



Study of the antiallergic activity of the leaves of *moringa oleifera* (moringaceae) in the albino mouse *mus musculus*

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Abstract

Allergic diseases are constantly growing; however the efficiency of classical treatments is not total. Thus, new therapeutic tools are considered such as phytotherapy. The objective of this study was to demonstrate the effect of the aqueous extract of *Moringa oleifera* in mice. The phytochemical study revealed the presence of poly terpenes/sterols, polyphenols, flavonoids, tannins and alkaloids but also the absence of quinones and saponosides. The acute toxicity study at a single dose of 2000 mg/kg bw by the oral route revealed that the aqueous extract of *Moringa oleifera* is not toxic and would have an oral LD₅₀ greater than to 2000 mg/kg bw. The anti-allergic effect of *Moringa oleifera* leaf E.T.A was evaluated by observing the number of scratching in allergic mice treated orally with two different doses of this extract. A reduction of the number of scratching in mice treated with the extract was observed. This was confirmed by hematological analysis where a considerable increase in the number of immune cells and a decrease in the recruitment of these cells to inflammatory sites were observed. This confirms that the aqueous extract of *Moringa oleifera* has a dose-dependent antiallergic activity.

Keywords: *Moringa oleifera*; Antiallergic activity; Antihistaminic activity; Immune cells

1. Introduction

Allergy or hypersensitivity reaction is the consequence of an excessive immune response of the body following the encounter with a foreign substance namely the allergen [1]. Commonly known reactions of allergy are eczema, hives, allergic rhinitis, asthma attacks as well as food allergies which are presented as diseases. It is also a phenomenon of pathological exaggeration of the immune response, particularly the inflammatory responses, to an antigen that is generally foreign to the body. The purpose of inflammatory responses is to neutralize and eliminate the intruder and then repair tissue damage. Nevertheless, in the case of allergy, these tissue lesions are poorly or not repaired due to the immune imbalance giving rise to allergic inflammatory diseases of varying severity [2].

The prevalence of allergic diseases has been steadily increasing worldwide in recent years [3]. According to WHO [4], Nearly 30% of the world's population is affected by this disease compared to 3.8% in 1968 and by 2050, one in two people will suffer from this disease. It is now the fourth chronic disease in the world after cancer, AIDS and cardiovascular diseases [2].

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To cope with this disease, treatments have been put in place such as specific immunotherapy which has proven its effectiveness in allowing the avoidance of allergens, pharmacological treatments such as antihistamines and corticosteroids [5]. In addition, although pharmacological treatments relieve symptoms, they are accompanied by notable side effects such as liver cancer, pancreatic cancer [2] etc... Also in developing countries, the population faces many difficulties in treating allergic diseases including difficult access to health centers, lack of qualified personnel, expensive cost of treatments, lack of hospital infrastructure [6]. To face all these difficulties, several studies are conducted on the therapeutic power of plants against allergic diseases. Indeed some plants such as *Rubus suavissimus* [7], *Acorus calamus* and *Moringa oleifera* [8] have shown significant antihistaminic activities.

Thus, *Moringa oleifera* (Moringaceae), belonging to the Moringaceae family, was used to study its anti-allergic activity in the albino mouse *Mus musculus*. It is a plant widely used in the treatment of many pathologies because of its multiple therapeutic effects [9 ; 10]. *Moringa oleifera* is a valuable tree with several virtues. It is used as a nutritional supplement [11] but also as an anti-inflammatory in the treatment of acute inflammatory conditions [12].

2. Material and methods

2.1. Material

2.1.1. Plant material

As plant material, leaves of *M. oleifera* collected at Université Felix Houphouët Boigny in September 2020 and identified at the Centre National Floristique (CNF) were used.

2.1.2. Animal material

Thirty-one (31) female mice of Swiss strain, eight (08) weeks old and weighing between eighteen (10) and twenty-two (22) grams, were used for the different studies. Six (06) nulliparous and non-pregnant mice were used for the acute toxicity test (OECD 423) and twenty-five (25) mice for the pharmacological test. All these mice were raised in the vivarium of the Ecole Nationale Supérieure (ENS). The rearing environment was characterized by a photoperiod and a natural temperature suitable for the well-being of the mice. The food consisted of bread, pellets manufactured by IVOGRAIN and water. The mice were placed in polypropylene cages with a wire mesh roof and a wood shavings bedding on the bottom. These cages were regularly cleaned and the bedding was changed daily.

2.1.3. Chemical material

Egg white containing ovalbumin, maalox was used as a supplement containing aluminum hydroxide and AERIUS containing desloratadine known as a drug treating allergic diseases.

2.2. Methods

2.2.1. Extraction

After harvesting the leaves of *M. oleifera*, they were dried in an air-conditioned room at 16°C for three weeks. After drying, they were ground to obtain a fine powder which was used for extraction. Fifty (50) grams of fine powder was mixed with 1L of distilled water in a blender. The mixing was done once every three minutes on three occasions. The resulting solution was filtered twice through a white cloth and five times through absorbent cotton to remove residues. The filtrate obtained was put in the oven at 50°C for forty-eight (48) hours to obtain a dry extract [13].

*Yield calculation:

The extraction yield is the amount of extract obtained from the plant powder. It is expressed as a percentage and calculated according to the following formula:

$$R = \frac{m}{M} \times 100$$

R: extraction yield; **m:** mass the extract; **M:** mass of the fine powder.

2.2.2. Phytochemical screening

A qualitative phytochemical screening was carried out on the dry extract samples obtained following the aqueous extraction. Analytical techniques described in the works of [14 ; 15] were used to highlight the presence of chemical compounds such as polyterpenes, polyphenols, flavonoids, tannins, alkaloids, quinones and saponosides.

2.2.3. Acute toxicity

The acute toxicity of the plant was carried out with the extract at the dose of 2000 mg/kg bw on female mice. The mice were fasted before administration of the extract. Food intake was suppressed and water intake was maintained for 3 to 4 hours. After the fasting period, the animals were weighed and the extract was administered. Then, they were again deprived of food for 1 to 2 hours in order to observe their behavior through the following signs: apathy, excitement, breathing disorders, refusal of food, oral and/or nasal bleeding, abdominal pain (contortion), convulsion, trembling, diarrhea, coma and death.

This observation was made per mouse for the first 30 minutes and then regularly for all mice for the first 24 hours after treatment. The individual weight of each animal was taken once every two days for 14 days of observation [16].

2.2.4. Induction of the allergic reaction

The induction of the allergic reaction was done with 25 mice divided into five batches of 5 mice. Lot 1 included the control mice, gavaged with 10mL/kg body weight of NaCl 9‰. Batches 2; 3; 4 and 5 contained the mice given 0.15mL of egg white; 0.05 mL of maalox and 0.05 mL of NaCl. The mixture of these three substances constituted the allergic solution that was administered intraperitoneally. The first sensitizing dose was administered on day 0. The second and third sensitizing doses of the allergic solution were administered on day 3 and day 6. Mice were individually weighed throughout the induction period.

Effect of *M. oleifera* extract on allergic mice

The anti-allergic effect of *M. oleifera* extract on mice was studied on mice that reacted favorably to allergy induction. They were grouped into four batches and one batch of healthy mice. Batch 1 consisted of negative control (NC) mice that were administered distilled water at 10 ml/kg body weight. Batch 2 consisted of positive control (PC) mice that were also administered distilled water at 10 ml/kg body weight. Batch 3 included mice, referent controls (RC), who received the desloratadine molecule at a dose of 5 mg/kg body weight. Lot 4 included mice treated with *M. oleifera* E.T.A. at a dose of 200 mg/kg body weight and lot 5, mice treated with *M. oleifera* E.T.A. at a dose of 400 mg/kg body weight.

Observation of the number of scratches of the animals was done with a camcorder in an isolated, lighted area for 15 minutes. It started at the time of the third sensitizing dose and ended on the last day of the pharmacological study 30 minutes after the sensitizing dose.

Determination of the level of different immune cells

The determination of the level of the different immune cells was performed immediately on the blood samples taken on the last day of the pharmacological study in tubes containing the anticoagulant Ethylene-Diamine-TetraAcetic (EDTA). The analyses were done according to the Numerical Blood Formulation (NBF).

2.2.5. Statistical processing

The statistical processing of the results was carried out using GraphPad Prism 8 software. The analysis of variance (ANOVA) was used to compare the means obtained. This software was also used to draw the graphs.

3. Results

3.1. Phytochemical screening

The results of the photochemical analysis allowed to highlight the presence or absence of some groups of compounds of therapeutic interest. The presence of chemical compounds such as polyterpenes, polyphenols, flavonoids, tannins and alkaloids was relatively abundant. An absence of quinones and saponosides was observed.

3.2. Acute toxicity

The oral administration of 2000 mg/kg of body weight (bw) of E.T. A of *M. oleifera* to the animals did not result in death or clinical signs of any form of toxicity. During the 14 days of observation, there was no significant difference in weight gain in mice given the extract at a dose of 2000 mg/kg bw compared to controls (Figure 1).

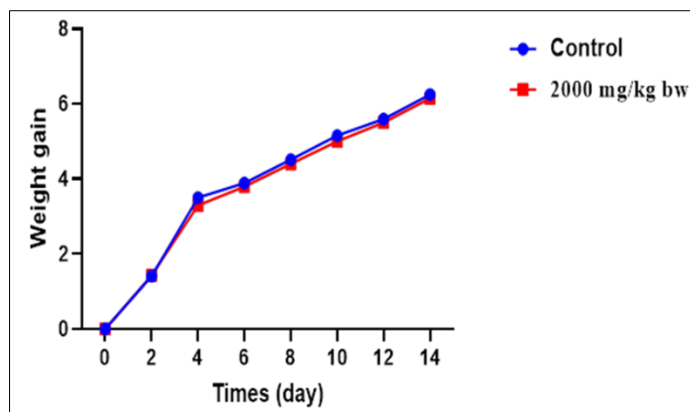


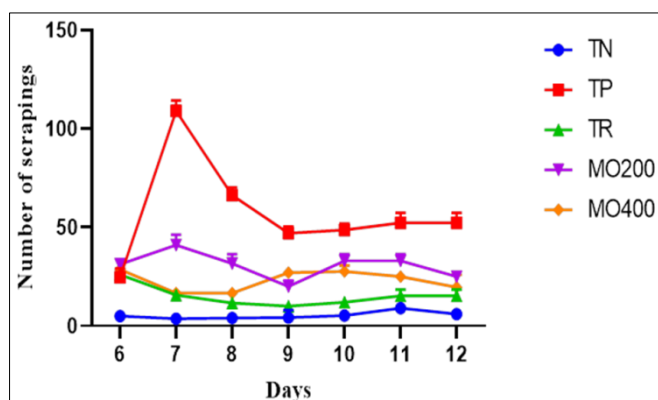
Figure 1 Variation in weight gain of mice as a function of time

3.3. Induction of the allergic reaction

The results of the induction were used to demonstrate the presence or absence of allergy in the mice. The clinical sign taken into account in this experiment was the number of scratches evaluated 30 minutes after each experiment. Figure 2 shows the number of scratches of the mice during the pharmacological study.

Allergic signs began on day 6, the last day of induction, and ended on day 12 of the pharmacological study. On day 6, a highly significant difference ($p < 0.0001$) was observed between the number of scratches in treated mice (27.67 ± 0.004) and in control mice (5.33 ± 0.68).

Furthermore, on the 7th day of the experiment, there was a highly significant increase ($p < 0.0001$) in the number of scratches of the allergic control mice, from (24.67 ± 0.001) on the 6th day to (109 ± 0.006) on the 7th day and then to (47 ± 0.002) on the 9th day. This number did not change much until the last day of the experiment (52.33 ± 0.004).



TN: Negative control mice receiving distilled water at 10 ml/kg body weight; PT: Positive control mice receiving distilled water at 10 ml/kg body weight; RT: Referent control mice received desloratadine molecule at a dose of 5 mg/kg body weigh; MO200: Mice treated with *M. oleifera* E.T.A. at 200 mg/kg body weight; MO400: Mice treated with *M. oleifera* E.T.A. at 400 mg/kg body weight.

Figure 2 Variation in the number of scrapings after treatment with the different doses of *M. oleifera* and the desloratadine molecule

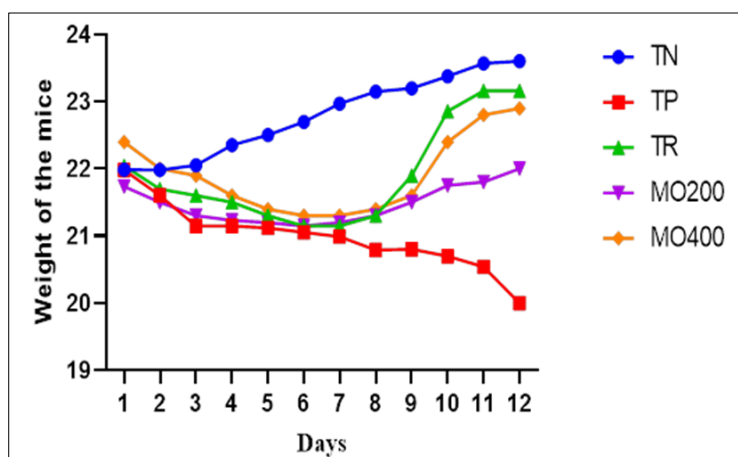
During the experiment, the number of scratching of mice treated with the reference molecule decreased from (26 ± 0.01) on day 6 to (15.67 ± 0.02) on day 7 and to (15.33 ± 0.01) on day 12. The percentage reduction in the number of scratching relative to the reference molecule was 69.56%.

For the mice treated with the plant extract at a dose of 200 mg/kg bw, a slight increase in the number of scratches was observed on day 7. This number increased from (31.33 ± 0.003) on day 6 to (41 ± 0.02) on day 7. It then decreased to (25 ± 0.045) on day 12. For mice treated with the plant extract at the dose of 400 mg/kg bw, the number of scratching decreases from (28.67 ± 0.067) on day 6 to (23 ± 0.01) on day 7 and then to (19.67 ± 0.001) on day 12. The percentage reduction in the number of scratching in mice treated with 200 and 400 mg/kg bw was 25.33% and 45.76%, respectively.

3.4. Effect of *M. oleifera* extract on allergic mice

Figure 3 shows the changes in weights of mice during the pharmacological study. The weight of control (TN) mice was stable throughout the observation with a mean weight of (21.99 ± 0.01) on day 0 and (23.6 ± 0.01) on day 12. However, in the mice that received ovalbumin and aluminum hydroxide, there was a decrease in weight throughout the induction of allergy until the second day of pharmacological treatment, which corresponds to day 8 of the experiment in treated rats. Also, the weight of mice treated with desloratadine (TR) and mice treated with 200 and 400 mg/kg CP of the aqueous extract, decreased from (22.04 ± 0.01) ; (21.74 ± 0.02) and (22.40 ± 0.01) to (21.15 ± 0.02) ; (21.20 ± 0.03) and (21.3 ± 0.003) grams on the 6th day respectively. From day 7 to day 8 of pharmacological treatment, the weights increased from (21.3 ± 0.03) ; (21.3 ± 0.04) and (21.4 ± 0.007) grams to (21.90 ± 0.03) ; (21.5 ± 0.012) and (21.60 ± 0.01) grams, respectively (Figure 3). From day 8 onward, there was an increase in the weight of desloratadine-treated mice and mice treated with 200 and 400 mg/kg CP with respective weights of (23.16 ± 0.05) ; (22.00 ± 0.01) and (22.90 ± 0.1) grams on day 12 (Figure 3).

In the untreated allergic mice (TP), a decrease in weight was observed throughout the experiment. Their average weight decreased from (21.98 ± 0.001) on the first day to (20 ± 0.01) on the last day (Figure 3), a reduction of 1.98 grams.



TN: Negative control mice receiving distilled water at 10ml/kg body weight; PT: Positive control mice receiving distilled water at 10 ml/kg body weight; RT: Referent control mice received desloratadine molecule at a dose of 5 mg/kg body weight; MO200: Mice treated with *M. oleifera* E.T.A. at 200 mg/kg body weight; MO400: Mice treated with *M. oleifera* E.T.A. at 400 mg/kg body weight

Figure 3 Effect of total aqueous extract and the molecule desloratadine on the weight development of allergic mice

3.5. Determination of the rate of different immune cells

The effect of the extract on the immune cells during the allergic reaction was observed on the 12th, last day of the experiment. For this study, a blood sample was taken from each mouse of the different batches. The results are presented in figures 4; 5; 6; 7; 8.

At the level of white blood cells or leukocytes, there was a significant decrease ($p < 0.01$) in the number of leukocytes in the untreated allergic control mice (TP) which is $(8.67 \pm 0.63 \cdot 10^3 / \mu\text{L})$ compared to the healthy control (TN) $(12.69 \pm 0.5 \cdot 10^3 / \mu\text{L})$. There was also a significant difference ($p < 0.01$) in the leukocyte count between allergic mice treated with the extract at 200 mg/kg bw $(15.37 \pm 0.06 \cdot 10^3 / \mu\text{L})$ and 400 mg/kg bw $(18.37 \pm 0.4 \cdot 10^3 / \mu\text{L})$ and PTs. Regarding the leukocyte count of allergic mice treated with the reference molecule (RT) $(26.48 \pm 0.3 \cdot 10^3 / \mu\text{L})$, there was also a significant difference ($P < 0.0001$) between RT and TP (Figure 4).

The neutrophil count of allergic mice $(11.17 \pm 0.2 \cdot 10^3 / \mu\text{L})$ was significantly higher ($p < 0.0001$) than that of healthy control mice $(6.33 \pm 0.13 \cdot 10^3 / \mu\text{L})$. They were $(5.47 \pm 0.1 \cdot 10^3 / \mu\text{L})$, $(15 \pm 0.02 \cdot 10^3 / \mu\text{L})$, $(11.59 \pm 0.3 \cdot 10^3 / \mu\text{L})$, and $(13.27 \pm 0.2 \cdot 10^3 / \mu\text{L})$, respectively, for untreated and molecule- and extract-treated allergic mice at 200 and 400 mg/kg bw. Thus,

the neutrophil count of the untreated mice was significantly low ($p < 0.0001$) compared to that of the mice treated with the reference molecule and the extracts of the 200 and 400 mg/kg doses of bw (Figure 5).

The monocyte count of allergic mice ($5.26 \pm 0.3 \cdot 10^3 / \mu\text{L}$) was significantly higher ($p < 0.0001$) than that of healthy control mice ($3.2 \pm 0.5 \cdot 10^3 / \mu\text{L}$). It was ($2.067 \pm 0.01 \cdot 10^3 / \mu\text{L}$), ($7.8 \pm 0.2 \cdot 10^3 / \mu\text{L}$), ($4 \pm 0.6 \cdot 10^3 / \mu\text{L}$), and ($6.33 \pm 0.1 \cdot 10^3 / \mu\text{L}$), respectively, for untreated allergic mice, molecule-treated mice, and extract-treated mice at doses of 200 and 400 mg/kg bw (Figure 6).

The eosinophil levels of healthy control mice, untreated allergic mice and mice treated with the molecule and extract at 200 and 400 mg/kg bw were ($0.26 \pm 0.01 \cdot 10^3 / \mu\text{L}$), ($0.20 \pm 0.01 \cdot 10^3 / \mu\text{L}$), ($0.26 \pm 0.02 \cdot 10^3 / \mu\text{L}$), ($0.22 \pm 0.004 \cdot 10^3 / \mu\text{L}$), and ($0.23 \pm 0.0025 \cdot 10^3 / \mu\text{L}$) respectively. The observed difference was not highly significant as shown in Figure 7.

The basophil levels of healthy control, untreated allergic mice and those treated with the molecule and extract at 200 and 400 mg/kg bw were ($0.23 \pm 0.02 \cdot 10^3 / \mu\text{L}$), ($0.20 \pm 0.001 \cdot 10^3 / \mu\text{L}$), ($0.3 \pm 0.01 \cdot 10^3 / \mu\text{L}$), ($0.2 \pm 0.04 \cdot 10^3 / \mu\text{L}$) and ($0.23 \pm 0.001 \cdot 10^3 / \mu\text{L}$), respectively. There was no significant difference between the different treated batches and the healthy control batch Figure 8.

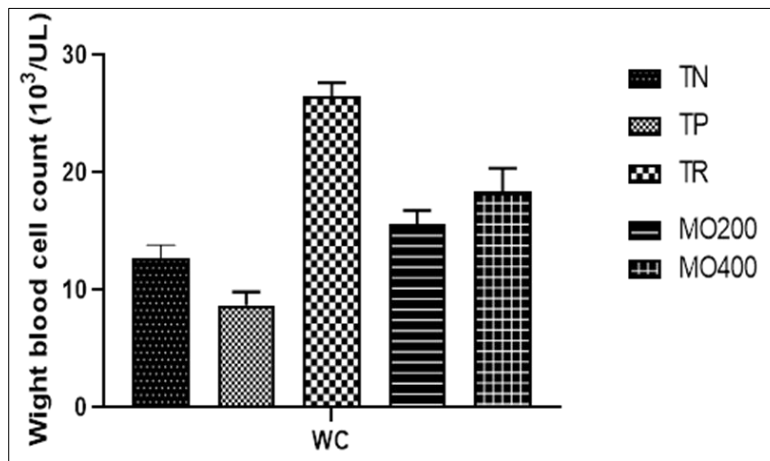


Figure 4 White blood cell levels in the blood of mice treated with different doses and the reference molecule

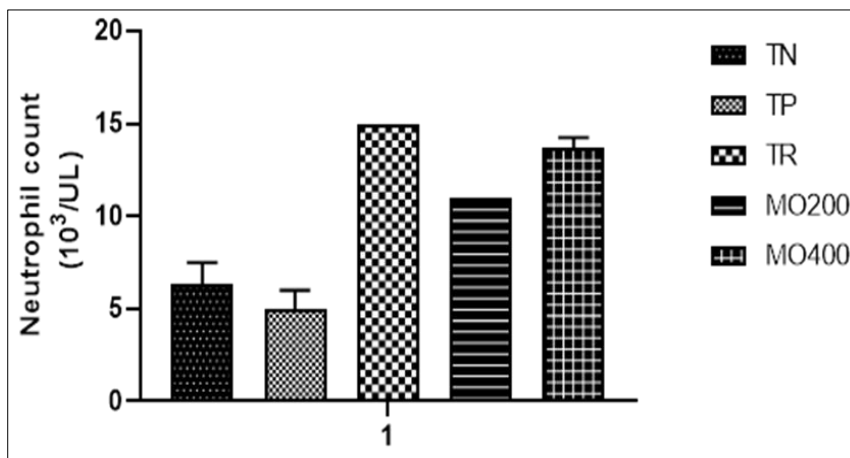


Figure 5 Neutrophil levels in the blood of mice treated with different doses and the reference molecule

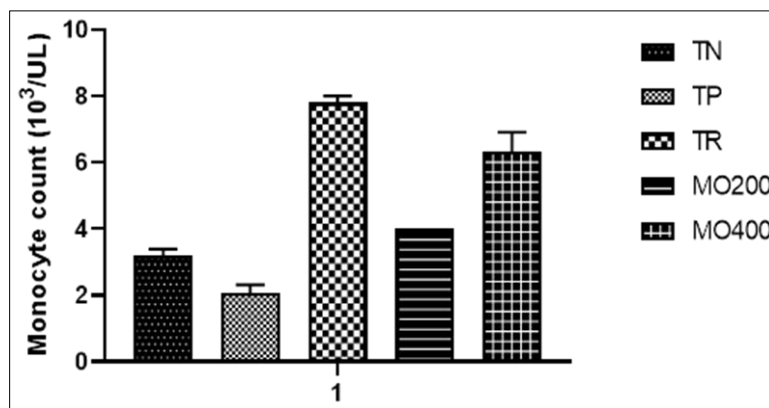


Figure 6 Monocyte levels in the blood of mice treated with different doses and the reference molecule

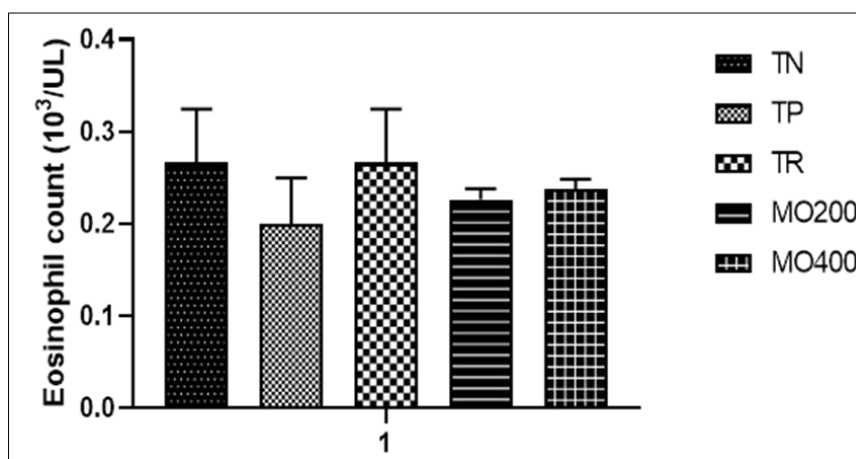


Figure 7 Eosinophil levels in the blood of mice treated with different doses and the reference molecule

4. Discussion

The results of the phytochemical screening showed the presence of secondary metabolites such as polyphenols, tannins, polyterpenes, flavonoids, alkaloids. Quinone derivatives and saponosides were not found in this extract. These results are similar to Sabah and Amina, [17]. On the other hand, Bennett *et al.* [18] found quinone and saponoside derivatives in *M. oleifera* leaves in addition to these chemical groups. This variability in results found at the level of these major groups of bioactive molecules can be explained by the different environmental and seasonal conditions [19]. It can also be explained by plant genetics, drying method, leaf maturity stage [20], and different extraction methods used [21]. These chemical compounds in the extract also have antioxidant [22], anti-inflammatory, anti-allergic, hepatoprotective and antispasmodic properties [23 ; 24]. This would explain its common use in traditional medicine.

This study consisted in evaluating the toxicity of the plant. The acute toxicity results of *Moringa oleifera* E.T.A in mice showed that E.T.A administered orally resulted in no mortality, no clinical signs at the single dose of 2000mg/kg bw. These results are similar to those of Awodele *et al.*, [25] on the total aqueous extract of *M. oleifera* leaves. According to these authors, the LD₅₀ would be 6400mg/kg CP by the oral route. These results are in agreement with the OECD 423 guideline which would indicate that the LD₅₀ would be higher than 5000 kg/mg of bw.

Allergy induction by the allergen showed clinical signs such as itching and weight loss. The work of Perrier [26] also showed that the induction of allergy to animals resulted in excessive weight loss and itching. These results were also observed in animals induced to allergy by egg ovalbumin by epicutaneous route according to the work of Wavrin and *al.*, [27]. Indeed, there was a significant increase ($p < 0.0001$) in the itch rate in untreated allergic mice (27.67 ± 0.004) as well as a weight loss compared to healthy control mice (5.33 ± 0.68). The latter observation could be explained by an intense immune response resulting from the release of biologically active mediators such as histamine by IgE-activated mast cells and basophils [28; 29].

As for the effect of E.T. A and the reference molecule in allergic mice, there was a significant ($P < 0.0001$) reduction in the rate of itching in mice treated with the extract compared to untreated allergic mice (positive controls). The reference molecule desloratadine has a higher rate of reduction in the number of scratching than the different doses of E.T. A. The reduction rate result of desloratadine is significantly similar to hydrocortisone (74%) [30], which is an anti-allergic drug. Regarding E.T.A, it has a dose-dependent effect with a maximum at the 400 mg/kg bw dose. These results are similar to those of Shiwen et al, [30] when treating an allergic reaction with glycyrrhizic acid. Thus, E.T.A from *M.oleifera* leaves would behave like glycyrrhizic acid allowing the regulation of TH cell differentiation. This decreases the high level of TH2 related cytokine (IL-4) secretion to restore the balance of the TH1 / TH2 immune system. Also Treben, [31] in this work on the anti-allergic effect of bramble (*Rubus suavissimus*) showed that the antihistamine activity of bramble was due to the presence of flavonoids. Therefore, the antihistaminic effect of *M.oleifera* extract could be explained by the activity of secondary metabolites.

Hematological analysis indicated a significant reduction in leukocytes, neutrophils and monocytes in untreated allergic mice. These results are similar to those of Halbwachs-Mecarelli, [32] during pre-sensitization to ovalbumin in mice. This reduction of immune cells could be explained by the increased recruitment of immune cells to the site of entry of the allergen which is characteristic of an inflammation installed after a hypersensitivity reaction. Also another study on the mouse model exposed to *Aspergillus* showed similar results on the rate of leukocytes, monocytes and neutrophils [33]). In the mice treated with the different doses and the reference molecule, an increase in the rate of leukocytes, monocytes and neutrophils was observed, although the rate of immune cells in the batch treated with the reference molecule was higher than in the different doses of E.T.A. from *M. oleifera* leaves. These results are similar to the results of the work of Amouzou and Maïga, [2] who showed the effect of plant mixture extract on the allergic reaction. Indeed, this increase in the rate of immune cells would be due to the increase in immunogenicity or the increase in immunogenicity of the allergen and a decrease in the recruitment of cells to inflammatory sites. This suggests that E.T.A. from *Moringa oleifera* leaves would be at the origin of this increase in the immune response, and therefore would have an immunogenic power.

5. Conclusion

Phytochemical screening of *Moringa oleifera* leaf extract revealed the presence of polyterpenes/sterols, polyphenols, tannins, alkaloids, flavonoids and an absence of saponosides and quinones. The acute toxicity study of the total aqueous extract of *M. oleifera* showed that this extract, when administered orally, was not toxic with an LD₅₀ between 2000 mg/kg bw and 5000 mg/kg bw ($2000 < LD_{50} < 5000$ mg/kg bw).

When administered to allergic mice, E.T. A of *Moringa oleifera* leaves showed antihistaminic properties. Thus, its ability to inhibit the allergic reaction would be suggestive of the presence of chemical compounds with antihistaminic potential. This anti-allergic property could also be due to the immunogenic capacity of the extract in the organism, i.e. its capacity to accentuate the immune response in order to defend the organism against antigens.

Compliance with ethical standards

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- Biosciences Training and Research Unit;
- Medical Education and Research Unit.

Disclosure of conflict of interest

All authors have seen and approved the manuscript as submitted. All authors participated in the work in a substantive way and are prepared to take public responsibility for the work. All authors of the manuscript have no conflict of interests to declare. The manuscript submitted to the journal is not copied or plagiarized version of some other published work. All the data taken from other sources is written in authors own language and properly cited. The text, illustrations, and any other materials included in the manuscript do not infringe upon any existing copyright or other rights of anyone.

Statement of ethical approval

The animal material used for this study, were used according to the Organisation for Economic Co-operation and Development (OECD) guideline 423.

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