dosage of 50 mg/kg. Group 2 acted as the anemic control group and was given 5ml/kg of distilled water. Group 3 was given 100 mg/kg of ferrous gluconate. Groups 4 to 6 were given 500 mg/kg of MOAE, 500 mg/kg of CPAE, and a combination of 500 mg/kg of MOAE and 500 mg/kg of CPAE, respectively. The treatment was administered orally for 28 days, following which the PCV, Hb, and WBC count of the animal models were examined. The results demonstrated that chloramphenicol caused a statistically significant reduction in PVC and Hb, indicating anemia, as well as a significant rise in WBC count. MOAE and CPAE resulted in a statistically significant rise in PCV and Hb levels, along with a reduction in white blood cell (WBC) count. The hematinic impact seen when the extracts were given together was significantly greater ($p< 0.05$) than when either extract was given alone. The extracts of *Moringa oleifera* and *Carica papaya* showed an additional blood-building impact when given together. This observation may be beneficial for managing anemia.

***Keywords*:** *Moringa oleifera*, *Carica papaya*, Additive, Haematinic, Chloramphenicol, Wistar rats

Introduction

Anemia is a medical illness marked by a substantial drop in the red blood cells, concentration of hemoglobin, and volume of packed cells. 1 The decline in all three results from three possible conditions: lower generation of RBCs, greater destruction of RBCs, and excess loss of blood. [2] Various factors can lead to anemia, these factors encompass genetic illnesses, environmental influences such exposure to specific medications or poisons, and infections caused by bacteria, viruses, and fungi.[3] As anemia advances, several key symptoms start to appear. Symptoms may include weakness, dyspnea, and malaise. A more severe type of anemia results in symptoms like confusion, dizziness, fainting, and paleness.[3] The significant value of therapeutic plants has been well recognized throughout history.[4–6] These ancient botanical marvels still fascinate academics and practitioners today. The World Health Organization acknowledges medicinal plants for their abundance of chemical metabolites, which possess therapeutic properties and can serve as crucial components for chemo-pharmaceutical semi-synthesis. [7]

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Moringa oleifera, a rapidly growing tree from the Moringaceae family, is native to Northern India but flourishes in tropical and subtropical areas worldwide. [8,9] It is commonly found around farms and complexes in Somalia, frequently used as a natural barrier. In this area, it acts as both a nutritious green vegetable alternative and a component in several medicinal methods.[9] Recent focus has been on the pharmacological properties of *Moringa oleifera* due to its extensive use in treating various illnesses.[10] The ethanol extract from the plant's leaves has shown strong antifungal effects against various dermatophytes. [11] Moreover, the methanol extract has exhibited a sedative impact on the central nervous system, whilst the aqueous extract has displayed potential in reducing fertility. *Moringa oleifera* is a crucial resource for managing diabetes mellitus, supported by clinical evidence of its hypoglycemic benefits. [10]

Carica papaya, a member of the Caricaceae family commonly referred to as the pawpaw tree, has distinctive traits in its biological habitat.[12] Experiencing swift development, this plant usually grows in an erect, mostly unbranched manner, reaching heights of 7-8 meters with a trunk diameter of approximately 20 centimeters. [12] The presence of a large amount of latex throughout its structure enhances its ability to withstand and adapt to various situations[12]. Originating from areas across Asia and sub-Saharan Africa, like Somalia, this plant has attracted significant interest due to its diverse healing powers. [13]

Traditional medicine has utilized different parts of the *Carica papaya* plant for their effectiveness in treating a wide range of health issues.[13,14] The recorded therapeutic properties encompass amoebicidal, antibacterial, anti-inflammatory, and laxative effects. The range of numbers is from 15 to 17. In Nigeria, it is employed for the treatment of upper respiratory tract ailments and uterine tumors, however in Ivory Coast, it is utilized as a remedy for mental health disorders.[12] In Trinidad, it is used to treat scorpion stings and

hypertension, whereas in Cote d'Ivoire and Sama, it is used for toothaches.[12] Mexico has incorporated it into tuberculosis treatment procedures, while Honduras and Turkey use it for liver diseases and constipation due to its laxative properties.[12,18] It is praised for its effectiveness in treating arthritis and rheumatism in the Philippines.[19] In West Africa, it is used to fight against diarrhea and dysentery.[19]
This study aims to investigate the hematinic effects of aqueous leaf extracts from *Moringa oleifera* and *Carica papaya*, both individually and in combination, on rat models. The main goal is to determine if there are any beneficial pharmacological interactions, such as synergistic, potentiating, or additive effects, in the given formulations.

Materials and Methods

The chemicals used in this study were obtained from Sigma Chemical Co. Ltd (USA). Ferrous gluconate (ThermoFisher Scientific, USA.) was bought from Mogadishu.

Experimental Animals
A group of healthy adult Wistar rats, of different genders, weighing between 120g and 190g were gently placed in cages and exposed to natural light cycles for 7 days to help them adapt to their new surroundings. During the acclimatization phase, the animals had a high-quality meal (Growers Mash, AfriMash) for rodents and had access to clean water. Ethical clearance was sort and received from the ethical reviewing committee with the ID:MED/RES/23-26.

Plant Collection and Identification
The leaves of *Moringa oleifera* and *Carica papaya* were gathered from a natural area in Mogadishu, Somalia from the period of December, 2023 – January 2024. The plants were recognized using a standard identification key,[20,21] and herbarium codes / voucher code were assigned for *Moringa oleifera* (MO-SOM1) and *Carica papaya* (CP-SOM1)

Preparation of Extracts
The leaves of *Moringa oleifera* and *Carica papaya* were dried for five days and then pulverized individually using a blender.[7] Extraction was conducted using the cold maceration method. Each batch of pulverized leaves weighing 1500 grammes was steeped in distilled water individually for 48 hours while being gently shaken. The extracts were acquired with the use of Whatman filter paper. The extracts were concentrated using a water bath set at 60^{o}c.[22] The study will refer to the extracts of *Moringa oleifera* as MOAE and *Carica papaya* as CPAE.

Toxicity Screening
Lorke's technique was used to examine the LD_{50} of the plant extracts.[23] The Lorke's method is a two-phase procedure employed to determine the lethal dose (LD50) of a substance. In Phase 1, a total of nine animals were divided into three groups. Each group is given doses of 100 mg/kg and 500 mg/kg, respectively. These animals are continuously monitored for a complete 24-hour period to observe their behaviour and identify any probable occurrences of mortality. During Phase 2, three animals were employed, with each animal allocated to an individual group. The animals were administered doses of 100 and 500 mg/kg, which were of the same magnitude, and their condition was observed for 24 hours. The LD50 was calculated by establishing the dose at which no fatalities occur (D0) and the dose at which all individuals perish (D100).

Induction of Anemia
Anemia was created by administering chloramphenicol orally at a dosage of 50 mg/kg for a period of 2 weeks. Anemia was confirmed by the markedly reduced packed cell volume (PCV) compared to untreated rats.[22]

Hematinic Screening
Thirty-six rats were used in the investigation, 30 of which had anaemia and 6 did not.
Group 1: Control (Distilled water and 5ml/kg po).
Group 2: Chlorophenicol (50mg/kg, po)
Group 3: Chlorophenicol + Ferrous gluconate (100mg/kg, po)
Group 4: 500 mg/kg of MOAE)
Group 5: 500 mg/kg of CPAE).
Group 6: 500 mg/kg of MOAE and 500 mg/kg of CPAE).
The treatment regimen was given once daily for 28 days. During the treatment period, all animals were provided with water and standard rodent pellets. Blood samples were taken before and after administering the extracts and reference medication via venipuncture to evaluate PCV, Hb, and WBC values.[22]

Statistical Analysis
The dataset was accompanied by the mean and the standard error of the mean (SEM). To assess statistical disparities, we employed a one-way analysis of variance (ANOVA). Subsequently, the Tukey-Kramer and Student-Newman-Keuls multiple comparison tests were conducted. Results were deemed statistically significant if the p-value was below 0.05.

Results and Discussion

During the acute toxicity investigations, no fatalities or indications of toxicity were observed at dosages of up to 5000 mg/kg for both MOAE and CPAE. Using Lorke's approach, it was determined that the oral LD50 for each extract was greater than 5000 mg/kg, indicating a substantial level of safety as represented in Table 1-2.Tables 3-5 display the impact of the water-based leaf extracts from *Moringa oleifera* and *Carica papaya* on the blood-related characteristics of anemic Wistar rats. Rats treated with chloramphenicol showed a substantial decrease in PCV and hemoglobin levels compared to non-anemic control group ($p< 0.05$). Chloramphenicol resulted in a substantial increase in white blood cell (WBC) count when compared to the group without anemia ($p< 0.05$). After 28 days of treatment, the leaf extracts of *Moringa oleifera* and *Carica papaya* resulted in a significant rise in PCV (packed cell volume) and Hb (hemoglobin) levels, as well as a substantial decrease in WBC (white blood cell) count. The concurrent administration of the extracts yielded a significantly enhanced impact ($p< 0.05$) in comparison to their solo administration. Orthodox medicine often prescribes several types of iron, such as ferrous sulphate and ferrous fumarate, to cure anemia.[1] However, these treatments have numerous side effects including nausea, vomiting, abdominal pain, gastric discomfort, and constipation.[2] Therefore, natural-derived active compounds for treating anemia have garnered global interest from both patients and researchers as a potentially beneficial alternative therapeutic option. This study aimed to investigate the hematinic properties of the leaves of two medicinal plants, *Moringa oleifera* and *Carica papaya.* Rats were given individual doses of aqueous leaf extracts from *Moringa oleifera* and *Carica papaya*, as well as a combined dose to investigate potential positive pharmacological interactions.
Anemia is characterized by three primary mechanisms: bleeding, reduced production of red blood cells, or increased breakdown of red blood cells. In this scenario, the main function of red blood cells is to convey oxygen from the lungs to various tissues in the body.[3] Therefore, any disorder that impacts the population, structure, or function of the red blood cells could be harmful to life.[24] In this study, following the administration of chloramphenicol, a potent antibiotic recognized for its propensity to induce severe idiosyncratic bone marrow damage,[25] Anemia was induced, as evidenced by the reduction in PCV (packed cell volume) and hemoglobin levels in rats. The rats' leukocyte counts significantly rose following administration of chloramphenicol.
Chloramphenicol is categorized as a top allergenic medication that affects the immune system, leading to a reduction in the number of white blood cells.[25] Treatment with *Moringa oleifera* and *Carica papaya* extracts separately resulted in a notable rise in PVC and Hb levels and a reduction in WBC count in anemic rats. The findings

supported earlier research indicating that the rise in PCV and hemoglobin levels in rats following treatment with extracts were clear indicators of recovery from anemia.[6,21] The combination of these two extracts led to a substantial increase in PVC and Hb levels, as well as a drop in WBC count. The observed hematinic effects of these extracts may be attributed to enhanced erythropoiesis.[21, 26] The extracts exhibited additive effects, indicating a positive pharmacological interaction that could be further investigated for improving anemia.

Table 1: Acute Toxicity of the Aqueous Leaf Extract of *Moringa oleifera* in Wistar Rats

Phase	Group	Treatment (mg/kg)	D/T	Observed Sign of Toxicity
I	1	MOAE (10)	0/3	-
	2	MOAE (100)	0/3	-
	3	MOAE (1000)	0/3	-
II	1	MOAE (1600)	0/1	-
	2	MOAE (2900)	0/1	-
	3	MOAE (5000)	0/1	-

Key: D= death, T=Total number of animals used

Table 2: Acute Toxicity of the Aqueous Leaf Extract of *Carica papaya* in Wistar Rats

Phase	Group	Treatment (mg/kg)	D/T	Observed Sign of Toxicity
I	1	CPAE (10)	0/3	-
	2	CPAE (100)	0/3	-
	3	CPAE (1000)	0/3	-
II	1	CPAE (1600)	0/1	-
	2	CPAE (2900)	0/1	-
	3	CPAE (5000)	0/1	-

Key: D= death, T=Total number of animals used

Table 3: Effect of the Administration of Aqueous Leaf Extracts of *Moringa oleifera* and *Carica papaya* on PCV of Chloramphenicol-induced Anaemia in Wistar Rats

Treatment	Pre- treatment (g/dl)	Post- treatment (g/dl)
Non-anaemic control (5ml/kg dist. H_2O)	-	43.18 ± 4.34^{c}
Anaemic control (5ml/kg dist. H_2O)	16.4±1.08	17.8 ± 1.21^{a}
Ferrous gluconate (100 mg/kg)	17.7±1.21	32.4 ± 4.26^{b}
MOAE (500 mg/kg)	16.3±1.45	25.5 ± 3.15^{ab}
CPAE (500 mg/kg)	17.8±1.99	28.1 ± 3.91^{ab}
MOAE (500mg/kg) + CPAE (500 mg/kg)	16.0±1.23	40.8 ± 4.73^{c}

The data is presented as the mean ± standard deviation with a sample size of 6. Mean values with different alphabetic superscripts in the column are significantly different at a significance level of P<0.05.

Table 4: Effect of the Administration of Aqueous Leaf Extracts of *Moringa oleifera* and *Carica papaya* on Haemoglobin (g/dl) of Chloramphenicol-induced Anaemia in Wistar Rats

Treatment	Pre-treatment (g/dl)	Post- treatment (g/dl)
Non-anaemic control (5ml/kg dist. H_2O)	-	11.9 ± 1.96^{c}
Anaemic control (5ml/kg dist. H_2O)	8.7±0.29	6.3 ± 0.31^{a}
Ferrous gluconate (100 mg/kg)	7.5±0.27	9.7 ± 0.28^{b}
MOAE (500 mg/kg)	8.6±0.45	8.1 ± 0.51^{ab}
CPAE (500 mg/kg)	7.1±0.26	9.6 ± 0.46^{b}
MOAE (500mg/kg) + CPAE (500 mg/kg)	7.6±0.57	11.5 ± 0.23^{c}

The data is presented as the mean ± standard deviation with a sample size of 6. Mean values with different alphabetic superscripts in the column are significantly different at a significance level of P<0.05.

Table 5: Effect of the Administration of Aqueous Leaf Extracts of *Moringa oleifera* and *Carica papaya* on White Blood Count ($x10^3/mm^3$) of Chloramphenicol-induced Anaemia in Wistar Rats

Treatment	Pre- treatment (g/dl)	Post- treatment (g/dl)
Non-anaemic control (5ml/kg dist. H_2O)	-	4.1 ± 0.66^{a}
Anaemic control (5ml/kg dist. H_2O)	7.5±0.23	7.6 ± 0.23^{b}
Ferrous gluconate (100 mg/kg)	7.4±0.46	3.8 ± 0.45^{a}
MOAE (500 mg/kg)	7.8±0.83	3.9 ± 0.81^{a}
CPAE (500 mg/kg)	7.6±0.48	3.8 ± 0.23^{a}
MOAE (500 mg/kg) + CPAE (500 mg/kg)	7.9±0.95	4.1 ± 0.56^{a}

The data is presented as the mean ± standard deviation with a sample size of 6. Mean values with different alphabetic superscripts in the column are significantly different at a significance level of P<0.05.

Conclusion

When *Moringa oleifera* and *Carica papaya* extracts are given together, they show an additional impact in improving blood hematological parameters and whole blood volume. This insight may be valuable in the treatment of anemia.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

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

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