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Evaluation of the effect of an aqueous extract of *Moringa oleifera* (Moringaceae) leaves on the biochemical and blood parameters of Cobb 500 broiler chickens

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Abstract

To better utilize *Moringa oleifera* leaves, a pharmacological study was conducted on the aqueous extract of the plant's leaves in the context of animal health. Thus, phytochemical analysis of the aqueous extract of *Moringa oleifera* leaves revealed the presence of sterols, polyterpenes, polyphenols, flavonoids, quinones, and catechin tannins. The acute toxicity test conducted according to OECD 423 showed that the aqueous extract of *Moringa oleifera* leaves is not toxic when administered orally as a single dose at 2000 mg/kg body weight. Administration of this extract resulted in an increase in the live weight of chickens and a reduction in the feed conversion ratio. Measurement of biochemical markers showed that the aqueous extract of *M. oleifera* leaves reduced urea and AST levels in chickens. Regarding the immune system, this extract increased white blood cell counts, neutrophils, and lymphocytes in broiler chickens. The leaves of this plant could therefore be used as an immunostimulant in broiler chickens.

Keywords: Broiler Chickens; *Moringa oleifera*; Biochemical; Blood

1. Introduction

Livestock farming is a fundamental sector in a country's economic development and helps meet the population's need for animal protein. Unfortunately, this industry faces many disease-related problems, leading to a high mortality rate (65–70%) among chicks aged 0 to 4 weeks, as well as a decline in performance [1]. To address this situation, developing countries are turning to traditional medicine. Indeed, medicinal plants offer credible alternatives for resolving public health issues. According to [2], more than 80% of the African population relies on plants for their healthcare needs. Furthermore, medicinal plants are important for drug research and development [3]. Furthermore, in animal health, these medicinal plants play a significant role among livestock farmers in developing countries [4]. In Côte d'Ivoire, several studies have demonstrated the benefits of using plants in animal health [5]. Among the medicinal plants of interest is *Moringa oleifera*, a tropical plant with multiple uses. It is used as a supplement in animal feed [4], in agroforestry, and in industry [7]. It is also known for its antioxidant, anticancer, and anti-inflammatory properties [8].

In light of the above, the overall objective of this study was to explore the potential of *Moringa oleifera* leaves by evaluating the effect of an aqueous extract of *Moringa oleifera* leaves on the biochemical and blood parameters of broiler chickens

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2. Materials and Methods

2.1. Biological materials

2.1.1. Plant material

The plant material consisted of fresh leaves of *Moringa oleifera* (Moringaceae) collected in the town of Abobo in July 2022, identified and authenticated at the National Floristic Center of Félix Houphouët Boigny University under the number UCJ012875.

2.1.2. Animal stock

The animal stock consisted of one-day-old *Gallus gallus domesticus* chicks of the Cobb 500 strain, weighing an average of 38 g, sourced from IVOGRAIN. This strain was chosen for its high-quality meat, its widespread consumption in Côte d'Ivoire, and its rapid production cycle.

2.2. Equipment

2.2.1. Farming equipment

A waterer and a feeder were used to feed the chickens. A scale was used to weigh the chickens and the feed. Three types of feed were used, depending on the chicks' age.

2.2.2. Veterinary Products

The veterinary products used were Covit (an antibiotic), Stressmix (a vitamin-based stress reliever), vaccines for Gumboro disease, Newcastle disease, and infectious bronchitis, and a disinfectant such as Virkon.

2.3. Preparation of the aqueous extract

The leaves were rinsed with distilled water and then dried in the laboratory at room temperature, away from direct sunlight, for two (2) weeks. They were ground into a fine powder using a grinder. A quantity of 100 g of powdered plant leaves was dissolved in one liter of distilled water, homogenized, and macerated using a flask containing a magnetic bar placed on a magnetic stirrer for twenty-four hours at room temperature at a speed of 3000 rpm. The resulting macerate was filtered successively through poplin cloth, then through cotton wool, and finally through Whatman paper. The filtrate was dried in an oven at 40°C in the Biochemistry Laboratory to obtain the dry extract. The resulting extract was stored at 4°C for 72 hours [9].

2.4. Phytochemical Screening

The characterization of the various chemical groups was performed using the colorimetric techniques described in [10] and [11].

The chemical compounds of interest are sterols and polyterpenes, alkaloids, polyphenols, flavonoids, saponins, and tannins.

2.5. Study of the acute toxicity of the aqueous extract of *Moringa oleifera*

The acute toxicity study was conducted in accordance with Organization for Economic Cooperation and Development (OECD) Guideline 423 [12]. The study involved nine (9) chickens, six (6) of which were used for the test and three (3) as controls. From day 1 to day 21, the chickens received antibiotics and vaccines in accordance with recommended prophylaxis. On day 21, the chickens were divided into two groups of three (3) chickens each. Thus, the first group received the aqueous extract of *Moringa oleifera* at a single dose of 2000 mg/kg body weight, and the other group received distilled water. The chickens were fasted overnight prior to the administration of the extract. After the fasting period, the animals were weighed. They were then administered the extract at a dose of 2000 mg/kg body weight and were again deprived of food for 3 to 4 hours.

The chickens were observed individually at least once during the first 30 minutes and regularly for 24 hours following treatment. Particular attention was paid during the first 4 hours and daily for 14 days following administration of the extract. All chickens were observed at least twice a day to record any signs of toxicity. Observations focused on changes in feathers, beak, eyes, tremors, convulsions, diarrhea, sleep, and coma

2.6. Preparing the Building

Before the chicks arrived, the broiler house underwent a deep cleaning. This involved removing all movable equipment from the room, followed by a thorough wash with water and disinfection using Virkon. A few days before the chicks arrived, the floor of the building was covered with wood shavings. The waterers and feeders were also disinfected with Virkon. Light bulbs were installed to provide nighttime lighting in the building.

2.7. Arrival and allocation of chickens into groups

Forty-eight (48) one-day-old chicks from IVOGRAIN were transported to the farm located at the Bingerville Central Veterinary Laboratory. The chicks were randomly divided into 3 groups of 16.

- Control group 1: received the antibiotic COVIT at a dose of 1 g/L in drinking water
- Group 2: the chicks received EAMO at a dose of 0.5 g/L in drinking water
- Group 3: chicks received EAMO at a dose of 1 g/L in drinking water

2.8. Evaluation of zootechnical parameters

Zootechnical parameters, including average live weight (ALW), feed conversion ratio (FCR), mortality rate (MR), and relative organ weights, were calculated using the following formulas.

2.8.1. Average live weight (ALW)

The average live weight is the ratio of the sum of the weights of the individuals in a given batch to the number of individuals in that batch.

verage live weight = Total weight of the chickens in a given flock / Number of

2.8.2. Feed Conversion Ratio (FCR)

This is the ratio of the average amount of feed consumed over a given period to the corresponding average weight gain during that period.

FCR = amount of feed consumed / (final live weight – initial live weight)

2.8.3. Relative organ weight

This is the ratio of the weight of the organs to the body weight, multiplied by 100

Relative weight (g) = (relative weight of the organ (g) / body weight (g) * 100

2.8.4. Slaughter of Animals

At the end of the treatment, three (03) chickens from each batch were slaughtered. Blood was collected in EDTA tubes for hematological analysis and in dry tubes for biochemical analysis. The organs—liver, heart, kidneys, gizzard, and bursa of Fabricius—were removed and weighed to calculate their relative weights.

2.9. Evaluation of Biochemical and Blood Parameters

2.9.1. Biochemical Analysis

Blood collected in dry tubes (red tubes) was centrifuged at 3,000 rpm for 5 minutes. The resulting serum was collected and stored at -20°C for the measurement of biochemical parameters, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, and creatinine, using the Cobas C 311 autoanalyzer

2.9.2. Hematological Analysis

Blood collected in purple tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA) was used to measure white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb), and hematocrit (HCT). The mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), neutrophils, and

lymphocytes were also measured. All these hematological parameters were analyzed using an automated analyzer (SYSMEX XN 1000).

2.10. Analysis of Results

The various data collected were statistically analyzed using GraphPad software, version 8. Values are presented as mean ± standard error. The significance level of differences between means was assessed using the t-test at a 5% significance level.

3. Results

3.1. Phytochemical composition of *Moringa oleifera*

A phytochemical study of the aqueous extract *of Moringa oleifera* (EAMO) revealed the presence of several chemical compounds, including sterols, polyterpenes, polyphenols, flavonoids, quinones, and catechin tannins.

3.2. Acute Toxicity

Administration of a single dose of 2000 mg/kg body weight of EAMO resulted in no mortality among the treated chickens. Observation of the chickens 30 minutes after administration, and then at regular intervals over the next 14 days, revealed no clinical signs of toxicity.

3.3. Effect of the extract on zootechnical, biochemical, and blood parameters

3.3.1. Zootechnical parameters

Feed conversion ratio

The feed conversion ratio for chickens treated with 0.5 g/L and 1 g/L of EAMO was 1.29 and 1.31, respectively, while that of the control group was 1.60. Statistical analysis of these values shows that the feed conversion ratio of the treated chickens was significantly lower (P>0.05) than that of the control group (**Figure 1).**

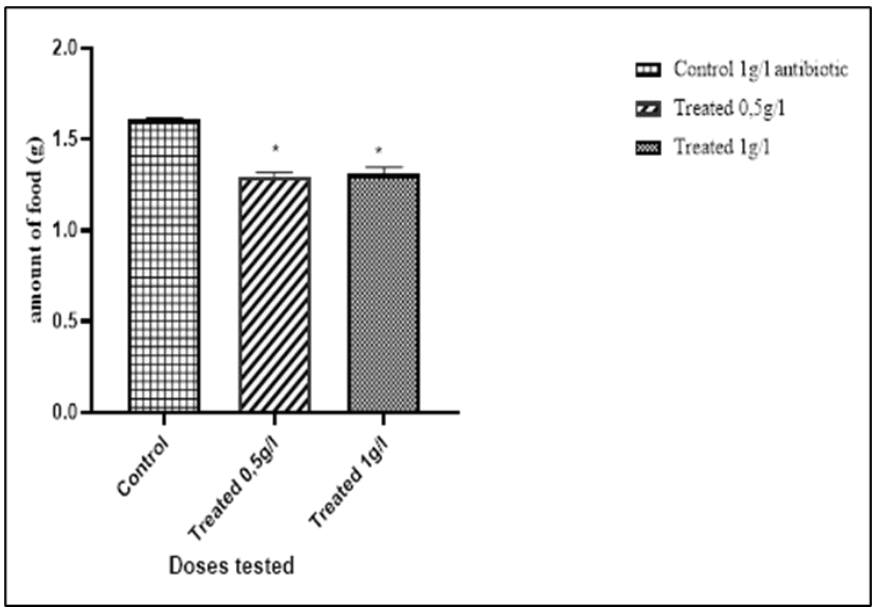

Figure 1 Changes in the feed conversion ratio of chickens following administration of different doses of EAMO

3.3.2. Average Live Weight

The average live weight of the treated chickens was 1140 ± 0.1258 g and 1280 ± 0.0675 g at doses of 0.5 and 1 g/L of EAMO, respectively. In contrast, the average live weight of the control group was 1011 ± 0.0356 g. Statistical analysis shows that the average weights of the treated chickens are significantly higher (P < 0.05) than that of the control group (**Figure 2**).

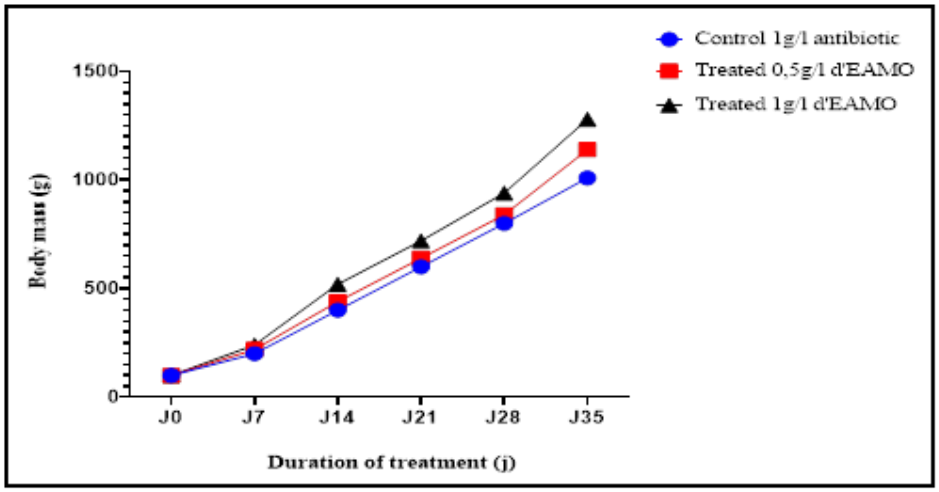

Figure 2 Changes in body weight of chickens during the treatment period

Mortality rate

During the rearing of the chickens, no mortality was observed in the three experimental groups.

3.3.3. Relative organ weights

The results showed that the relative weights of the liver, kidneys, heart, and gizzard in the treated groups were lower than those in the control group. For the liver, the values were 18.75 ± 1.060 and 18.96 ± 0.8467 at doses of 0.5 and 1 g/L of EAMO, respectively, for the treated groups, and 19.81 ± 0.6021 for the control group. For the kidneys, these values are 0.2100 ± 21.60 and 0.2200 ± 21.59 at the respective doses of 0.5 and 1 g/L of EAMO for the treated batches and 0.2800 ± 21.53 for the control batch. The heart weight of the treated batches is 5.817 ± 15.99 and 5.867 ± 15.94 at respective doses of 0.5 and 1 g/L of EAMO, and 6.167 ± 15.64 for the control. For the gizzard, the values are 17.55 ± 4.260 and 17.59 ± 4.223 for the treated batches at respective doses of 0.5 and 1 g/L of EAMO, and 18.01 ± 3.797 for the control batch. Meanwhile, the relative weight of the bursa of Fabricius in the batches treated with doses of 0.5 and 1 g/L of EAMO was higher (0.6467 ± 21.16 and 0.7500 ± 21.06) than that of the control batch (0.5867 ± 21.22). Statistical analysis shows no significant difference ($P > 0.05$) between the relative weights of these different organs in the treated chickens compared to the controls (**Figure 3**).

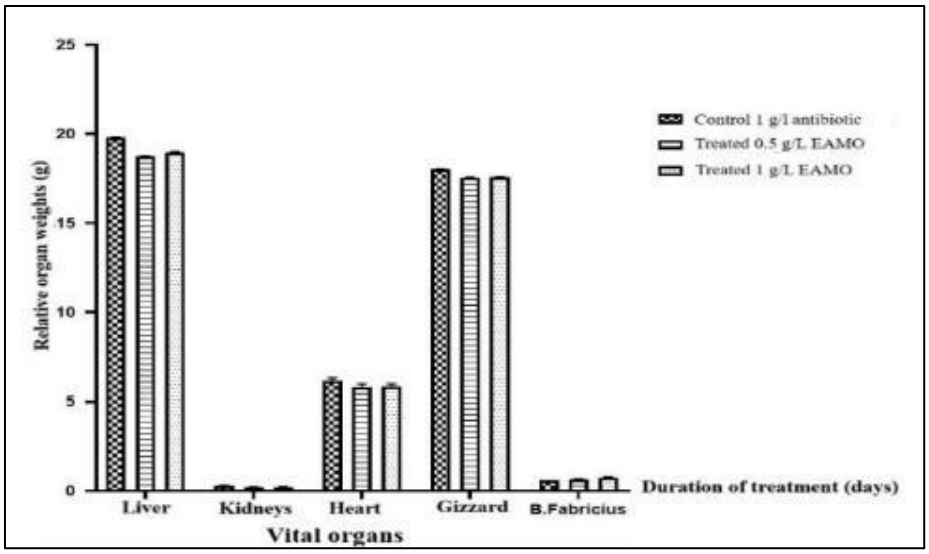

Figure 3 Effect of Moringa oleifera aqueous extract on organ weight

3.4. Effect of the aqueous extract on biochemical and blood parameters

3.4.1. Effect of EAMO on biochemical markers

Analysis of the results showed that the levels of urea, AST (aspartate aminotransferase), ALT (alanine aminotransferase), and creatinine in chickens treated with EAMO were lower than those in the control group. The urea level in the control chickens was 3.887 ± 0.8749 mmol/L, while those in the treated group were 2.213 ± 0.7823 and 2.220 ± 0.7792 mmol/L at doses of 0.5 and 1 g/L of EAMO, respectively. Regarding AST, the control group's level was 147.40 ± 67.07 U/L, while those of the treated groups were 133.51 ± 60.67 and 134.0 ± 60.85 U/L at doses of 0.5 and 1 g/L of EAMO, respectively. Regarding ALAT, the results showed that the control group's level was 6.533 ± 1.237 U/L, while those of the treated groups were 5.900 ± 0.9413 and 5.867 ± 0.9257 U/L at respective doses of 0.5 and 1 g/L of EAMO. Creatinine in the control group was 5.600 ± 0.8010 mmol/L, while in the treated groups it was 5.133 ± 0.5829 and 5.047 ± 0.5423 mmol/L at doses of 0.5 and 1 g/L of EAMO, respectively. Statistical analysis showed that only the urea and AST levels in chickens treated with the aqueous extract of *Moringa oleifera* were significantly lower ($P < 0.05$) than those in the control chickens (**Figure 4**).

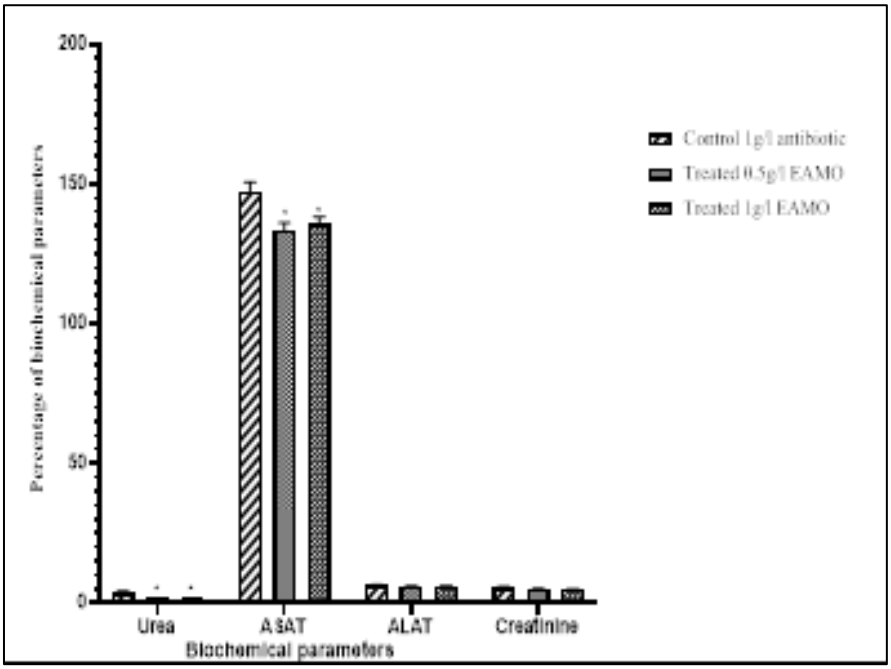

Figure 4 Effect of *Moringa oleifera* aqueous extract on biochemical parameters

3.4.2. Effect of EAMO on blood parameters

- Effect of EAMO on erythrocyte parameters

Administration of the aqueous extract of *Moringa oleifera* leaves showed that the erythrocyte parameters of the treated groups were lower than those of the control group.

For red blood cells (RBCs), the values for the treated groups were 2.250 ± 0.4000 and 2.367 ± 0.2833 at doses of 0.5 and 1 g/L of EAMO, respectively, and 2.650 ± 0.2383 for the control group. For hemoglobin (HGB), the values for the treated batches were 9.967 ± 7.317 and 9.767 ± 7.117 at doses of 0.5 and 1 g/L of EAMO, respectively, and 12.20 ± 9.550 for the control batch. Regarding mean corpuscular volume (MCV), these values varied for the treated groups from 114.3 ± 111.70 and 115.1 ± 112.5 at doses of 0.5 and 1 g/L of EAMO, respectively, to 116.1 ± 113.4 for the control group. The hematocrit (HCT) levels for the treated groups were 23.03 ± 20.38 and 24.78 ± 22.13 at doses of 0.5 and 1 g/L of EAMO, respectively, and 27.07 ± 24.42 for the control group. The mean corpuscular hemoglobin concentration (MCHC) was 7.100 ± 4.450 and 7.433 ± 4.783, respectively, at doses of 0.5 and 1 g/L of EAMO for the treated groups, and 8.767 ± 6.117 for the control group. Statistical analysis showed no significant difference ($P > 0.05$) between the treated batches and the control batch in terms of erythrocyte parameters (**Figure 5).**

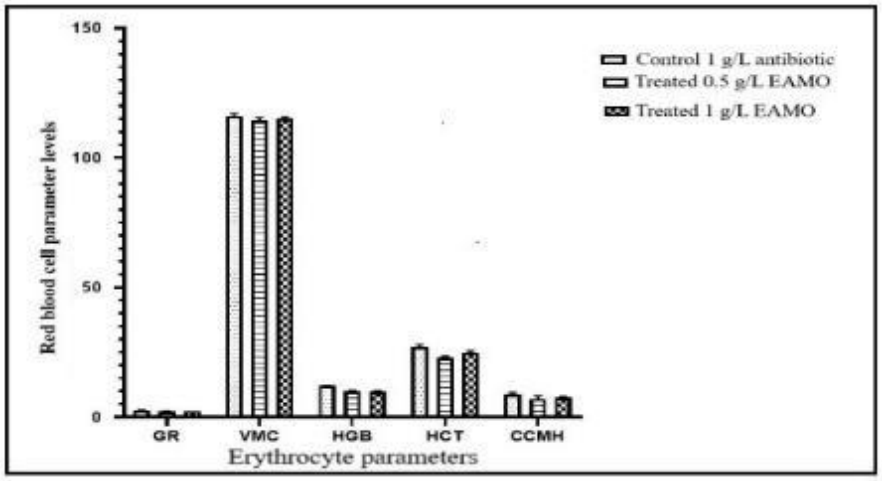

Figure 5 Effect of *Moringa oleifera* aqueous extract on erythrocyte parameters

3.4.3. Effect of EAMO on leukocyte parameters

Leukocyte parameters include white blood cells, lymphocytes, neutrophils, and platelets. The results showed that the levels of white blood cells, lymphocytes, neutrophils, and platelets in the treated chickens were higher than those in the control group. For white blood cells (WBC), the values were 26.85 ± 2.120 and 26.93 ± 2.197 for the groups treated with doses of 0.5 and 1 g/L of EAMO, respectively, and 24.73 ± 21.52 for the control group. Regarding lymphocytes (LYMP), the values were 6.267 ± 19.47 and 6.270 ± 10.46 for the groups treated with doses of 0.5 and 1 g/L of EAMO, respectively, and 4.920 ± 92.81 for the control group. For neutrophils (NEUT), these values are 14.22 ± 11.51 and 14.27 ± 11.47 for the batches treated with respective doses of 0.5 and 1 g/L of EAMO, and 12.10 ± 10.63 for the control batch. Statistical analysis showed a significant increase ($P<0.05$) in the treated batches compared to the control for these parameters. However, regarding platelet counts (PLT), those in the treated batches were lower (6.233 ± 18.10 and 6.217 ± 18.52) than that of the control batch (6.630 ± 18.53). Statistical analysis of these parameters did not show a significant difference ($P>0.05$) between these different groups in terms of platelet count (**Figure 6**).

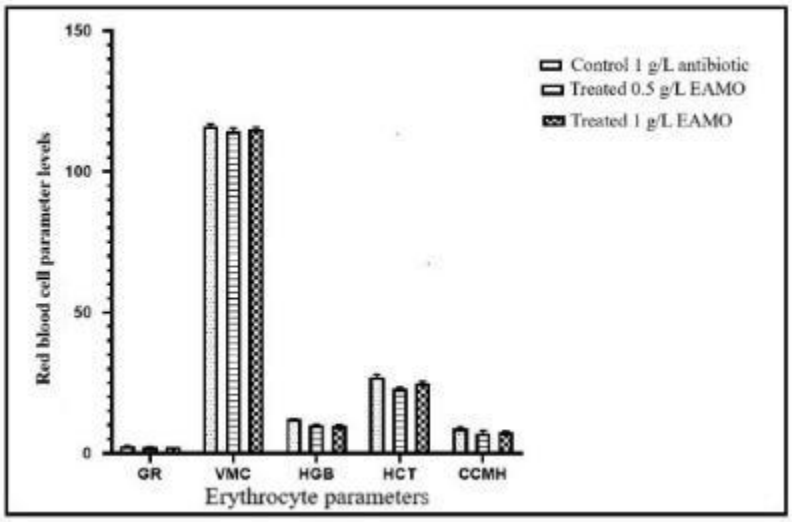

Figure 6 Effect of an aqueous extract of *Moringa oleifera* on leukocyte parameters

4. Discussion

Phytochemical screening of the aqueous extract of fresh *Moringa oleifera* leaves revealed the presence of sterols, polyterpenes, polyphenols, flavonoids, quinone compounds, alkaloids, and catechic tannins, as well as the absence of saponins. These results differ from those reported in [13]. Those authors, using an ethanol extraction, detected saponins and no catechic tannins in fresh *Moringa oleifera* leaves. This difference could be explained by the time of leaf harvest or by the solvent used for extraction. The study of acute toxicity via oral administration of the aqueous extract of

Moringa oleifera leaves, according to OECD Guideline 423 [12], showed that this extract caused no mortality in chickens at a single dose of 2000 mg/kg body weight. Consequently, the LD50 of the aqueous leaf extract is greater than 2000 mg/kg body weight in chickens. These results were consistent with those of [14], which showed that *M. oleifera* leaf powder was not toxic at a dose of 2000 mg/kg body weight. According to [15], a plant administered orally is considered non-toxic in animal testing when its LD50 is greater than 1000 mg/kg body weight. The results of this study revealed that the feed conversion ratio of the treated chickens was significantly lower ($P > 0.05$) than that of the control group, with values of 1.29 and 1.31, respectively, at doses of 0.5 and 1 g/L of EAMO, and 1.60 for the control group. According to [16], as the protein content of the feed increases, the feed conversion ratio decreases while weight gain increases. These results are similar to those reported in [17]. According to that author, the feed conversion ratio of Ross 308 broiler chickens treated with Zingiber officinale Roscoe powder was lower than that of the controls. The results revealed that the average weights of the treated chickens (1140 ± 0.1258 g and 1280 ± 0.0675 g) were significantly higher ($P < 0.05$) than those of the control group (1011 ± 0.0356 g). The improvement in live weight of the treated chickens could be explained by the high content of *M. oleifera* in vitamins (A, B, and C), calcium, iron, and protein. These results are consistent with those of [18]. According to the latter, *Moringa oleifera* leaf meal significantly increased ($P<0.05$) the body weights of the treated chickens compared to the controls. No mortality was observed during this study. These results would confirm the non-toxicity of the extract. Similar results were obtained by [19]. In that study, no mortality was observed after incorporating *M. oleifera* leaf meal into the chickens' diet. The results regarding the relative weight of the liver, kidney, heart, gizzard, lung, and bursa of Fabricius showed no significant difference ($P>0.05$) between the relative weights of these organs in the treated groups and the control groups. These results are consistent with those of [19]. According to this author, *M. oleifera* leaf meal showed no adverse effects on these same organs in chickens. Regarding biochemical parameters, the results revealed that urea and AST levels in the groups treated with EAMO at doses of 0.5 and 1 g/L, respectively, were significantly lower ($P < 0.05$) than those in the control group. Indeed, urea is a marker of renal function that indicates glomerular filtration rate, as well as the kidney's capacity for concentration and dilution [20]. The decrease in urea levels in chickens treated with the aqueous extract of *Moringa oleifera* leaves would suggest that this extract has a protective effect on the kidneys. The results of [21] differ from those of this study regarding urea levels. According to this author, the administration of ginger powder (Zingiber officinale Roscoe) showed no significant difference in urea levels . The difference between these results is likely due to the plant used, as it has different pharmacological effects. Regarding AST levels, the results showed that AST levels in chickens treated with EAMO were significantly lower ($P<0.05$) than those in the control group, with respective values of 133.51±60.67; 134.0 ± 60.85 (U/L) and 147.40 ± 67.07 (U/L). The aqueous extract of *Moringa oleifera* leaves did not show any toxic effect on liver function but rather a hepatoprotective action, which would explain the low AST levels. Indeed, AST is a marker of liver function. This decrease in AST in the treated chickens could be due to the hepatoprotective role of the flavonoids contained in the aqueous extract of *M. oleifera*. [7] observed in their studies that flavonoids had a protective effect on the liver. With regard to blood parameters, particularly erythrocyte parameters, the results showed no significant difference ($P > 0.05$) between the red blood cell count, mean corpuscular volume, hematocrit, and mean corpuscular hemoglobin concentration in the treated groups and the control group. These results could be explained by the fact that the aqueous extract of *Moringa oleifera* leaves does not have an adverse effect on erythrocyte parameters. These results corroborate those of [22]. According to this author, the incorporation of chili powder into the diet has no effect on hematological parameters. With regard to leukocyte parameters, the levels of white blood cells, lymphocytes, and neutrophils in the treated batches are significantly higher ($P<0.05$) than those in the control batch. This increase in white blood cells suggests that the aqueous extract of *Moringa oleifera* leaves enhances the subjects' immunity against diseases. These results are supported by those of [23]. These authors demonstrated that, following administration of an aqueous extract of *M. oleifera* leaves to laying hens, the white blood cell count increased in the treated groups, unlike in the control groups. Neutrophil counts showed a significant increase ($P < 0.05$) in chickens treated with the aqueous extract of *Moringa oleifera* compared to the control chickens. The increase in neutrophil levels may suggest that this extract possesses immune-boosting properties due to its tannins. Indeed, numerous studies have demonstrated the antimicrobial efficacy of tannins against various bacteria, viruses, and fungi [24].These results differ from those reported in [25], which observed lower levels of neutrophils following administration of *Allium sativum*, *Moringa oleifera* roots, and *Cymbopogon citratus* to *Isa Brown* chicks. This difference is likely due to the stressors experienced by broiler chickens. Regarding lymphocytes, the elevated levels in chickens treated with the aqueous extract of *Moringa oleifera* leaves suggest that this extract strengthens the immune system due to the saponins present in the extract. Indeed, research has shown that saponins isolated from plants used in traditional medicine possess antibacterial and antifungal properties [26]. These results contradict those of [23]. Those authors observed a decrease in lymphocytes following administration of the aqueous extract of *M. oleifera* leaves to laying hens. These differences may be related to the breed of experimental chickens.

5. Conclusion

In conclusion, the phytochemical analysis of the aqueous extract of *Moringa oleifera* leaves revealed the presence of certain chemical compounds that may explain its traditional use. The acute toxicity study showed that the aqueous extract of *Moringa oleifera* leaves is not toxic when administered as a single dose at 2000 mg/kg body weight. The extract increased the live weight of the chickens and reduced the feed conversion ratio. In terms of biochemical parameters, the aqueous extract of *Moringa oleifera* leaves reduced urea and AST levels in chickens. This extract increased white blood cell, neutrophil, and lymphocyte counts in chickens. The aqueous extract of *Moringa oleifera* leaves therefore appears to have strengthened the chickens' immune system. In light of these results, this plant could be used in the development of antimicrobial products for poultry farming.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflicts of interest regarding the publication of this paper.

Author's contribution

The first author collected, processed and drafted this article. The other authors read and corrected the manuscript. All the authors read and approved the final manuscript.

Statement of ethical approval

The animal tests were conducted in accordance with the Organisation for Economic Co-operation and Development (OECD) guideline 423

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